The Role of Cavitation in the Water Relations of Irrigated and Non-Irrigated Apple Trees.

by

Douglas W. Brough, B.Sc.

Ph.D.

University of Edinburgh

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Declaration.

This thesis has been composed by myself from the results of my own work, except where acknowledged to the contrary.

To Alison

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Abstract.

The aim of the present study was to assess whether diurnal and seasonal declines in stem density, reported by various authors, could be detected in apple trees, and whether they could be attributed to embolisms formed by cavitations in the xylem water columns. A secondary aim was to observe the effect of irrigation, overhead mist, and ground sprinklers, on plant water stress and its possible use in decavitation or in the prevention of cavitation.

Measurements of stem density, leaf water potential, leaf relative water content and transpiration were made in an apple orchard on a diurnal and seasonal basis throughout the 1982 growing season. To interpret any changes in stem density, a series of observations were conducted using an acoustic detection system to measure cavitation in conjunction with a gamma probe to measure stem density.

It was found that xylem density varied both diurnally and seasonally, a proportion of the variation being attributed to cavitation, and a proportion to changes in the amount of water held in "storage" tissues within the xylem. Irrigation studies indicated that overhead mist irrigation was more effective in reducing plant water stress than ground sprinkler irrigation, resulting in an increase in fruit growth and presumably a reduction in the level of cavitation within the misted trees.

Chapter (1) Introduction.

Several authors, Gibbs (1935), Clarke and Gibbs (1957), and Markstrom and Hann (1972) have reported that the stem water content of trees varies both diurnally and seasonally.

> "The variations are correlated with tension changes and no doubt also with transpiration. "
>
> (Gibbs 1957).

The conclusion that may be drawn from such studies, is that a proportion of water-filled xylem conduits within a tree may, under conditions of stress, undergo cavitation, so becoming gas-filled and thus non functional. The primary aim of the present study is to investigate changes in stem water content in apple trees, and assess the consequence of cavitation for the normal diurnal and seasonal cycles of the trees.

Cavitation of water columns within the xylem, is a phenomenon whose very existance is still considered by many to be a matter of conjecture, due to the lack of any sensor for detecting unequivocally, the individual embolisms in the living plant.

The mechanism by which water ascends from the soil, through a plant to the atmosphere, has been the subject of much discussion since the beginnings of plant physiology. Various theories have been proposed since the work of Hales in the early eighteenth century, with the most widely accepted being that of Dixon and Jolly (1894) and Dixon (1914) known as the "Cohesion Hypothesis". In the modern interpretation, this hypothesis proposes that transpirational water loss from the cell walls of the leaf sub-stomatal cavity lowers the cell water potential, and gives rise to a gradient of water potential along continous water columns. These continous water columns, within the xylem, extend from the soil to the cells of the leaf and exist due to the cohesive nature of water molecules which tend to "cling" together by the process of hydrogen bonding. It is along this gradient of water potential that water uptake takes place.

Two major assumptions are contained within this theory,

a) that continous columns of water, able to withstand tensions without cavitating, exist between the roots and leaf cells,

b) the surface tension forces of the air-water interfaces in the pores of the cell walls, exceed the tensions of the water column, and so prevent the entry of air into the xylem. The necessity for continous water columns poses problems when, in experiments involving overlapping sawcuts through a tree, the tree continues to grow, despite the appearance of all the conduits having been severed. The theory can be sustained however if one accepts that water can travel laterally through the xylem and bypass any embolisms or cuts.

The tensions that water columns can withstand before cavitating is also a matter of much speculation. Although water is a liquid, it possesses significant tensile strength, caused by the attraction between the molecules, itself the result of hydrogen bonding. Thus in a capillary tube, a column of water can exist which is entirely supported by a meniscus, due to the surface tension effects at the air-water interface. Calculations of the theoretical tensile strength of pure water, i.e. the tension at which a column will fracture, range from 621 to 1850 MPa, depending upon the method of calculation (Milburn 1979), tensions which are never reached within the xylem of a living plant. These calculations have led various authors to state that cavitation cannot occur within the xylem under normal conditions of growth (Oertli 1971), and therefore the presence of embolisms, as indicated by experimental evidence which will be discussed later, can only be explained by some other mechanism than simply exceeding the tensile strength of the column. If it is accepted that the water within a xylem vessel is not "pure", but contains dissolved gases, for example carbon dioxide from respiration of the living cells in the xylem medullary rays, then a mechanism for column fracture may be provided by some form of nucleation fracture. The theory of nucleation fracture proposes that a cavitation begins as a bubble containg either a gas or essentially a vacuum, which forms either in a bulk liquid (homogeneous nucleation), or at a solid-liquid interface (heterogeneous nucleation), and grows as the fracture occurs.

Let us first consider homogeneous nucleation. There are two main sites for bubble formation within a bulk liquid: free gas bubbles which arise at a surface and migrate into the bulk phase, and bubbles which form de novo (Pickard 1981). The former is thought to be important in rapidly moving liquids (Winterton 1977), but will not be important within the xylem, due to the lack of a suitable "surface", and the "slow moving" flow of water through the vessels. De novo formation of bubbles is a statistical possibility (Pickard 1981). Where density fluctuations are present in a liquid, there is a probability that the liquid might vapourise at the fluctuation and so produce a growing bubble. These fluctuations are however a property of fast turbulent flow, which does not exist within the xylem, and so are unlikely to be the cause of cavitation in plants.

An alternative to bulk phase nucleation is that of interface or heterogeneous nucleation, where fractures occur due to the expansion of gas nuclei, which exist trapped in conical cavities in a solid surface which is in contact with a liquid. A hydrophobic crack with a diameter of 0.14 μm, can contain a gas nucleus which will nucleate at room temperature and a tension of only 1 MPa (Pickard 1981), conditions which readily occur under the normal course of events. Unfortunately the conditions for heterogeneous nuclei and sites appears hard to fulfil within the xylem. From earliest differentiation the xylem should be water filled so that nuclei will not be available for embolism, and any subsequent entry of air should be prevented by the ultrafiltration properties of the membranes. It is possible however that,if as proposed earlier, the xylem sap contains dissolved gases from respiration, then these dissolved gases might come out of solution under certain circumstances and be trapped in "cavities" in the xylem walls and so become heterogeneous nuclei.

Many of the opponents of the theory of xylem cavitation propose the theoretical tensile strength of water as a conclusive argument for denying the existence of gas embolisms within the xylem of a living plant. It has been indicated that cavitation may depend, not upon theoretical calculations of cohesive strength, but upon the ease of bubble formation. Various mechanisms for bubble formation have been shown to exist in model systems.

a) The absorbtion of ionising radiation is known to reduce tensile strength (Greenspan and Tschiegg 1967), and so set up nucleation. This mechanism has been experimentally evaluated in living plants (Milburn 1973a), and found to be undetectable.

b) Tribonucleation is the mechanism where cavitation is induced when two solid surfaces, immersed in a liquid under tension, are rubbed together. This system appears unlikely to occur in plants, as two solid surfaces do not "rub together" inside a xylem vessel.

c) The "bends" mechanism occurs when a diver who has been subjected to high gas pressures returns to the surface too quickly, so that the dissolved gas in the blood nucleates in the vessels and tissues. The occurence of bends in divers does not normally occur below 0.2 MPa, but as homogeneous nucleation, upon return to atmospheric pressure, requires a dissolved gas pressure in excess of 130 MPa (Hemmingsen 1970), some other mechanism to bring about the bends must be involved.

Although the mechanisms for bubble formation described above cause embolisms in model systems, their relevance to xylem cavitation is questionable. Despite the theoretical problems concerning the mechanism for cavitation, experimental evidence would tend to suggest, that at least a proportion of xylem conduits undergo embolism under conditions of water stress. Crafts (1939) reported that although the water columns of dissected xylem vessels were originally intact, embolism occurred when they were allowed to lose water. Experiments involving the use of dyes in the transpiration stream indicated abnormal patterns of water transport in trees which had been subjected to water stress (Greenidge 1955a), which might indicate cavitation of xylem conduits under stress. Using apparatus for detecting cavitation acoustically, Milburn and Johnson (1966) proposed a relationship between water stress levels in leaves and the occurrence of "clicks", which were purported to be shockwaves resulting from vibrations within the xylem conduits. As tension builds up within the xylem vessels due to water stress, the conduit walls are drawn inwards forming an "hourglass" shape. The energy which is then stored within the walls is released when the vessel cavitates so causing the clicks. Johnson (1977) presented photographic evidence showing the "hourglass" vessel walls when the sap was under tension, whereas the walls of turgid cells were "straight". Other possible explanations for the cause of these clicks were put forward by Milburn and Johnson and will be discussed later.

In an attempt to discover which vessels, if any, cavitate within a stem, Milburn and MacLennan (1978) used a vacuum injection technique in a stressed stem. In this system water filled vessels were identified by cutting the stem under a warm gelatine solution containing indian ink, so that the conducting vessels would draw up this solution and be stained black. When this solution has solidified, cavitated conduits can be injected under vacuum with a different coloured gelatine solution, so that the stem can be sectioned and the cavitation pattern investigated. As cavitation cannot be observed directly in the living plant, a method for investigating embolism formation within intact xylem is required to support or disprove the "acoustic probe" experiments.

In Plantago (Milburn and McLauchlin 1974), Ricinus (Milburn 1975), and Apple (West and Gaff 1976), cavitation, as detected by the acoustic probe, occurs at rather low tensions i.e. 0.5 - 1.5 MPa, which commonly occur within plant systems on moderately sunny days in the field. Thus cavitation may be a normal occurrence in the "life" of a plant. If cavitation is indeed a normal event in the diurnal cycle, then some mechanism must exist to replace or repair the embolised vessels.

Once an embolism has been initiated within the xylem, and provided sufficient tension exists, the bubble will expand until the conduit element is filled. The bubble is prevented from expanding further by the pit membranes, where menisci will form in the pores and so prevent further movement of the air-water interface. Various mechanisms to "repair" this event have been proposed.

a) If the xylem conduit is narrow, as in a conifer tracheid, once the sap tension has lowered to around 0 MPa, the effect of surface tension could overcome the internal pressure of the bubble. This would cause the bubble to redissolve into solution, and restore the conduit. Larger vessels could not be restored in this way, and require some other mechanism.

b) Root pressure (Kramer 1969, Milburn and Davies 1973 and Zimmermann 1971), is the term given to the positive pressure generated at the cut surface of a stem, and has been proposed as a source of pressure for decavitation. In general, these pressures are of the order of 0.1, 0.2, or even up to 0.6 MPa (White 1938), which, although they might not be sufficient to refill a cavity at the top of a very tall tree, would be capable of fully pressurising a two meter tall apple tree. Root pressure is also associated with the "bleeding" phenomenon exhibited by many species including *Malus*, which might give some indication of the considerable pressurisation of an apple tree which exists for a good proportion of the growing season (Palmer pers. comm.).

c) Injection i.e. water entering the xylem via the cuticles and the stomatal pores of wet leaves is proposed as a mechanism for cavitation reversal (Daum 1967). It is unlikely that this system could be the sole mechanism for decavitation as it would require rain or dewfall on a daily basis. Inspection of apple leaves, which are known to readily inject when floated on water, did not show injection to any great extent, during periods of mist irrigation.

d) Living parenchymatous ray tissue within the xylem, might permit the transfer of water to embolised vessels (Edwards 1980). This mechanism might occur if diurnal variation in water potential was accompanied by a phase lag in osmotic adjustment of the parenchyma. When transpiration is reduced, the low osmotic potential would induce water movement into the cells, which would be expelled when osmotic adjustment was made, thus providing a small positive pressure for decavitation.

When a cavitation event first occurs, the bubble may contain some water vapour, or to all intents and purposes, a vacuum, which will eventually be replaced by diffusion, with other gases dissolved in the xylem sap. If a positive pressure is applied, by whatever means, before significant diffusion occurs, then this pressure will reduce the size of the bubble until it dissolves, and thus restores the vessel.

If cavitation is a direct result of water stress, and this stress can be relieved by reducing transpiration, or by providing a sufficient water store to meet transpirational demand, then cavitation within a tree or other plant may be reduced by various irrigation techniques.

In the present study, two methods, an overhead misting system and a ground sprinkler system are employed. The application of mist in the field has been tested by several authors including Carolus (1971) in the U.S.A., and Goode (1979) in England. This technique is not aimed at increasing the soil moisture content, but rather lowering of plant temperatures by evaporation, and in warmer climates eg. in California, raising the air humidity within the canopy, and so facilitating a decrease in transpiration. The second system sprinkles water over an area of approximately one meter square, around the base of a tree, thus allowing direct manipulation of the soil water content.

The primary aim of the present study was to see whether the seasonal and diurnal variations in stem water content, reported by Gibbs (1935), Clark and Gibbs (1957) and Markstrom and Hann (1972) could be detected in orchard grown apple trees, and if so, whether they could be attributed to the presence of embolisms caused by cavitations. In this work, the changes in water content of the xylem were measured using a beam of gamma radiation.

The attenuation of a beam of gamma rays has been used by several authors to measure density changes in various materials such as soil (Gurr 1962, Groenvelt, de Swart and Cisler 1969), and living plants (Olszyczka 1979 and Edwards 1980). The use of gamma radiation to measure the density of plant stems will be discussed more fully in a later chapter.

Having developed techniques which might monitor the levels of cavitation within the tree, and the proposed cause of cavitation i.e. water stress, the second aim of this study was to compare the relative efficiencies of the two irrigation systems under study in relieving water stress and so the formation of cavitations. The effects of cavitation, if any, upon the growth and productivity of the apple trees was investigated. To this end shoot extension growth and crop yield, both in size of fruit and total crop weight, were measured throughout the growing season on the irrigated and non irrigated trees. These experiments will be discussed more fully in a later chapter.

Chapter (2) Experimental Plot Design.

The field experiments were carried out during the 1982 growing season in a hedgerow apple orchard at East Malling Research Station, Kent. The experimental orchard plot was composed of five alternate rows of Cox's Orange Pippin and Golden Delicious apple trees, both on M9 rootstock, planted in 1975 (figure 2.1). The trees were planted 1 m apart, in bare soil strips, 2.4 m wide, with grass swards 1.7 m wide between the rows (all dimensions are approximate). It was decided to restrict the study to the middle two rows of Golden Delicious, as the Cox trees had suffered badly from Cox "disease" and were not suitable for the experiment. Foliage commenced approximately 0.5 m above ground level, and by mid June, formed almost a continous hedge, extending to a height of between 2.0 and 2.5 m , and a width of 2.0 m. For statistical interpretation of the experiment, the orchard plot was divided into four randomised blocks, each of which contained three plots of nine trees, one plot for each of the three experimental treatments, i.e. ground sprinkler irrigation, overhead mist irrigation, and non irrigated controls. As it had been decided not to use the Cox, this gave a total of 72 experimental trees, 24 under each irrigation treatment within the plot. The irrigation blocks were separated from each other by at least one, and in most cases two or three guard trees, to prevent cross "contamination" of treatment.

Both irrigation systems, supplied by Watermen Irrigation Ltd., Suffolk, were fed by filtered mains water, reduced to approximately 0.4 MPa pressure, via separate control systems and flow meters. The ground sprinkler system was manually controlled by a stopcock,

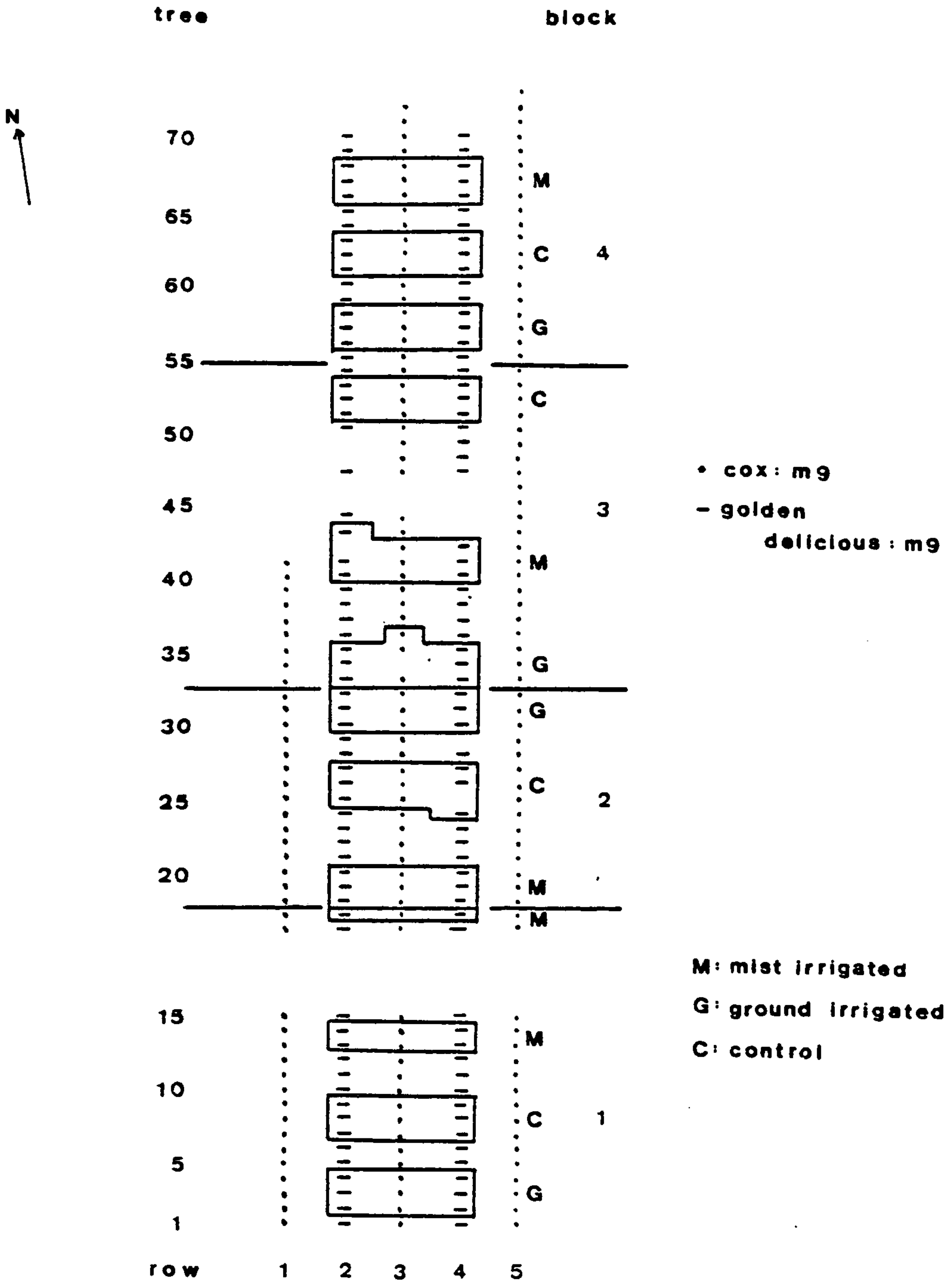

Figure 2.1
Orchard Plot Layout.

and supplied water to each of the twenty four experimental trees by means of a regulated Mantaz nozzle/spike. This nozzle produces a "fan" of water over an area of approximately one meter square around the base of a tree (plate 2.1), at a rate of 40 l/hr. Sufficient water was applied each morning to the plot to replace that lost by evapotranspiration during the previous twenty four hours. The amount of water "lost" was calculated using a computer evaluation of the Penman evapotranspiration formula (Berry 1964), as used at East Malling (modification to original program by G. White), with appropriate adjustment being made for rainfall.

The ground irrigation system is designed to keep the soil at field capacity so that the transpirational demands of the trees would not exceed the available water supply and so set up stress. The alternative approach to the problem is to reduce transpirational demand to a level where the naturally available water supply is not exceeded. Using overhead mist irrigation it is possible to reduce the driving force for transpiration i.e. the leaf to air difference in water vapour pressure. This reduction is achieved by reducing leaf temperature by evaporative cooling, and by increasing the relative humidity of the air within the canopy. It is also noted, that if the leaves within the canopy are totally covered by a film of water during the misting cycle, then for that period of time no transpiration will take place.

Each of the experimental trees were irrigated by two Mist Whizzler nozzles, each with an output of 35 l/hr, which produce "clouds" of water "mist" (plate 2.2) within the canopy. These nozzles were supported on Bamboo canes which were placed so as to cover the maximum area of foliage with the mist. Initial trials

Plate 2.1

The ground sprinkler irrigation system. A Mantaz regulated nozzle/spike delivering 40 L/hr over an area of approximately 1 m^2.

Plate 2.2

The overhead mist irrigation system showing the "mist cloud" produced within the canopy.

with the system used a timed misting period of 5 seconds in every 20 minutes, between the hours of 10.00 am and 4.00 pm, the normal times of high transpirational demand. The control system used was based round an automatic Cloud 9 controller (Bermad, Israel), but was found to be unsatisfactory for our requirements. A timed irrigation system cannot take account of changing environmental factors, so that the same level of irrigation is applied whether the transpirational demand is high or low. This inflexibility of the system can either waste water or perhaps more importantly defeat the object of irrigation by supplying insufficient water for effective evaporative cooling. It was therefore decided that some form of "demand" control system be used in our experiments.

A demand control system was constructed using a "model leaf" (figure 2.2) which was placed within the canopy to act as a "wetness" sensor. When the irrigation system is initially switched on, the mist droplets build up upon the surface of the sensor and bridge the gaps between the sensor contacts. When the circuit is then completed a current is passed to a "driving" circuit within the controller, which operates a solenoid valve in the water supply and turns off the mist. After a period of time, depending upon environmental conditions, the sensor will dry out, breaking the circuit, and so causing the mist to be switched on once more. It was found during the course of the experiments that the sensor would remain wet for between 15 and 40 minutes depending upon environmental conditions, and that a proportion of the canopy, generally those areas directly under and beside the mist nozzles, remained wet throughout the day.

In this form the mist system would operate whenever the sensor

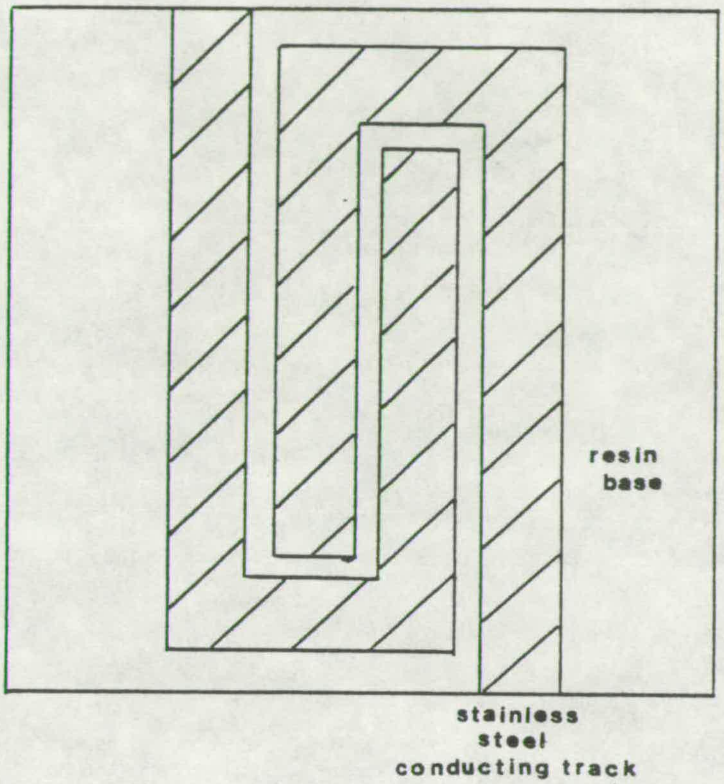

Figure 2.2

Model Leaf: Wetness sensor for the
irrigaton control system.
Actual Size.

was "dry". We only require misting during times of high transpirational demand, and so a further addition to the control system is necessary. It was decided to limit mist operation to periods when air temperature was above 17 ^{o}C and the radiation load above 300 W m^{-2}, and so thermal and light "switches", i.e a thermistor and a light dependant resistor, were built into the circuit (figure 2.3). With this control system a very fine covering of water droplets was sprayed onto the leaves of the canopy (plate 2.3) as required during times of high transpirational demand, to reduce that demand. A proportion of the mist spray reached the soil as "run-off", but as the misting intervals were generally quite short, determined by the wetness sensor, it was felt that the effects of the run-off on soil moisture status would be small. No attempt to measure the proportion of mist that reached the soil as run-off was made.

This reduction in transpiration will only be achieved if the majority of the leaves under stress are cooled by the mist spray. To test the efficiency of the system in "wetting" the canopy, fifteen groups of five leaves were tagged at fixed points in several canopies, and inspected for their degree of "wetness" after a normal misting interval, under various environmental conditions. The results of this study will be given later.

So that we might have some indication of how efficiently we could hold the ground irrigated blocks at field capacity, and prevent water loss by reducing transpirational demand in the mist blocks, the soil moisture content of the plot was determined on a weekly basis using the Neutron Probe technique. The theory and operation of this instrument will be fully discussed in a later chapter.

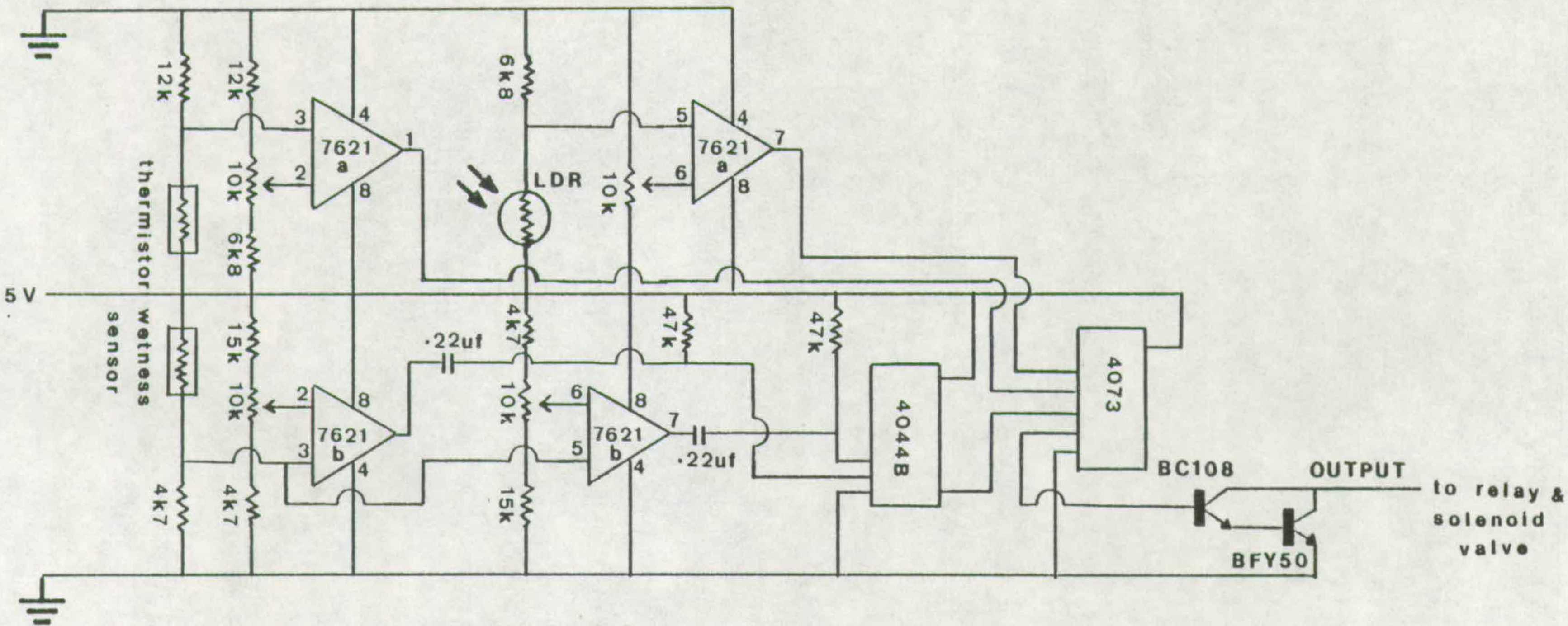

Figure 2.3

Irrigation Control Circuit Diagram.

Plate 2.3

Fine water droplets deposited upon the canopy by the mist system.

Chapter (3) The Detection of Cavitation.

(3.1) The Acoustic Probe.

(3.1.1) Background Theory and Literature Review.

As previously stated, the main technique for studying cavitation in the living plant, in previous investigations, has been that of acoustic detection (Milburn and Johnson 1966). In this system a leaf was impaled upon a sensitive acoustic probe, formed by attaching a pin to the diaphram of a microphone, and allowed to lose water. The output from the probe was amplified using a domestic "Hi-Fi" amplifier, with the result that "clicks" could be heard, via a pair of headphones, which were thought to indicate individual cavitations.

Various lines of evidence have been presented to support the proposal that the major source of these clicks is the occurrence of cavitation.

a) The rate of click producton is related to the rate of water loss; treatments which stop transpiration or supply water to the leaf, cause click production to stop (Milburn and Johnson 1966, Milburn 1973a and b).

b) Mechanical distortion of the tissue, designed to cause tissue fracture, does not result in detectable clicks (Milburn and Johnson 1966).

c) Click production is reversible; if a leaf is allowed to recover full turgidity, so as to stop click production, clicks can be heard if stress is reapplied (Milburn and McLauchlin 1974).

d) Clicks can be detected in isolated vascular bundles (Milburn and McLauchlin 1974).

e) Treatments which either remove gas from cavitated vessels, or accelerate gas dissolution speed up both recovery of turgor and the propensity to produce clicks (Milburn and McLauchlin 1974).

Using the same detector system as Milburn and his various co-authors, West and Gaff (1976) conducted experiments on isolated apple leaves in an attempt to correlate plant clicks with leaf water status, measured using a pressure bomb. Similar work has been carried out by Crombie (1983), where cavitation "profiles" i.e. click frequency vs. leaf water potential or relative water content have been determined for several species.

(3.1.2) Equiptment and Experimental Design.

The original detection system possessed many disadvantages. One of the most serious was the suseptibility to interference from background noise, which tended to "blanket" the sound of the clicks and make them difficult to detect. To overcome this problem, a new amplification system was designed (Anderson unpub.), to remove background noise electronically (fig 3.1.1). In this design, two ceramic "Hi-Fi" pickups (BSR X-5H) are used as detectors, with the diamonds in the styli being replaced by stainless steel pins. One probe is implanted into the petiole of a leaf, or the xylem of a branch or stem, and detects both clicks and background noise. The other "pickup" is suspended in the air, near to the first, and records only background. The signals from these probes are passed to a pre-amplifier , a main amplifier and filtration circuit, and then to an anti-coincidence cicuit which "subtracts" one signal

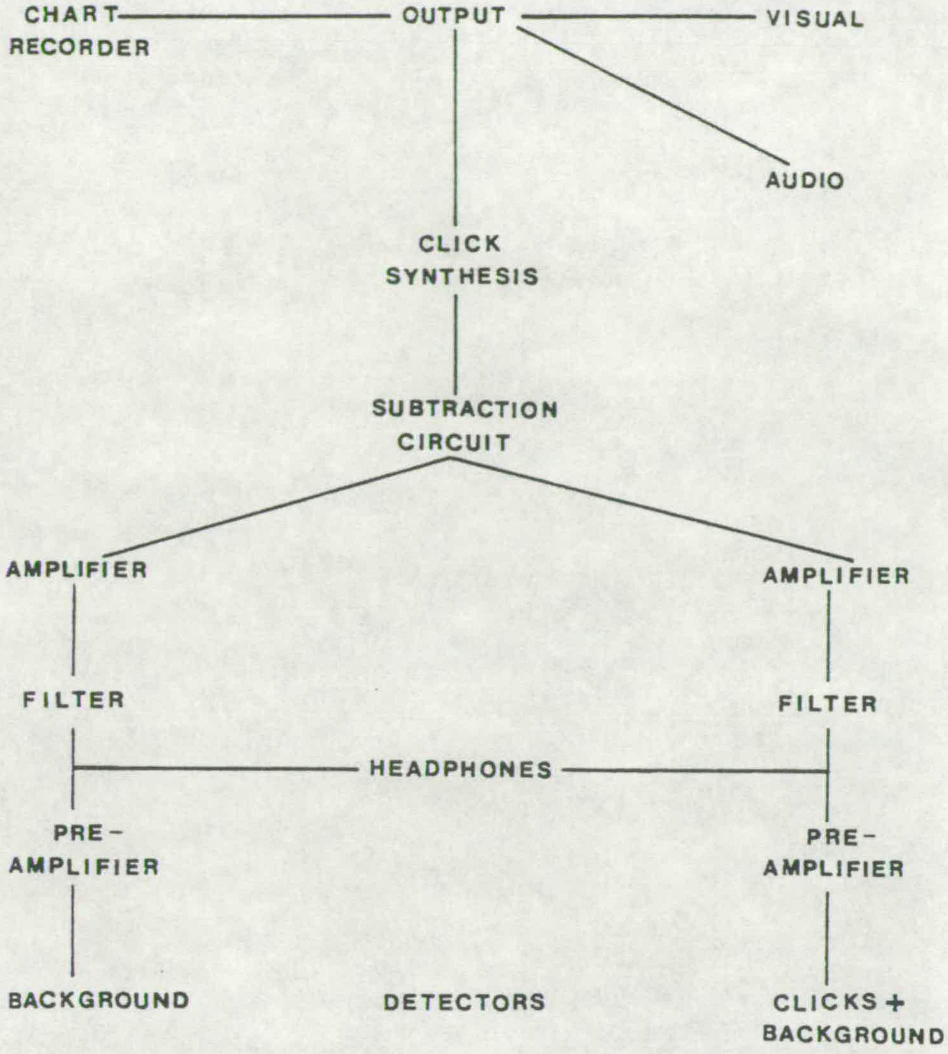

Figure 3.1.1.

Cavitation Acoustic Probe Circuit.
(for full circuit diagram see
Crombie 1983)

from the other so leaving a pure "click signal". Using descriminator circuits the system can be tuned so that only the click signals are detected. The frequency response of the pickups was crudely tested, by exciting the probe with vibrations from a sonic transducer crystal, connected to a signal generator. The pickup was found to be sensitive to frequency up to the output limit of the generator i.e. 100 kHz. Thus we may conclude, that a wide range of acoustic phenomena are detectable, including those in the ultrasonic wavebands, at least to 100 kHz. The output from the detection system can be monitored aurally, visually or recorded on a chart recorder for future analysis.

Although this system was designed, in theory, to eliminate background noise, it was found that the system was not capable of dealing with high levels of noise, and therefore was not suitable for field use. By enclosing the "pickups" and the plant material inside a soundproof cabinet, it was found that the background noise could be reduced to an acceptable level, thus allowing the acoustic probe to be used in the laboratory.

This "soundproof" cabinet, external dimensions 2 m x 1.5 m x 1 m was constructed with an outer wall of 20 mm thick chipboard, and an inner hardboard wall, with the 100 mm cavity being packed with wood shavings. The cabinet was supported on low density foam and placed on a trolley to isolate it as far as possible from noise conducted through the floor. It was found that when the pickups were placed in the cabinet, the average level of background in the laboratory was not detected inside the box. To provide a light source within the cabinet to stimulate water loss, two car headlight bulbs, powered by batteries, were placed near the plant material, as mains powered light sources produced electrical interference which could not be totally eliminated.

Using this system an experiment was designed to determine whether

a) There was a correlation between the rate of click production and water sress, as determined by leaf water potential.

b) There exists a correlation between the rate of click production and xylem density as determined by the gamma probe.

To this end a gamma probe clamp was attached to an apple branch some 35 mm in diameter and 0.5 m in length and the attenuation of the gamma beam determined. This value was taken as the reference point for subsequent calculation of any percentage change in density (Δp). The branch was then severed and placed in the soundproof cabinet and the frequency of clicking measured. Measurements of click frequency, attenuation count, and leaf water potential, measured with a pressure bomb, were taken at 30 minute intervals over a 24 hour period in an attempt to establish a correlation between these three variables.

(3.2) The Gamma Probe.

(3.2.1) Background Theory.

To test the hypothesis that the "clicks" detected by the "acoustic probe" originate from cavitations, a second technique was employed to measure the extent of the resulting embolisms. This technique relies upon the attenuation of a beam of gamma radiation to detect diurnal and seasonal variation in the density of the xylem within tree stems. Gamma ray systems have previously been used to study the internal water content of various materials eg, soil (Groenvelt, de Swart and Cisler 1969) and trees (Olszyczka 1979 and Edwards 1983).

Gamma rays are a form of electromagnetic radiation which interacts with the electrons and nuclei of individual atoms of matter. The dominant interaction process at low energies i.e. less than 0.5 MeV, is photoelectric absorbtion, where a gamma ray will transfer its energy to an electron within an atom, which then leaves that atom. This electron can susequently interact with other atoms to produce ionisation. At medium energy levels, 0.5 to 1.0 MeV, Compton scatter is the dominant process, where the collision of a an orbital electron with a gamma photon will cause the photon to lose part of its energy to the electron, and the photon's path to be altered. If the gamma photon has an energy greater than 1.0 Mev, then it can interact directly with the electromagnetic field of an atomic nucleus, forming an electron-positron pair (Olszyczka 1979).

When a parallel monoenergetic beam of gamma rays with a flux density Io, traverses an absorber of thickness t, normal to the beam, then the flux density of the emergent beam is reduced to the value I, where

$$I = Io\ e^{-Mt/p}$$

M = Mass attenuation coefficient

t = Absorber thickness

p = Density of the absorber.

Thus if Io, M and t are known, then the value of p can be calculated from I. Or, if M and t are constant, any observed change in I can be interpreted as a change in p.

In practice the beam of gamma rays is collimated to reduce the effects of scatter which tends to increase the number of photons reaching the detector and so apparently decreasing the value of the attenuation coefficient.

(3.2.2) Equipment and Experimental Design.

The gamma source selected for our investigations was a 45mCi bead of Americium 241 of 60 KeV energy (Amersham International, Bucks.), which was held within a brass housing lined with a cylinder of lead 25 mm diameter. Although the relatively low energy levels associated with the photons from Americium 241 render these very susceptible to the problems of "back scatter", (Cattrel pers. comm.) it was chosen over other sources such as Caesium 137, which, although possessing advantages such as higher energy photons for greater penetration, requires over twenty kilograms of lead shielding for complete operator safety. This level of shielding makes Caesium 137 rather unsuitable for a portable field instrument, for unsupported attachment to small trees.

The Americium source holder (plate 3.2.1) was held on one side of a yoke (plate 3.2.2), by means of a fail-safe bayonet lock, so that accidental exposure of the operator to the source could not occur when the system was not in use. On the opposite side of the yoke is the photon detector system, composed of a DM-1 photomultiplier tube and a sodium iodide Crystal Scintillation Detector, (Nuclear Enterprises, Edinburgh), which was linked to a Scaler Ratemeter, model PSR6, (Nuclear Enterprises, Edinb.). To maximise the resolution of the system, the number of counts in any one measurement should be as large as practically possible. To facilitate long counting times the ratemeter was modified from a five to a six digit display by constructing a separate 6 digit L.C.D. display unit which can be connected to the scaler and timer circuits of the ratemeter in parallel with the existing display when required.

The collimation of the beam is accomplished both by the design of the source holder, and by a separate detector collimator fitted over the iodide crystal. This collimator is formed by two 5 mm thick lead discs, 25 mm diameter, which are held apart within a 5 mm thick lead lined brass tube, (fig 3.2.1). The collimation system was tested by passing the gamma beam through a series of holes, of increasing diameter, drilled in a 10 mm thick lead shield, until a diameter was reached when the count rate measured at the detector equalled that in air. Using this technique the effective beam diameter was found to be 4.4 mm. To test the scatter of this beam dental film badges were placed in the optical path, immediately adjacent to the ends of the collimators and at

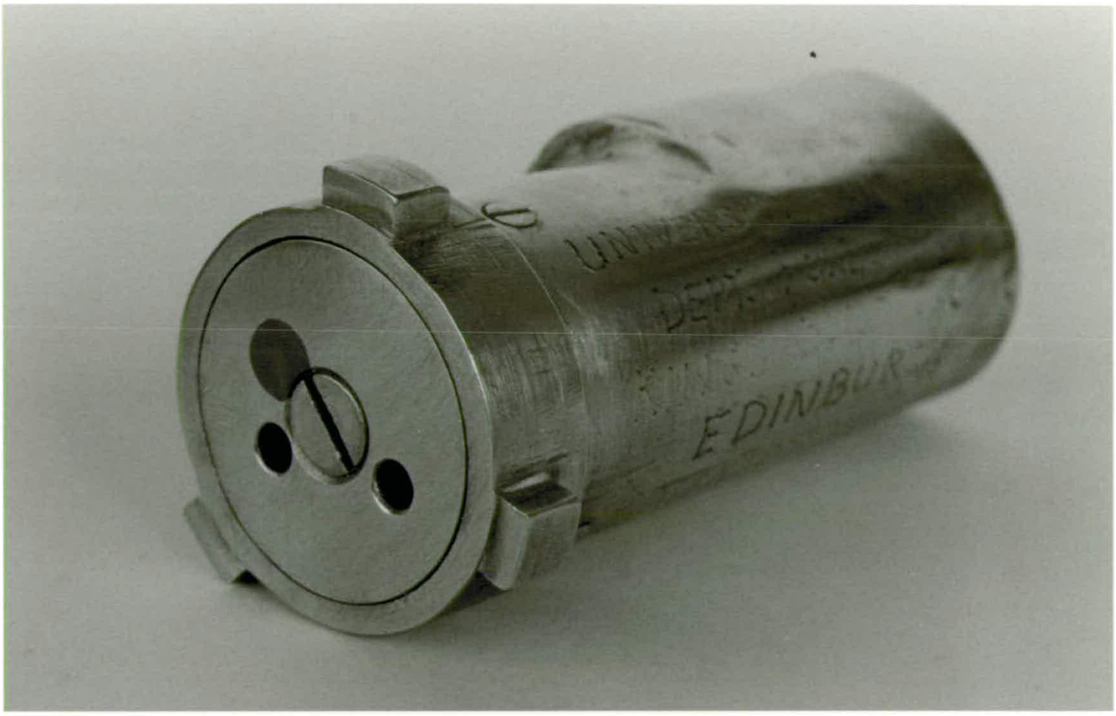

Plate 3.2.1

The Americium 241 gamma source holder/collimator.

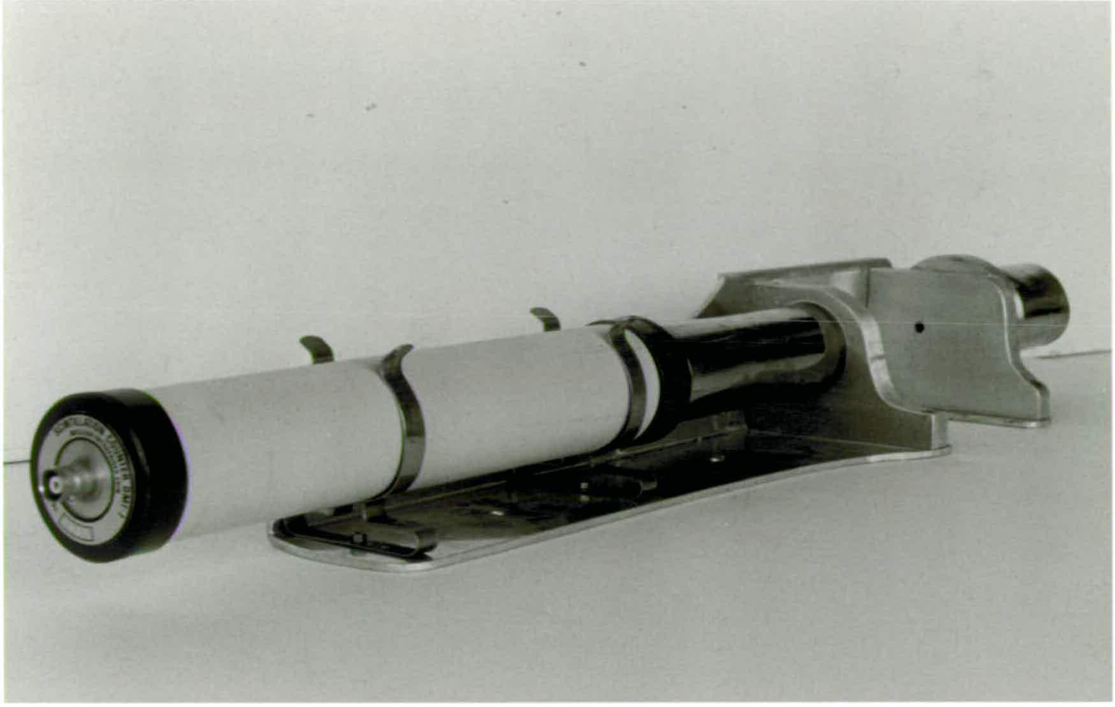

Plate 3.2.2

The gamma probe, incorporating source holder, detector and collimator.

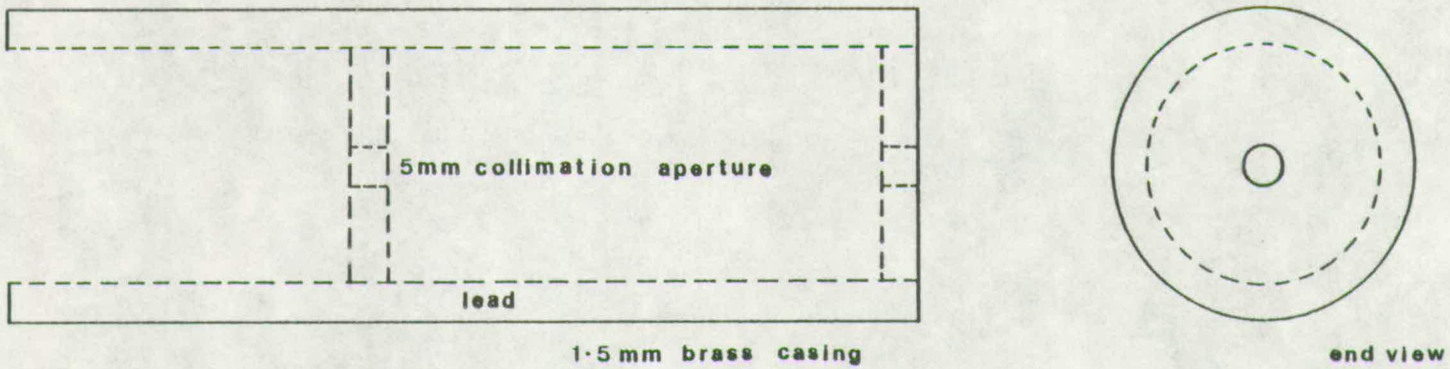

Figure 3.2.1.

Gamma Probe Detector Collimator.

(actual size)

the mid-point of the yoke. The badges were exposed to the beam for six hours and then developed. It was found that all the films showed an intense black spot of 4.4 - 4.5 mm diameter, with no significant signs of a "halo" round the image which would indicate scatter.

The "Gamma Probe" was attached to a stem by means of a nickle-plated steel triangular clamp (plate 3.2.3). This clamp is fastened permanently to the tree and permits rapid and repeatable mounting of the probe to a fixed position on the stem. The triangular construction of the clamp permitted stem growth in the apices of the triangles so minimising any long term ill effects caused by the constriction of the stem. Trees which had clamps fixed to them for almost the complete 1982 growing season showed no signs of having been adversly affected by the clamps when compared with other similar trees in the plot.

To detect changes in the density of the stem xylem using the gamma probe, changes in the thickness of the xylem parallel to the beam must be recorded. This was achieved by using linear variable displacement transducers (L.V.D.T), the theory and operation of which will be discussed later.

To avoid any influence of changes in phloem density or xylem growth, the phloem and cambial tissues were removed from the beam path by cutting two "windows", 20 mm diameter, in the bark on either side of the stem. This ensured that the tissues in the path of the beam did not grow. By monitoring any changes in xylem thickness, any changes in density that occur within the xylem may be attributed unequivocally to real changes in the density of the xylem. Any embolism formation ought then to be detectable,

Plate 3.2.3

A triangular clamp for attaching the gamma probe to a stem, showing the "window" cut in the stem to remove the phloem and cambial tissues.

providing its extent is greater than the resolution of the system.

Calibration of the gamma probe was achieved using a variable mass "rig", (plate 3.2.4) which fitted within the yoke in place of the stem. This rig was composed of a perspex block, whose mass approximates that of a 50 mm apple tree, plus five thin perspex slides which could be removed one by one, so decreasing the mass of perspex within the beam path. Perspex was chosen to form the calibration rig, as both perspex and water are closely similar in their interaction with gamma rays,(Cattrell pers. comm.). Counts of gamma radiation as detected by the crystal detector, form a Poisson distribution, with a standard deviation $\sqrt{N}$, where N is the number of counts in any one measurement. Typically the count recorded through an apple stem, in a three minute period, was 350,000 giving a standard deviation of 592, and a 95% confidence interval of +/- 1159. Using the calibration system, it was found that a 1% change in stem mass was represented by a 2847 change in total count (figure 3.2.2), so that with a three minute counting time, the system could resolve changes in stem density of approximately 0.8%.

It was found that the detection system, in particular the ratemeter, was very susceptible to changes in ambient air temperature (figure 3.2.3). As this temperature coefficient was a major source of error within the system, the meter was held at a constant temperature by placing it within a temperature controlled cabinet. Details of the construction and operation of this cabinet are given in chapter 4.1.2. By holding the ratemeter at a fixed temperature and by conducting a calibration with the perspex rig before each measurement, to correct for the temperature sensitivity

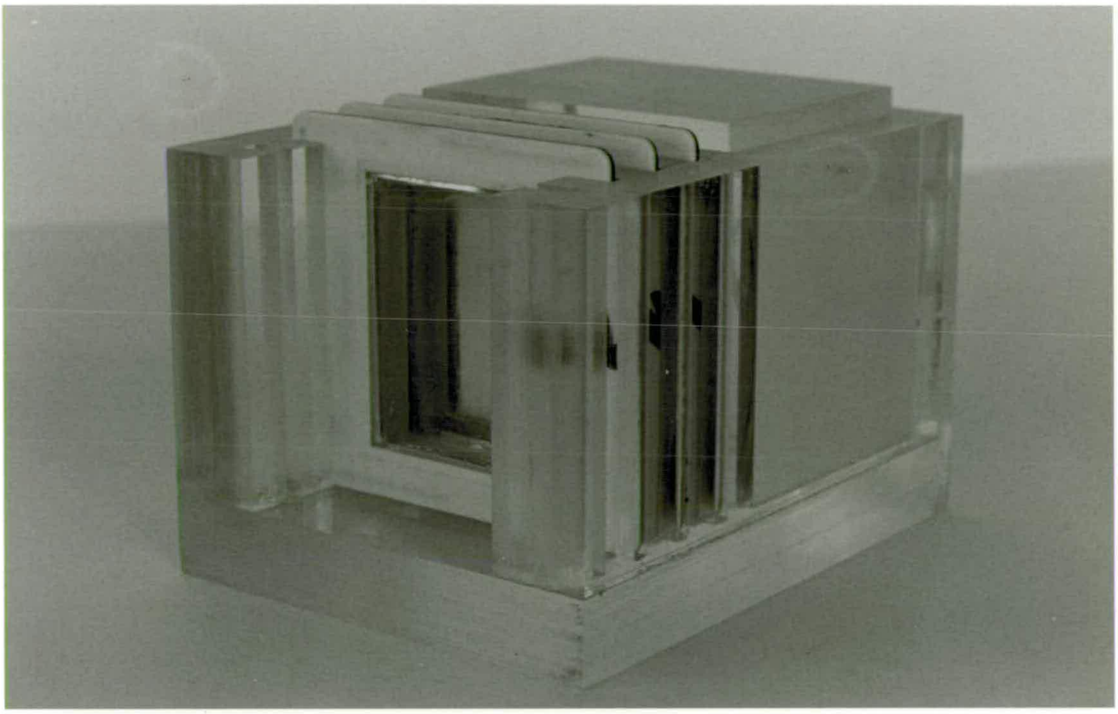

Plate 3.2.4.

The gamma probe calibration rig.

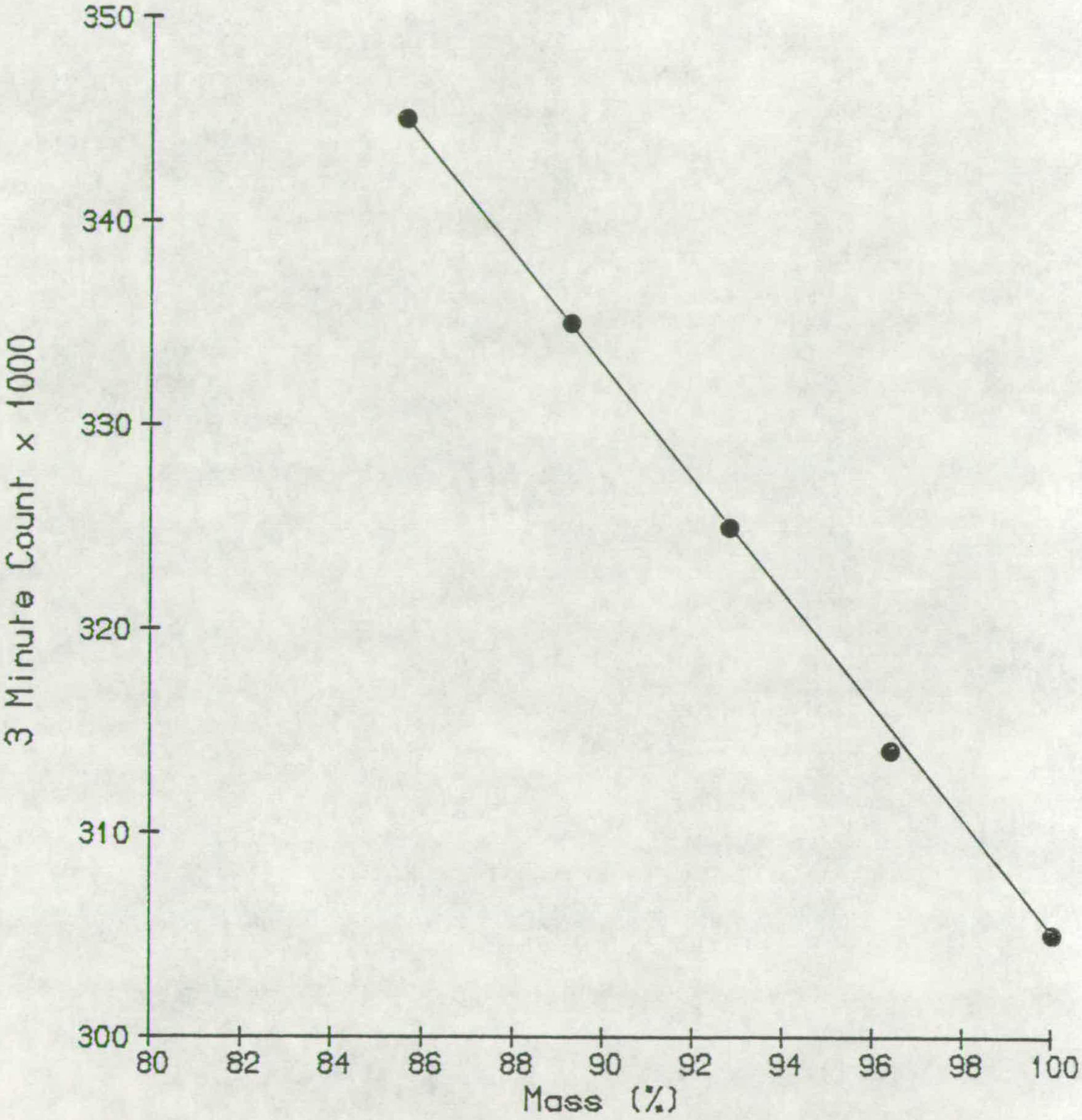

Figure 3.2.2.

Typical Gamma probe Calibration

Curve. (at 29.8 oC)

2847 counts = 1% change in mass

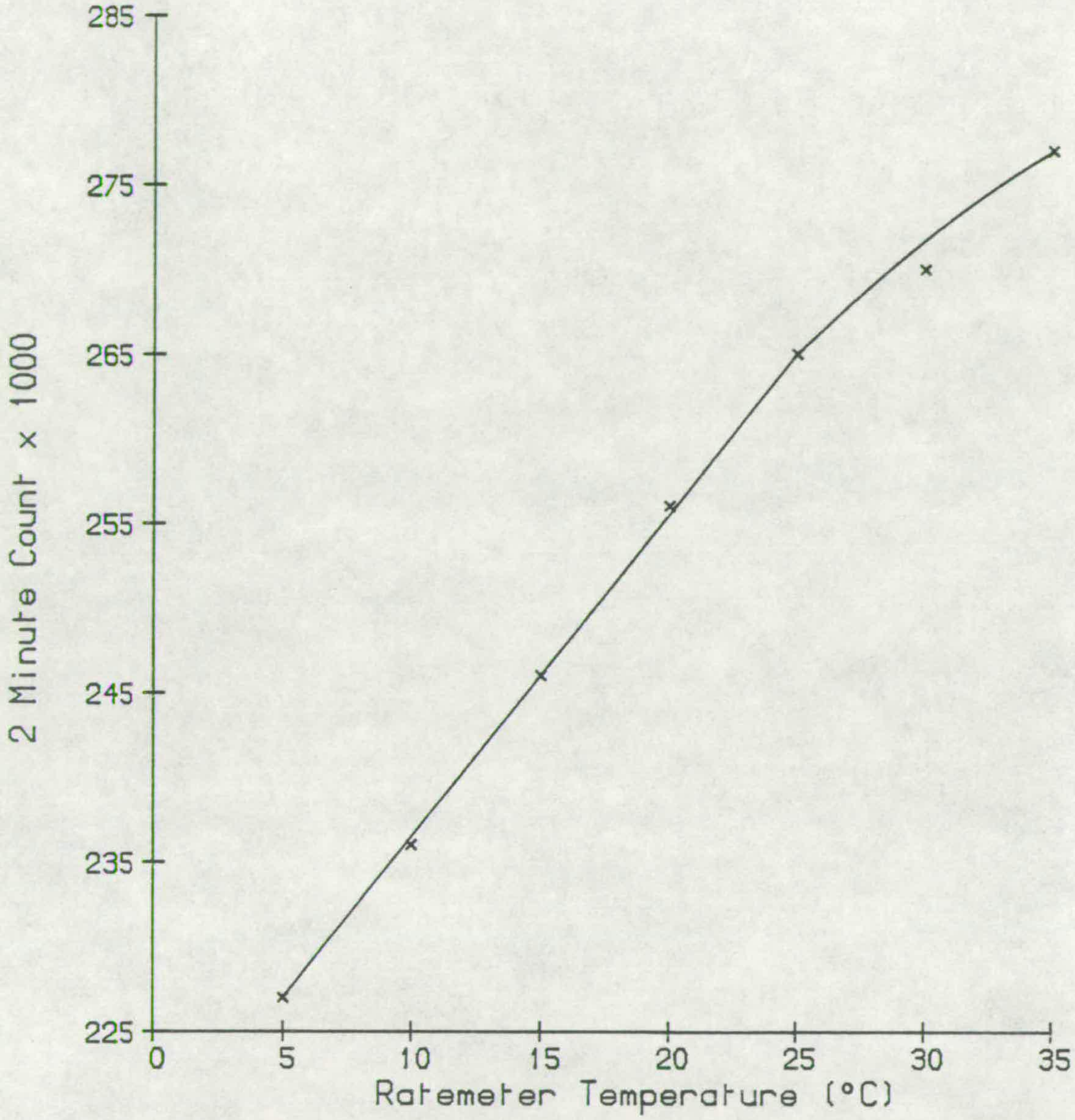

Figure 3.2.3.

Gamma Probe Ratemeter Temperature Response Curve.

of the remainder of the system, it was felt that any changes in count rate measured, would be an accurate indication of xylem density changes.

The diurnal variation in xylem density was determined by hourly measurements using the gamma probe, commencing at dawn, and ending just before sunset. At each time interval gamma photons were counted for three minutes,

a) Through 1 to 5 slides in the calibration rig,

b) Through each of the experimental trees under investigation.

Using the dawn count as a reference point, the changes in attenuation were used to find the percentage change in density, relative to this dawn measurement. Hereafter this will be refered to as percentage change in density (Δ p). Seasonal variations in xylem density were calculated in a similar fashion, with measurements being made at dusk, as it was felt that this was perhaps the simplest and most convenient point in the diurnal cycle to determine. Attenuation was recorded on each of the experimental trees on each occasion, with the count rates obtained on the 17/6/1983 being used as the reference point for determination of Δ p.

(3.3) Stem Diameter Measurement.

(3.3.1) Background Theory and Literature Review.

It is well known that stem diameter undergoes diurnal changes. These changes are related to leaf water status, itself dependent on those environmental variables which drive transpiration, especially the net radiation input into the canopy (Klepper 1971).

If the diameter of the xylem varies with water stress then, as described in chapter (3.2.1) the attenuation of a gamma beam passing through the xylem cannot only be attributed to a change in stem density. However Richards (1971), reported that most of the variation in stem diameter occurred exterior to the xylem i.e. in the phloem and cambial tissues, though a variation in the dimensions of the xylem was measurable..

(3.3.2) Equipment and Experimental Design.

To enable any changes in xylem dimension to be accurately measured, a system involving two matched linear variable displacement transducers (LVDT's) was developed, one transducer to measure xylem changes and the other to measure phloem and cambial tissues on the same stem.

Two stainless steel threaded rods (4 B.A.) were screwed through the stem to provide a stable and secure anchorage for the LVDTs, (Sangamo DG/5.0/S, Schlumberger, W.Sussex). To these rods was clamped the LVDT mounting block, made of perspex, by means of two steel blocks which bolt onto the rods (plate 3.3.1). The transducers were held in place in the mounting block, one resting

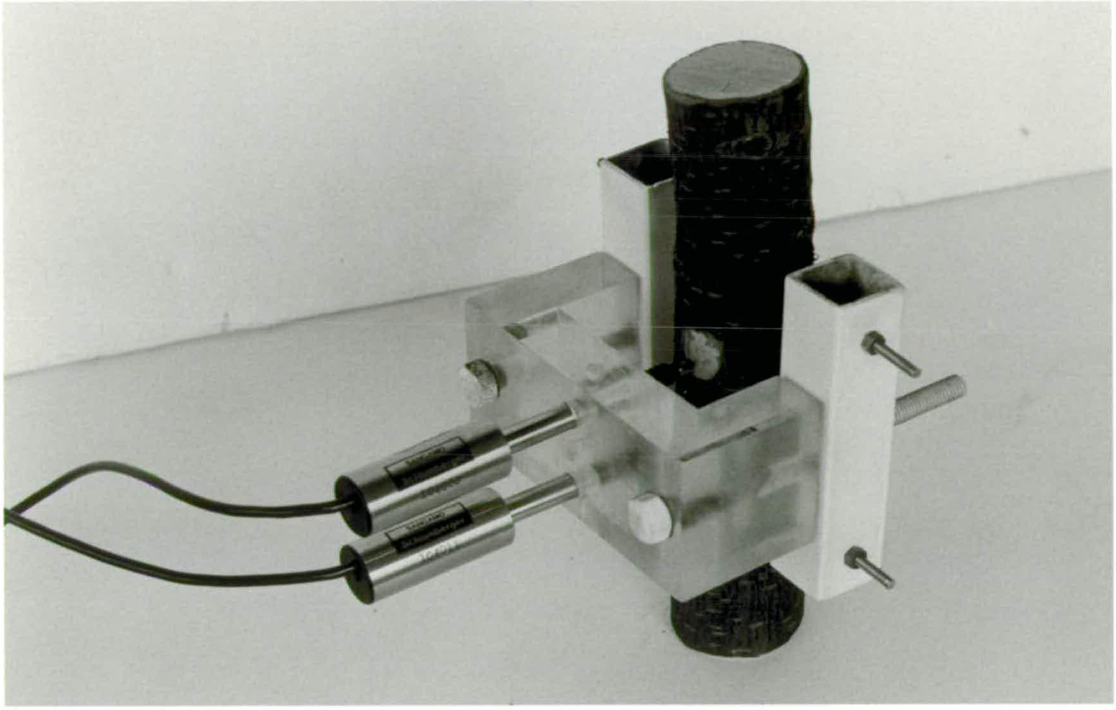

Plate 3.3.1

The LVDT stem measuring system, showing the sensors and mounting rig.

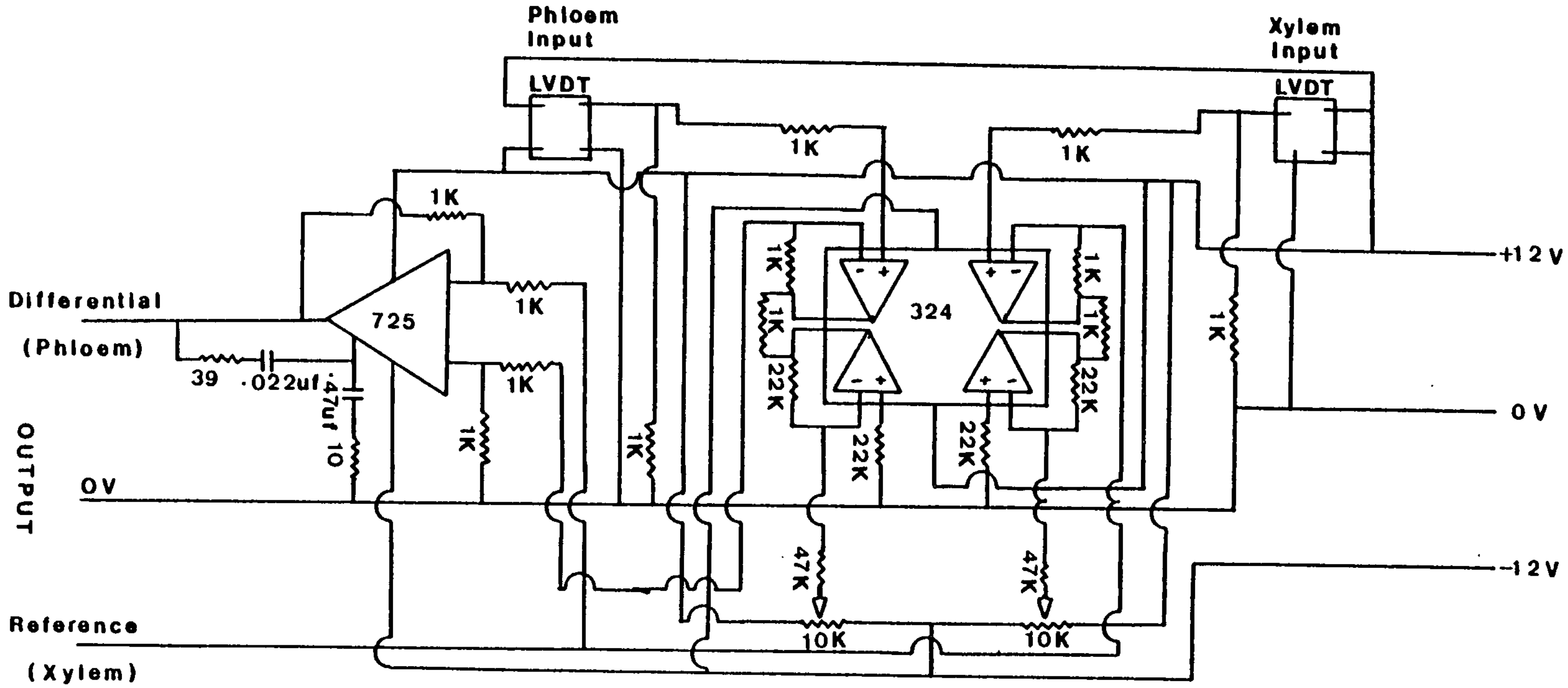

Figure 3.3.1.

Linear Variable Displacement Transducer (LVDT) Circuit Diagram.

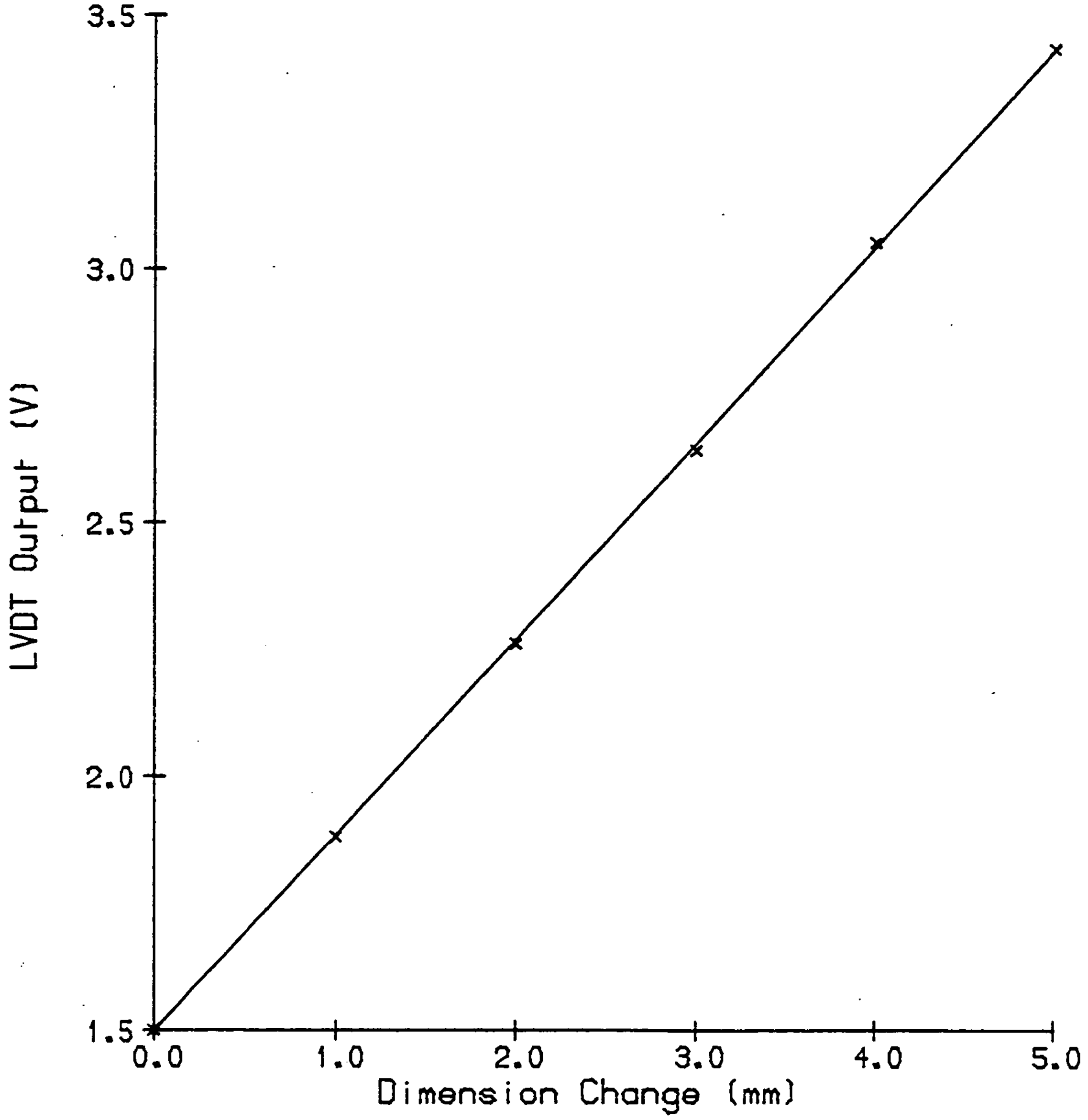

Figure 3.3.2.

LVDT Calibration Curve.

(at 20 oC)

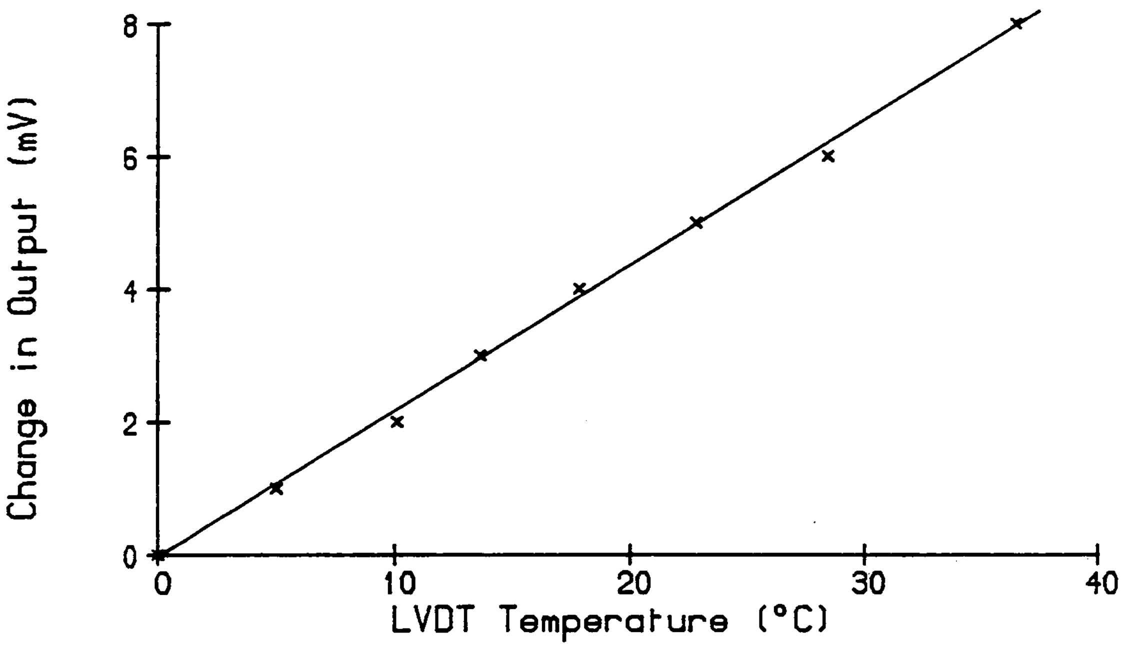

Figure 3.3.3.

LVDT Sensor Temperature

Response Curve.

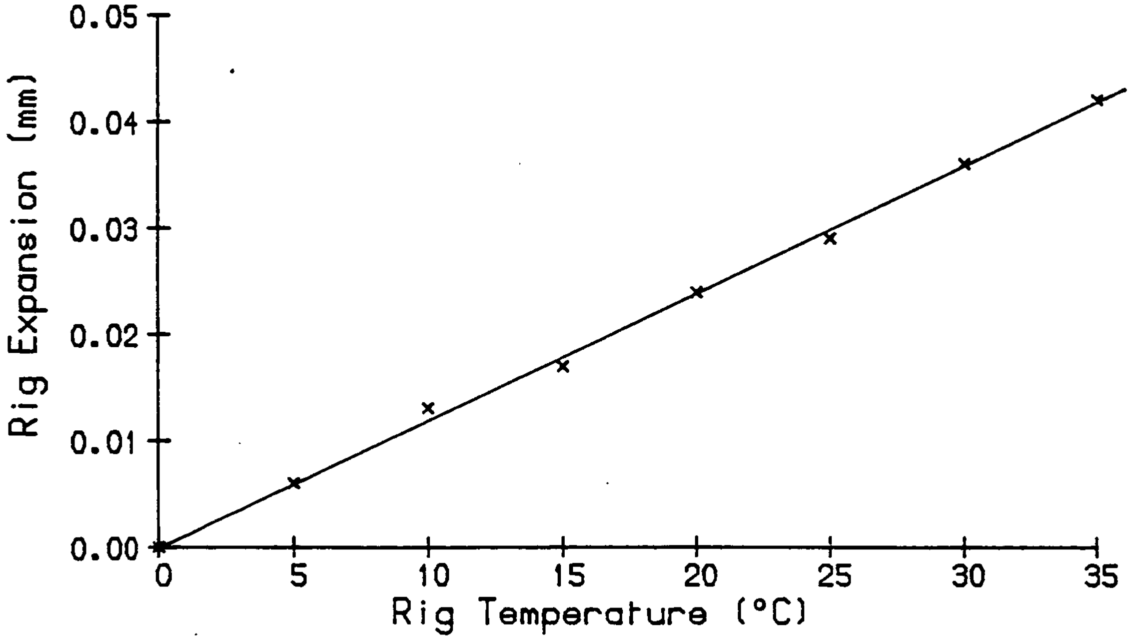

Figure 3.3.4.

LVDT Mounting Rig Temperature

Response Curve.

(3.4) Dye Uptake Experiments

(3.4.1) Background Theory.

Since the earliest beginnings of plant physiology, the uptake of dyes by living plants has been used to follow the path of water transport within the xylem. As no transport of water is possible through a region of embolisms, such regions if large enough, ought to be detectable by sectioning the stem after a period of dye transport. The use of dyes was therefore entertained as a supporting technique, enabling any large declines in stem density to be identified as large embolisms, and perhaps enabling their distibution within the stem to be pinpointed.

The difficulties of using dyes are several. Firstly, to examine the distribution of dye the stem must be cut off. In a commercial orchard this can only be done to a few trees. Secondly, the assumption must be made that the insertion of the dye does not disturb the pattern of water flow. Thirdly, as it is well known that the rate of water movement in outer zones of the xylem is faster than the inner, attention must be paid to the time course of transport. If insufficient time is allowed for dye uptake, then regions of slow transport may be mistaken for regions of no transport at all. Conversly if the time course is too long, then it is possible that non conducting regions may be stained due to diffusion of the dye into those regions from the conducting pathway.

(3.4.2) Equipment and Experimental Design.

As the sampling technique is destructive, observations can only be made by sampling at least two individuals either within a single treatment block at the beginning and end of the experiment, or between blocks at any convenient point in the course of the investigations. It was felt that due to the lack of replication that was possible, a comparison should be made between a mist irrigated and a control block at the end of the experimental period, thus allowing data collection for other experiments to be carried out on these trees right up to the day of sampling.

Dye was introduced into the xylem by first sealing a plastic container of some 0.3 m diameter and 0.4 m depth around the base of a 60mm apple ~~the~~ tree. A "water - tight" seal was achieved using "Blu-Tac" to plug any gaps. A 0.1% solution of decolourised basic fuchsin dye (Talboys 1955) was placed in this container and the stem cut off completely using a pruning saw, under the surface of the dye to exlude air, and the tree allowed to transpire. After approximately twenty minutes, dye was observed in the xylem of the topmost extension shoots,whereupon the tree was removed from the dye and allowed to "dry out" before being sectioned and the stained conducting areas determined.

Chapter (4) Measurement of Plant and Environmental Water Status.

(4.1) Transpiration

(4.1.1) Background Theory and Literature Review.

It is proposed that cavitations within the xylem of a tree result from water stress. This water stress is likely to be a function of the water flux through the tree and the cross sectional area of functional xylem within the stem. The driving force for this flux is the process of transpiration which is a function of the difference in water vapour pressure between the leaf sub-stomatal cavity and the surrounding environment, across the leaf boundary layer. The rate of transpiration of any leaf can be calculated from a solution of the energy balance of that leaf. The energy balance approach is based upon the First Law of Thermodynamics which states that energy can neither be created nor destroyed, i.e.

Energy Input = Energy Output + Energy Stored

We can write the energy balance of a leaf as

$$Rn = C + \lambda E + G + J + P \qquad (1)$$

Rn = Net radiation input to leaf or canopy,

C = Sensible heat flux,

λE = Latent heat flux, where λ = Latent heat of vapourisation of water,

G = Heat flux either to soil or via stem by conduction

J = Net flux of energy into physical storage,

P = Net flux of energy into biochemical storage,

all variables per unit leaf surface area.

Taking into account the relative importance of each term in equation (1), one finds that added together, the terms P,J and G for a relatively small and thin leaf such as an apple, are smaller than the major terms Rn and C. It is therefore possible to ignore these terms and simplify the equation as

$$Rn = C + \lambda E \qquad (2)$$

The rate of sensible heat loss is proportional to the difference between the leaf and air temperatures, and can be expressed as

$$C = \frac{\rho cp(Tl - Ta)}{rh} \qquad (3)$$

C = Sensible heat flux,

Tl = Leaf temperature in degrees Kelvin,

Ta = Air temperature in degrees Kelvin,

rh = Resistance to heat transfer by convection,

ρ = Density of air,

cp = Specific heat of air at constant pressure,

all per unit leaf surface area.

The resistance to heat transfer rh, is related to the boundary layer resistance to water vapour ra, inversely as a ratio of the diffusivities of heat and water vapour in air, which at 20 °C are 21.5 and 24.2 mm^2 s^{-1} respectivly, raised to the power 0.66 (Monteith 1973).

$$\frac{rh}{ra} = \left(\frac{24.2}{21.5}\right)^{0.66} = 1.12 \qquad (4)$$

For a laminar boundary layer, one would expect that ra and rh would be proportional (Grace 1980), but empirically it is found that the exponential is required. This is because the relationship depends not only on simple diffusion, but also on mass flow. A full explanation is contained in Monteith (1973 pgs. 106, 107).

For approximate purposes one can assume that rh = ra, or by measuring one the other can be inferred. If the leaf under investigation is hypostomatous, then a further correction must be made, as in such cases heat is lost from both sides of the leaf, while water is lost from one side only.

If we consider an actively transpiring leaf whose energy balance can be stated as,

$$Rn = \lambda E + \frac{\rho\, cp(Tl - Ta)}{rh} \qquad (5)$$

and a non-transpiring leaf, where

$$Rn^* = 0 + \frac{\rho\, cp(Tl^* - Ta)}{rh} \qquad (6)$$

Rn* and Tl* used to denote the different net radiation input and leaf temperature from that of the transpiring leaf.

Following Monteith (1973),

$$Rn = (1 - \rho\, l)(Stot + Se) + \varepsilon\, l(Ld + Le - Ll) \quad (7)$$

ρ l = Reflection coefficient of the leaf,

Stot = Total incident shortwave radiation,

Se = Shortwave reflected from the environment,

ε l = Emissivity of the leaf,

Ld = Downward flux of longwave radiation,

Le = Longwave radiation from the environment,

Ll = Longwave flux of fullwave radiation at leaf surface temperature (degrees Kelvin) $= \sigma Tl^4$, where σ = Stefan's Constant, all per unit leaf surface area.

Expanding equations (5) and (6),

$$(1 - \rho l)(Stot + Se) + \varepsilon_l(Ld + Le - \sigma Tl^4) = \lambda E + \frac{\rho cp(Tl - Ta)}{rh} \qquad (8)$$

$$(1 - \rho l)(Stot + Se) + \varepsilon_l(Ld + Le - \sigma Tl^{*4}) = \frac{\rho cp(Tl^* - Ta)}{rh} \qquad (9)$$

Combining the left-hand sides of these equations and simplifying

$$Rn^* = Rn - \varepsilon l\, \sigma(Tl^{*4} - Tl^4) \qquad (10)$$

By taking the emissivities of both leaves as 1, as we can assume that they behave almost as "black bodies" (Monteith 1973), solving equations (8) and (9) for rh, then equating and rearranging:

$$Rn = \lambda E + Rn^* \cdot \frac{\rho cp(Tl - Ta)}{\rho cp(Tl^* - Ta)} \qquad (11)$$

Replacing Rn* by equation (10) and solving for E,

$$E = \frac{1}{\lambda}\left(Rn - \frac{(Rn - \sigma(Tl^{*4} - Tl^4))(Tl - Ta)}{(Tl^* - Ta)}\right) \qquad (12)$$

Using this equation and measuring Rn (Wm^{-2}), the temperatures of the air, transpiring and non-transpiring leaves (Kelvins), it is possible to calculate the transpiration rate of any leaf. The transpiration rate of a large and well distributed sample of leaves within a canopy can be used as an estimate of the transpiration rate of the canopy as a whole. This method was used to determine the diurnal variation in transpiration rate of irrigated and non irrigated trees on four days, from sunrise to sunset, during the 1982 growing season.

During the course of these studies it was found that accurate measurement of the net radiation flux of a leaf in the field is very difficult, and it was decided that an alternative approach avoiding direct measurement of Rn should be employed in any future investigations. By calculating the boundary layer resistance ra, of a suitable sample of leaves, then by substituting rh ~~ra~~ into equation (6) and measuring Tl* and Ta, the net radiation Rn* can be found.Using equation (10) and substituting in Rn*, a value for Rn can be found simply by measuring Tl. Thus by proceeding in this way it is possible to estimate the transpiration rate of any canopy without direct measurement of net radiation.

The boundary layer resistance of a leaf can be obtained (after Dixon and Grace 1983), using a brass leaf model, equipped with an internal heater and temperature sensor,of the same size, shape and orientation as the actual leaf in the tree canopy. This technique assumes that all heat supplied to the model is gained by passing an electric current through the heating element embedded in the model, and is lost by convective heat transfer to the air, i.e.

$$C = I^2 r = \frac{\rho cp(Tm - Ta)}{rh} \qquad (13)$$

C = Convective heat loss

I = Current supplied to model

r = Resistance of the heating element

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Tm = Model surface temperature,

all per unit "leaf" surface area.

This approach depends on an absence of radiative exchange between the environment and the model, i.e. that the model has zero emissivity. In practice this situation is very dificult to achieve in the natural environment. A model with zero emissivity is impossible to construct. Moreover the determination of a model's emissivity is a rather difficult proposition. One could of course carry out the measurement of rh during the night when there would be no shortwave interception, but longwave interaction will still occur and so complicate the determination of rh. A variation in the original technique is therefore necessary.

The problems of radiation exchange can be solved by using two model leaves of exactly similar size, shape, reflective and emissive properties, and supplying a current to only one leaf. As the shortwave properties of both leaves are similar, the radiation fluxes to the models will differ only in the longwave component, due to the heat supplied to one leaf. With reference to equations (2),(3),(6),(7) and (8), where $(1 - \rho\ m)(Stot + Se)$ is replaced by Snet, and the longwave into the model by Li, the energy balance of the non-heated model can be written as

$$Snet + Li - \varepsilon\, m\sigma\, Tm^4 = \frac{\rho cp(Tm - Ta)}{rh} \tag{14}$$

and that for the heated model as

$$I\ r + Snet + Li - \varepsilon m\ \sigma Tm*^4 = \frac{\rho cp(Tm* - Ta)}{rh} \tag{15}$$

Subtracting equation (14) from (15) then

$$I^2 r - \varepsilon m\ \sigma(Tm^{*4} - Tm^4) = \frac{\rho cp(Tm^* - Tm)}{rh}$$

therefore

$$rh = \frac{\rho cp(Tm^* - Tm)}{I^2 r - \varepsilon m\ \sigma(Tm^{*4} - Tm^4)} \qquad (16)$$

Using this equation and the ratio of rh/ra of known value, ra can be substituted into equation (6) as previously described to obtain a value for Rn*, and thereafter the transpiration rate of the canopy.

Using this differential system the problems of radiative interaction are theoretically solved. In practice the heated model should be operated at a temperature only a few degrees above that of the real leaves in the canopy, so as to minimise errors caused by free convection, and therefore it would be desirable if radiation absorbtion by the models could be reduced. Reduction of absorbtion while maintaining good emissive properties can be achieved by coating the surface of the models with brilliant white zinc oxide paint, which has shortwave reflection and longwave emission coefficients of greater than 0.95 (Sestak, Catsky and Jarvis 1971). Unfortunately this surface is very susceptible to weathering, so for field apparatus another material is required. A model fulfilling all our requirements can be constructed by covering the surface of the model with aluminised mylar, (Melinex, I.C.I.. Herts.), which has shortwave reflection and longwave emission coefficients of 0.8 and 0.9 respectivly (Fuchs and Tanner 1965).

We have therefore shown, that by inserting appropriate models in the canopy, it should be possible to calculate the boundary layer resistance for the leaves under various environmental conditions using a heated model technique. Once values for the boundary layer resistance and the other parameters used in the solution of the energy balance equations are known, it is possible to determine the transpiration rates for a sample of leaves and therefore the canopy, as previously described.

To this end, ten model leaves were constructed, five of which were electrically heated whilst five remained unheated. To test the system these models were placed within a controlled environment wind tunnel and their boundary layer resistance determined over a range of windspeeds. The results were compared with published data. The system was evaluated for field use by using it to obtain a correlation between boundary layer resistance and windspeed within an orchard plot at East Malling during April 1983. Although a good correlation was obtained, it was not possible to use this correlation to calculate values of ra for the 1982 diurnal cycles as the canopies in April were too dissimilar from those in July and August.

(4.1.2) Equipment and Experimental Design.

To calculate the transpiration rate of a canopy as outlined in chapter (4.1.1), the net radiation flux of the canopy, the absolute temperatures of transpiring leaves and non-transpiring leaves within the canopy and ambient air must be accurately measured.

In measuring the temperature of the leaves the sensing system must be such that it does not a~~e~~ffect the energy balance of the leaves it is measuring. It is possibe to determine average whole canopy temperatures using remote sensing systems based on infra-red detection, but these techniques have large calibration problems due to drift, and were in any case not available for the present study. Alternatively, sensors such as thermistors or thermocouples can be attached directly to a sample of leaves within the canopy. If that sample is sufficiently large and well distributed, then the average temperature of that sample can be used as an estimate of the canopy temperature as a whole.

A thermistor is a semi-conductor whose resistance decreases with increasing temperature, but unfortunately this change is non-linear, except over small ranges. Thermistors can be very sensitive, but the smallest thermistor is relatively large when compared with fine thermocouples, and so would effect the energy balance of the leaf to a greater extent by disrupting the boundary layer and possibly blocking stomata. For this reason, fine thermocouples (0.2 mm dia.) constructed from 42 gauge copper and constantan wires (Dural Plastics, Australia), were used for all the leaf temperature measurements in the present study. These thermocouples and 20 mm of their lead wires were attached at various positions on the lamina, by coating them with "Sellotape" glue (Dixon 1982). This method of attachment allowed good coupling of the sensor and the leaf with minimal damage to the lamina and avoided heat conduction across the boundary layer.

Two sets of ten thermocouples, wired in parallel, were placed on ten transpiring, and ten non-transpiring leaves (coated with a thin layer of "Vaseline", Thorpe and Butler 1977), within each canopy. Each transpiring leaf was "paired" with an adjacent non-transpiring leaf, so that the average sample temperatures obtained were made on leaves with similar variation in exposure to wind and radiation.

A reference for the thermocouples was provided by a block of Aluminium (80 mm x 60 mm x 25 mm) which was held at a fixed temperature inside a temperature controlled cabinet. The block was channelled and drilled so that the reference junctions could be embedded into it, the lead wires being tightly held in the channels by means of a perspex cover which minimised heat conduction along the wires to and from the junctions (figure 4.1.1)

The temperature controlled cabinet (figure 4.1.2), 0.5 m x 0.3 m x 0.5 m in size, was formed of 12 mm thick plywood and lined with 25 mm thick expanded polystyrene insulation. An interior division divided the cabinet into a lower heated section comprising 70% of the total volume, and an upper section which allowed access via a lid, but prevented heat loss. The heating system consisted of a heat source (car indicator bulb (Lucas 12 V, 21 W), equipped with a reflector as a diffuser), whose output was controlled by a themometer contact switch (Grant Instruments, Cambridge, Model P1698 +/- 0.1 degrees tolerance). A fan (Micronel, model V581M, Radiatron Components Ltd. , Middlesex) with a capacity of 530 L of air per minute, stirred the air within the cabinet to prevent "hot spots". The circuit (figure 4.1.3) was so designed that current from a car battery could be passed to the heater via a relay, to protect the switch from excessive current, until a desired cabinet temperature was reached, when the thermal switch would operate and

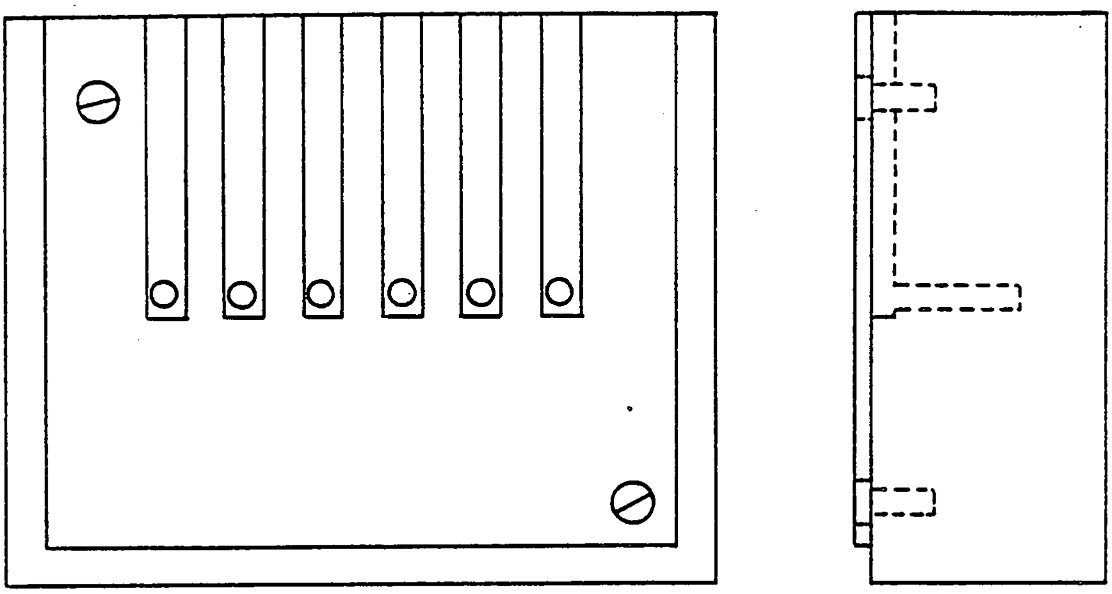

Figure 4.1.1.

Thermocouple Reference Block.

(scale x2)

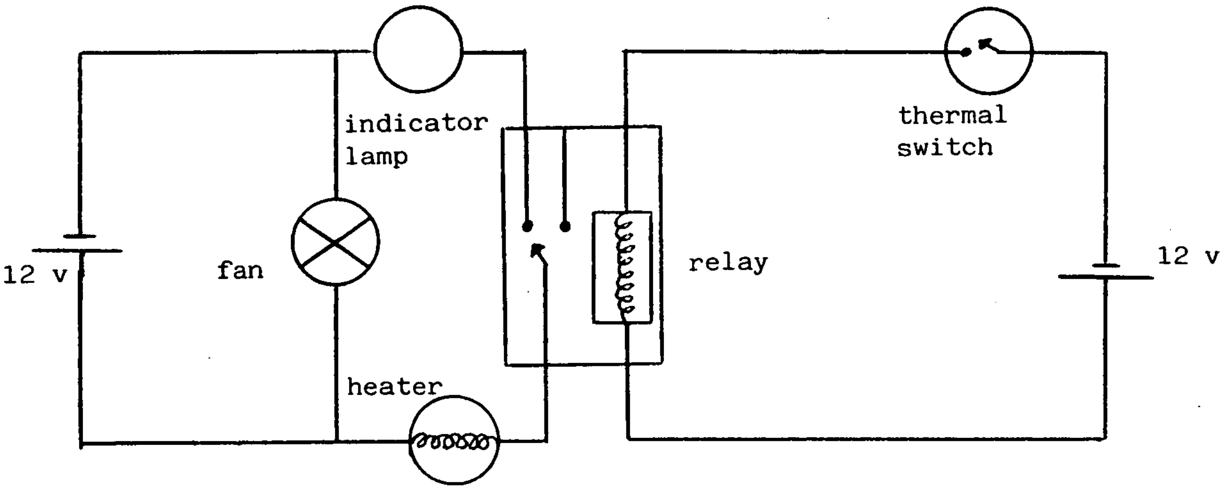

Figure 4.1.3.

Temperature Controlled Cabinet:
Temperature Control Circuit.

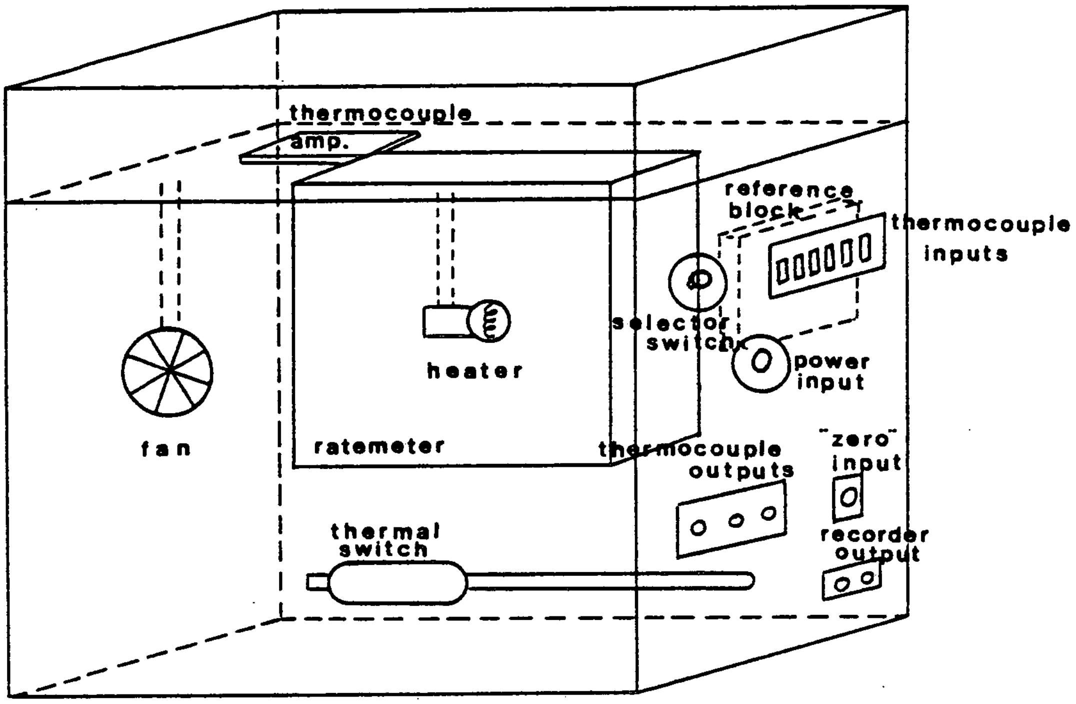

Figure 4.1.2.
Temperature Controlled Cabinet.

disrupt the flow of current to the heater. Using this system, the internal temperature of the cabinet was held at a constant 29.8 +/- 0.5 ^{o}C, with a 20 second time interval between minimum and maximum temperatures. Although the cabinet temperature varied by this amount, no detectable variation in the temperature of the thermocouple reference block was found over this interval due to its thermal mass. Performance was checked using a precision platinum resistance thermometer (Guildline, Ontario, Canada).

The "sensing" thermocouples were connected via copper-constantan plugs and sockets (Omega Engineering Inc. Conn. USA) to the junctions, with the resulting outputs being passed to a multi-position switch. This switch allowed each of the sensors to be selected in turn, and the output monitored on a thermocouple amplifier (Dixon 1982), or recorded on a chart recorder for future analysis. Outputs were also provided which bypassed the selector switch, so that all the channels could be monitored continously using a data logger.

The thermocouple amplifier used in this study was mounted inside the controlled cabinet, so that its temperature might be held at a fixed value to prevent errors due to drift. The amplifier was capable of resolving temperature differentials of +/- 0.05 ^{o}C. Also mounted within the cabinet was the PSR6 gamma ratemeter (chapter 3.2.2), so that its temperature could be controlled to prevent any temperature dependent drift in zero or sensitivity.

Ambient air temperature was measured using a standard 42 gauge thermocouple, referenced to the aluminum block, and placed within a ventilated psychrometer (Jones unpub.), which was positioned aproximately two meters from the canopies and at the same height.

The net radiation flux of the canopies was determined using a miniature Funk-type net radiometer (Solar Radiation Instruments, Victoria,Australia), which was held approximately 0.5 m above the canopy. Initial trials of the energy balance technique in determining the transpiration rate of small potted trees when compared with gravimetric measurements of actual water loss, indicated that a good correlation (figure 4.1.4) existed between the techniques if the value of net radiation used in the calculations was 0.75 of the actual value measured above the canopy. This factor departs from unity because the net radiometer does not sense the net radiation absorbed by the leaves under study, only that of the canopy surface as a whole, plus the ground. As some of the leaves are shaded from the sky by their neighbours, and so experience a reduced absorbtion of net radiation compared with those leaves "seen" by the radiometer, the factor is likely to be always less than unity. This however will depend upon canopy structure and leaf area index. It is possible to get values for this factor greater than one, depending upon the radiative properties of the ground relative to the leaves. As the present study was more concerned with relative differences in transpiration rate between the experimental treatments rather than absolute values, it was felt that the adoption of this factor was justified.

However, to obtain more accurate values of transpiration a modification of the energy balance technique was investigated as described in chapter (4.1.1). This modification allowed the calculation of the net radiation absorbed by the leaves in the canopy by first determining the boundary layer resistance of a sample of leaves using models. The models were constructed by

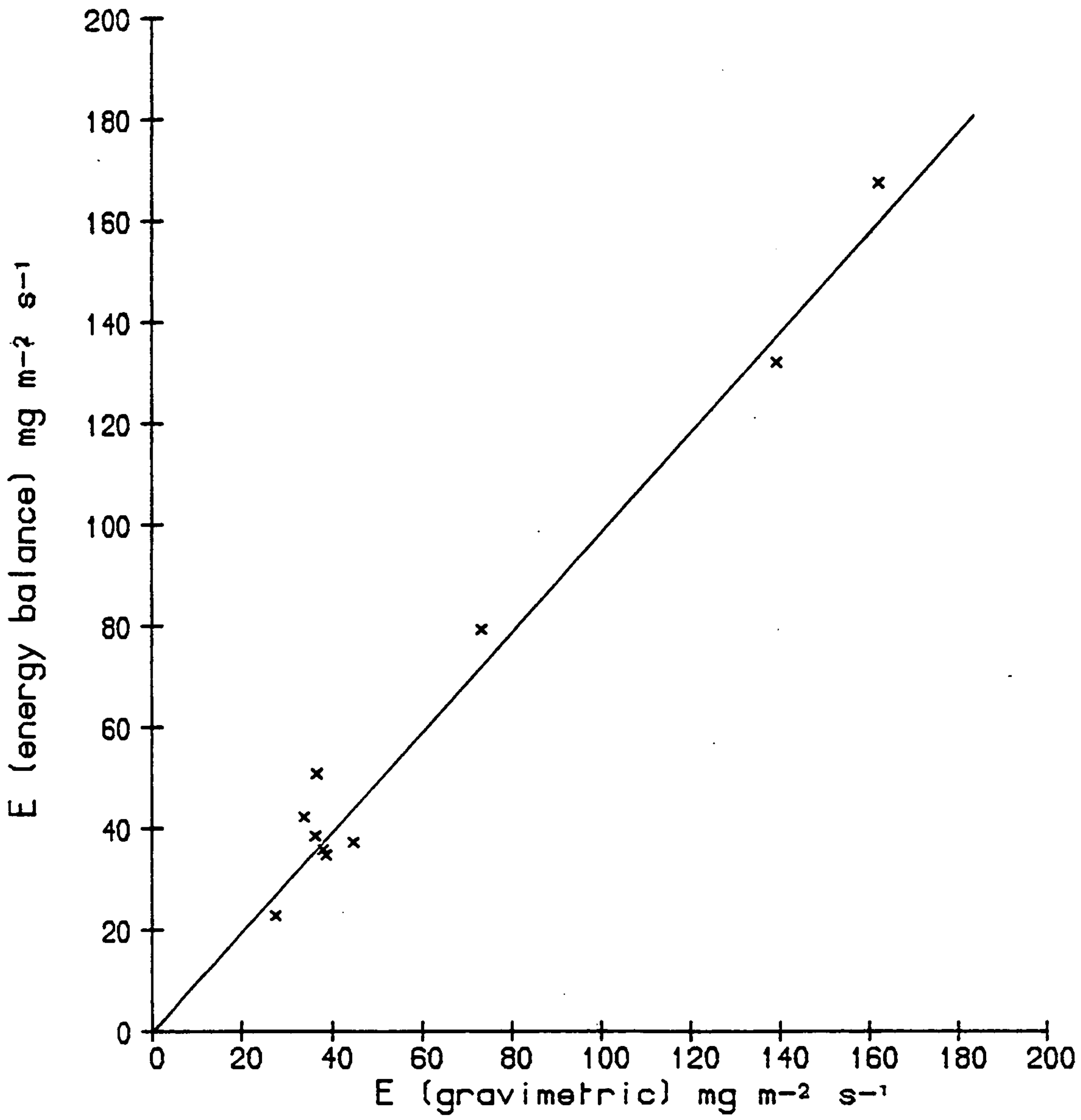

Figure 4.1.4.
The Relationship between Evapotranspiration Determined Gravimetrically and using the Energy Balance Technique.

tracing the outlines of apple leaves onto brass sheet, 0.25 mm thick. Two outlines of each leaf were sandwiched together using double-sided "Sellotape" and covered on both sides with aluminised mylar (plate 4.1.1). In the case of the heated models, 42 gauge insulated constantan wire was run in a tight "zig-zag" between the two outlines to form the heating element. The efficiency of this heating system was tested by placing five thermocouples at various positions on the model surface. No variation in temperature was detected across the area of the "leaf". A heating current from a car battery was passed to the heated models which were wired in parallel, via a current limiter to control the model temperature. The surface temperature of the heated and non-heated models were measured using two sets of thermocouples wired in parallel, one thermocouple on each side of each model.

Initial trials were carried out in a controlled environment wind tunnel using five pairs of models to calculate boundary layer resistance at a variety of windspeeds. A relationship was obtained, and the results compared with published data (figure 4.1.5), using non-dimensional groups i.e. Reynolds number Re, and Nusselt number Nu, so that the comparison would be independent of leaf size (Monteith 1973), where

$$Re = d/rh.k \quad \text{and}$$

$$Nu = ud/v$$

d = the characteristic dimension of the leaf (Monteith 1973)

rh = boundary layer resistance

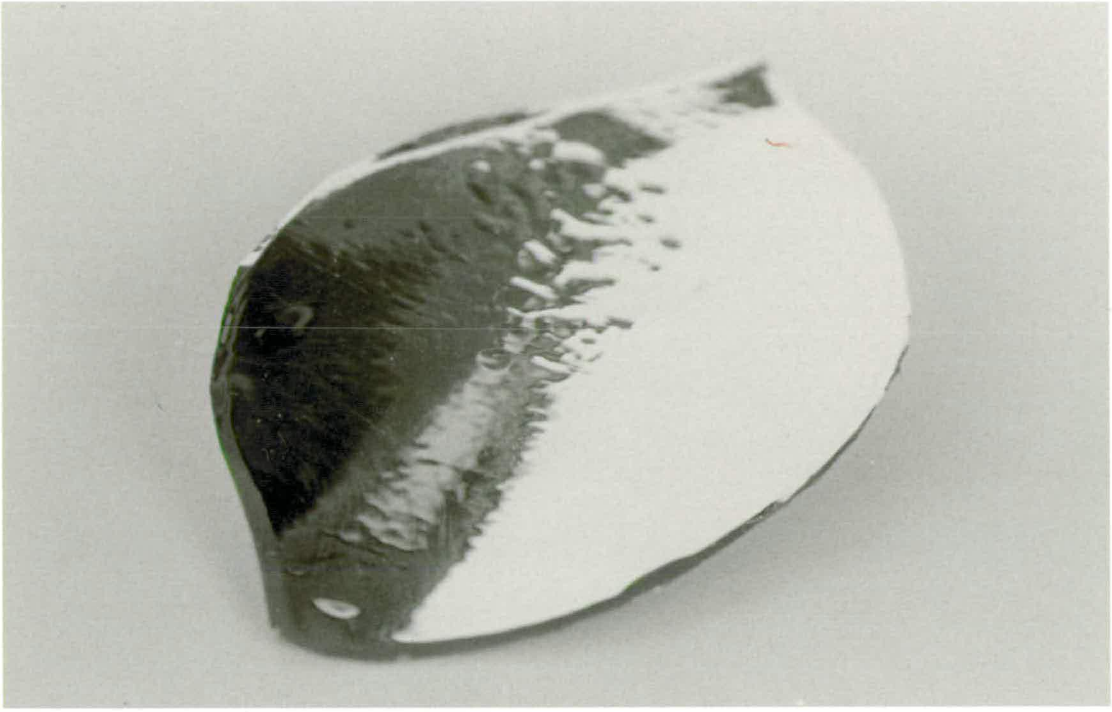

Plate 4.1.1

A heated leaf model used in the determination of boundary layer resistance.

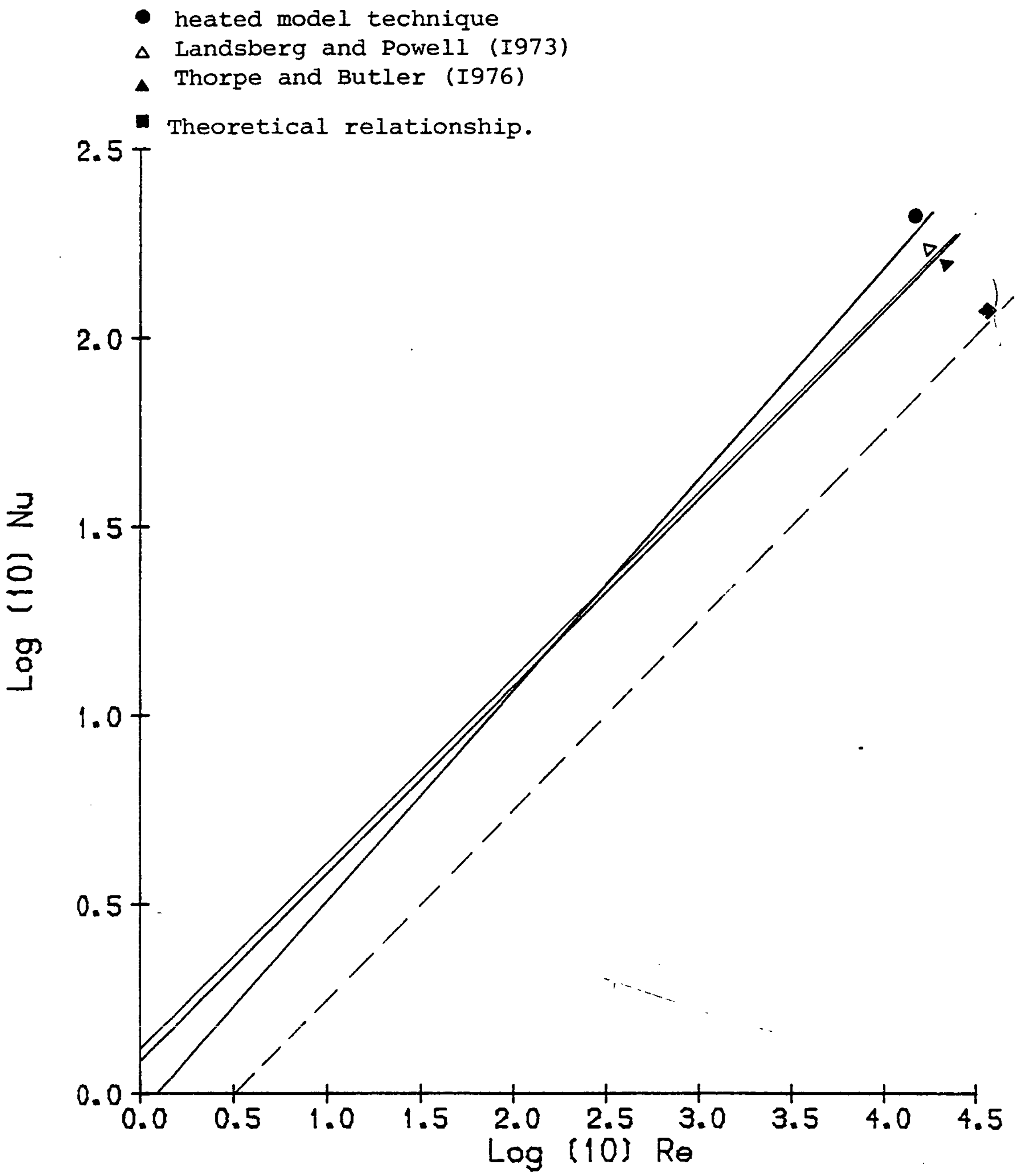

Figure 4.1.5.
The relationship between Boundary Layer Resistance and Windspeed, as indicated by Reynolds and Nusselt numbers, determined using a heated model technique, and compared with the results of Landsberg and Powell (1973), Thorpe and Butler (1976) and the theoretical relationship Grace et al (1980)

k = thermal diffusivity of air

u = windspeed

v = kinematic viscosity of air

The observed relationships were also compared with a relationship derived from theoretical considerations called the Polhausen relationship (Grace, Fasehun and Dixon 1980) where the relationship between boundary layer resistance is calculated for laminar boundary layers, where

$$Nu = 0.66\ Re^{0.5} \left(\frac{v}{k}\right)^{0.33}$$

The results of this study indicated that the relationship derived using the heated model agreed very well with the results of Landsberg and Powell (1973), and Thorpe and Butler (1976), though all three relationships were somewhat different from that calculated from theory. Subsequent field trials were carried out in an orchard at East Malling in April 1983, when values of boundary layer resistance were obtained over a thirty hour period and a correlation determined with windspeed, estimated from an anemograph trace, measured at a meterological station a few hundred meters from the models (figure 4.1.6).

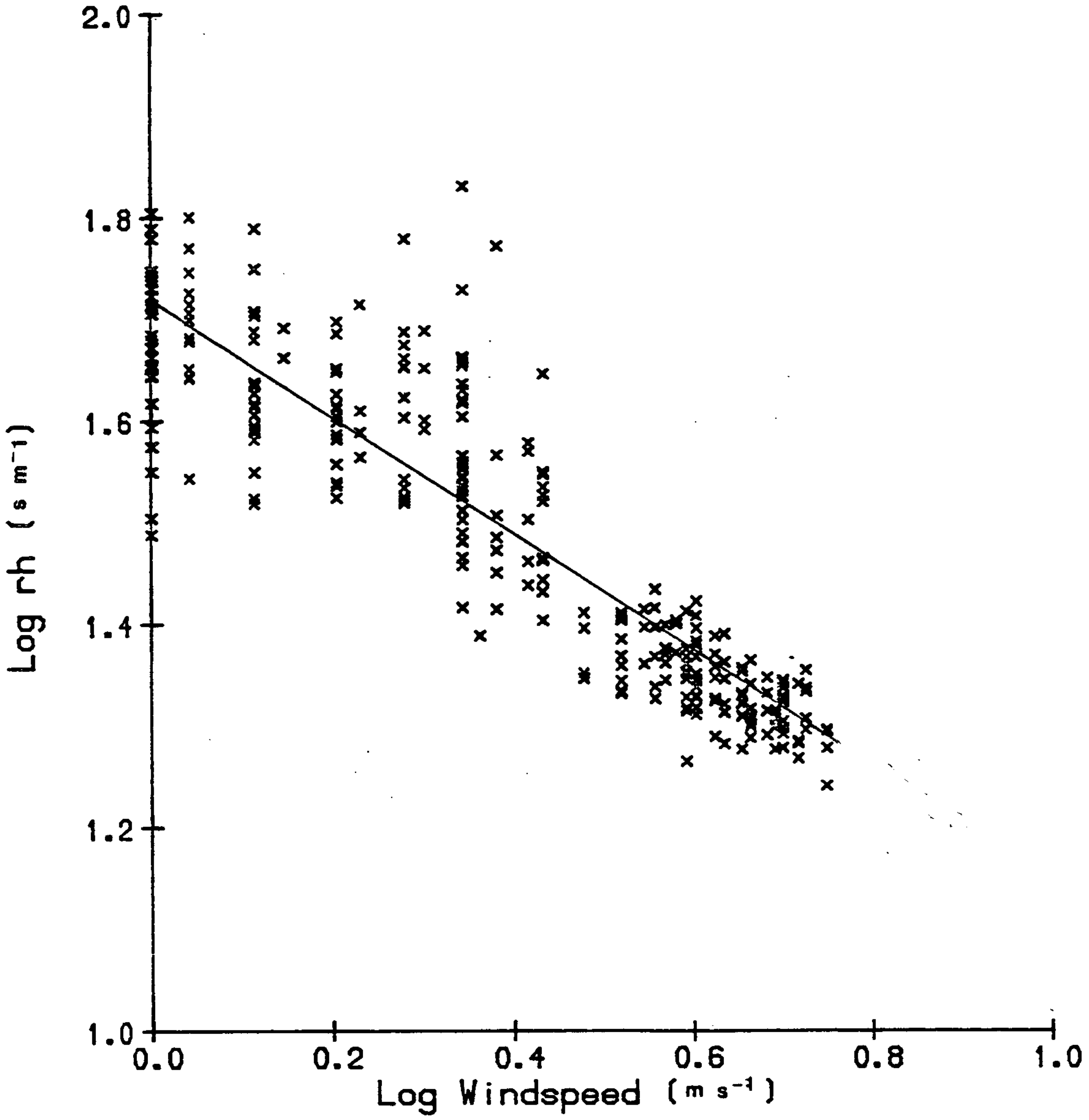

Figure 4.1.6.
The relationship between Boundary Layer Resistance and Windspeed, determined in the field using the heated model technique.

(4.2) Water Potential

(4.2.1) Background Theory and Literature Review.

Every physical and biological system has the capacity for doing "work"; this capacity being known as the Gibbs free energy of the system. If a system changes from one state to another such that the free energy decreases, then "useful work" can be done during the transition. Describing the process of transpiration in these thermodynamic terms then, as water evaporates from the cells of the leaf sub-stomatal cavity, it changes from a liquid to a gaseous state, changing the plant's free energy and allowing work, the transportation of water, to be done.

The contribution of water to the free energy of the system is known as the chemical potential of water. Thus the driving force for water transport can be described in terms of the difference in chemical potential between the plant and the atmosphere, across the leaf boundary layer and stomata. This difference in chemical potential is transmitted down the conducting pathway, setting up a gradient between the roots and leaves.

In order that a comparison of potential can be made between one system and another, an arbitrary standard of zero potential is defined as, the potential of pure free water at atmospheric pressure, and a temperature equivalent to that of the system under investigation (Milburn 1979). The chemical potential within a plant will differ from that of the reference due to factors such as hydrostatic pressure greater that one atmosphere, the presence of solutes dissolved in the xylem sap, and the presence of interfaces such as that between the sap and the xylem cell walls. This differential, when divided by the partial molal volume of the water within the system, is known as the Water Potential, Ψ (Slatyer 1967).

By defining water potential in this way, when water is freely available, Ψ is equal to zero, and becomes more negative as water content is reduced, eg. during periods of water stress. The units of water potential are defined in terms of energy per unit volume i.e. J m^{-3}. Expressing energy in Newton meters (1 Joule = 1 N m), the units of water potential are seen to be equivalent to those of pressure i.e. N m^{-2}. Using this definition, it is seen that the water stress experienced by a plant can be measured in simple pressure units i.e. the Pascal (1 Pa = 1 N m^{-2}).

It possible to measure water potential in several different ways. perhaps the simplest and most direct technique is that of the pressure "bomb" first proposed by Dixon (1914), but developed as a working system by Scholander et al (1965). When a leaf or branch is cut from a plant, the tension within the xylem will cause the sap to be drawn into the tissue away from the cut surface. If the leaf or branch is then sealed in suitable chamber with the cut end exposed, then pressure can be applied to the leaf, by means of a gas, until the sap is observed at the cut surface. At this point the applied positive pressure exactly balances the negative pressure or tension which existed within the tissue before excision. Although this system is destructive, on a large canopy this technique allows simple rapid determination of water potential.

The thermocouple psychrometer determines water potential by enclosing a sample of plant tissue within a sealed chamber, and allowing it to come to vapour pressure equilibrium with the air in the sample chamber. After equilibrium has been reached, the humidity of the air, which is determined by the water potential of the sample, can be measured after condensing a droplet of water on a thermocouple within the chamber by Peltier cooling. This droplet is allowed to evaporate, the rate of evaporation being dependent upon the humidity within the chamber. Evaporative cooling causes a temperature differential between the thermocouple and the chamber which can be measured with a microvoltmeter. This is known as psychrometric determination of water potential. It is possible to use a psychrometer as a hygrometer by using it in the dew point mode. In this system the thermocouple is cooled by Peltier cooling as before, but in this mode the temperature of the junction is held at that of the dew point where there is equilibrium between condensation and evaporation. The dew point temperature depression can then be measured as before. Both methods of measurement i.e. psychrometric and dew point should give the same value of water potential for a given tissue sample, after proper calibration. The dew point method is generally prefered, as this mode is much less affected by changes in ambient temperature than the psychrometric mode, and its sensitivity coefficient is almost twice that of the psychrometric method (Wescor Inc., Utah, USA). Using osmotic solutions of known water potentials, it is possible to calibrate the psychrometer to meaure the water potential of any plant tissue. This technique is very attractive in that the amount of plant tissue required for sampling can be very small, leaf discs 5 mm in diameter being commonly used. Unfortunately the system requires long equilibration times, for some samples, in excess of five hours. Thus for good statistical replication of an experiment many chambers would be required for frequent sampling over short time periods.

Dixon (1978) reported a linear relationship between the electrical impedance of a tree and its water potential, measured with a pressure bomb and a thermocouple psychrometer. Unfortunately this relationship only holds for any one plant tissue at any one sampling time, making it impossible to use absolute values of impedance to compare water potentials between individuals or the same individual over a time course (Dixon 1982).

Other techniques such as the Schardakov method, which involves immersing leaf tissue in sucrose solutions of varying concentration and determining the isotonic solution (Goode 1965), and correlation with leaf thickness, as measured by the attenuation of a beam of beta particles (Jones 1973a), have also been used with success to measure water potentials.

(4.2.2) Equipment and Experimental Design.

In the present study, water potentials were measured using a pressure bomb, designed and constructed in the Department of Forestry and Natural Resources, The University of Edinburgh. This device allowed rapid, simple determination of water potential up to values of 3 MPa. The maximum precision possible with this pressure bomb is around 0.01 MPa, but the actual precision achieved during use will be less than this due to problems associated with the determination of the end point, i.e. the point at which water just wets the cut surface.

Diurnal variation in water potential of a tree canopy was measured by averaging the potentials of three leaves taken from similar positions on extension shoots in different areas of the canopy. Measurement commenced before dawn, and ended just before sunset, with no more that six minutes elapsing during sampling of all three experimental canopies. Seasonal variation was determined in a similar fashion, choosing dusk as the standard time of day for measurement. This was a convenient point in the diurnal cycle to determine, so that diurnal fluctuation could be removed from the variation between sampling dates.

(4.3) Relative Water Content.

(4.3.1) Background Theory and Literature Review.

A commonly used indicator of plant water status is that of relative water content (RWC), where RWC is the water content, on a percentage basis, relative to the water content of the same tissue at full turgor (Barrs 1968). As previously stated, during times of water stress, water is drawn from the plant tissues maintaining an equilibrium between cell and xylem water potentials. Therefore, as water stress is related to absolute cell water content, the measurement of RWC is perhaps more relevant in many cases than that of water potential. It is noted however that RWC is a rather insensitive indicator of water status when only a small deficit exists (Hsiao 1973).

Calculation of RWC involves weighing a sample of plant tissue, to obtain the fresh weight (FW), and floating the sample, usually a leaf disc, on water for a period of time until full turgor is reached. This point is determined as the point where leaf water potential is equal to zero. The disc is then blotted dry, and the turgid weight, (TW), measured. The discs are then dried and the dry weight of the tissue, (DW), determined. Relative water content is then calculated as,

$$RWC = \left(\frac{FW - DW}{TW - DW}\right) \times 100\%$$

(4.3.2) Equipment and Experimental Design.

In the present study, diurnal and seasonal variation in RWC was measured as an average of three leaf samples, taken from similar positions on extension shoots, in different areas of a canopy. As apple leaf discs floated on water are prone to injection, i.e. water influx into the cellular air spaces, which would tend to give a false value for TW, whole leaves were used for RWC determination. Sample tubes, 100 mm x 25 mm, were filled with 15 ml. of water and weighed. The leaves were cut from the branches, and immediately placed in the tubes, so that the petioles were immersed in the water. The tubes were again weighed, and the difference in weighings taken as the fresh weight of the leaves. The tubes were placed in a fridge at a temperature of $5^{o}C$, to prevent growth, for a period of 36 hours to achieve full turgor, when the leaves were removed, blotted, and the turgid weight measured. The leaves were dried in a hot air oven for a period of 12 hours at $65^{o}C$, and again weighed to determine the dry weight.

A time course experiment was conducted to determine the optimum incubation time for the leaves to attain full turgidity. Excised leaves were placed in sample tubes, and incubated as before. Samples were removed at 30 minute intervals and weighed until a point was reached when no further increase in leaf weight was observed, i.e. full turgor had been achieved (figure 4.3.1).

Diurnal variation in RWC was measured at hourly intervals, commencing before dawn and ending just before sunset, while seasonal variation measurements were made at dusk, as previously described.

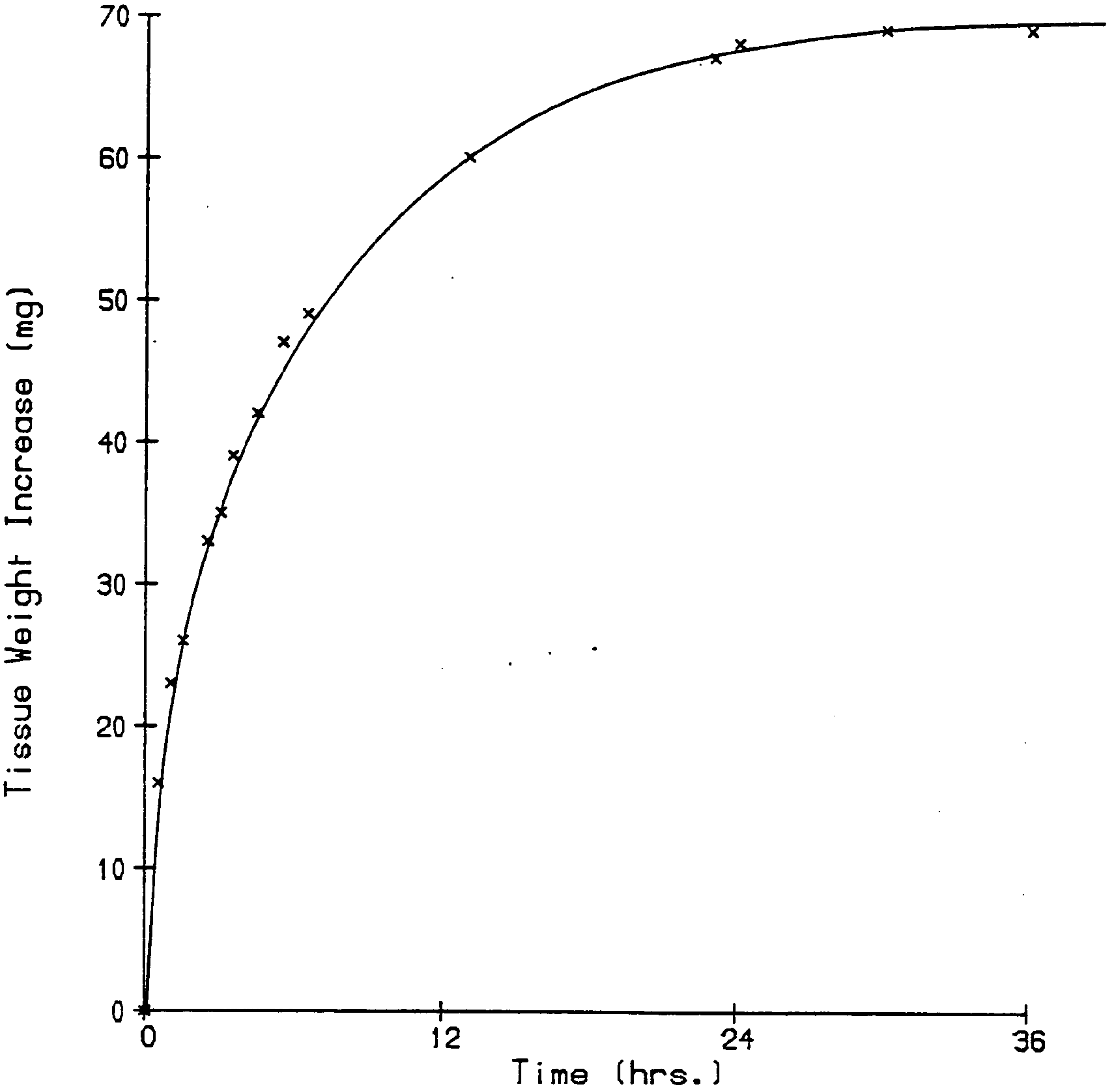

Figure 4.3.1.
Relative Water Content Determination:
Time Course for Golden Delicious
apple Leaves to attain Full Turgidity
at 5 oC.

(4.4) Soil Water Status.

(4.4.1) Background Theory and Literature Review.

The flux of water from soil to plant depends upon the difference between soil water potential and leaf water potential. If the soil water potential is higher, i.e. less negative, than the plant potential, then water will flow from the soil into the root system and into conducting pathway. Plant water stress develops when water loss by transpiration exceeds water absorbtion from the soil. Temporary mid-day deficits occur in rapidly transpiring plants because resistance to water movement from the soil through the roots causes absorbtion to lag behind transpiration, even in moist soils (Kramer 1969b). Long term and even more severe deficits develop when decreasing soil water potential and hydraulic conductivity cause decreased absorbtion. Thus diurnal cycles in water stress are controlled chiefly by transpiration, but severe long term deficits develop mainly because of decreasing availability of water.

Measurement of soil water status can be achieved by determining the soil water potential using psychrometers or tensiometers or the soil water content by electrical resistance methods or neutron scattering. The soil psychrometer is similar in principal to that used for leaf tissue (4.2.1), where a representative soil sample is placed in a closed container at a known constant temperature, and the water vapour pressure above the sample measured by the Peltier cooling technique already described. Commercial psychrometers are available which can be buried in the soil, and water potential measured in situ.

A soil tensiometer is essentially a porous ceramic pot filled with water, which is connected to some means of measuring pressure changes, such as a mercury manometer, vacuum gauge or pressure transducer. As the soil dries, water is extracted from the pot and a negative pressure registered. If the soil continues to dry, the pressure falls until a point is reached, around -0.8 MPa , when air is drawn through the pores of the pot and the tensiometer cavitates. (Milburn 1979). Thus a tensiometer will work only in the range 0 to -0.8 MPa, which would seem to be rather inadequate for most field uses.

Electrical resistance techniques, involving gypsum blocks, can measure water potentials over a range of approximately -0.05 to -1.5 MPa with a reasonable degree of accuracy (Bouget et al 1958). In this technique, two electrodes, embedded in a gypsum block, are buried in the soil and the resistance between them measured using an A.C. bridge.The resistance of the gypsum block varies with its water content, which is in equilibrium with the water content of the soil. Calibration of the system may be achieved by measuring the resistance of the block in soils of known water content, which can be determined gravimetrically or by using a pressure plate apparatus.

The pressure plate technique is similar to that of the pressure bomb, where a soil sample is compressed in a chamber using gas pressure, and the amount of water exuded at different pressures measured. This method enables a soil moisture release curve to be plotted so giving a correlation between applied pressure, equivalent to tension or soil water potential, and soil water content.

The estimation of total soil moisture content can be achieved by the technique of neutron scattering. In this system, "fast" neutrons are emitted from a suitable radioactive source which has been lowered into the soil via an aluminium access tube. These neutrons are slowed down by collision with hydrogen atoms, present mainly in the form of water, so that a "cloud" of "slow" neutrons is produced, whose flux density is proportional to the moisture content of the soil per unit volume. Detection of these slow neutrons is possible by using boron trifluoride proportional counters, where a nuclear reaction takes place between a slow neutron and the boron resulting in the emmission of an alpha particle which is registered as a discharge pulse. The measurement of the density of this slow neutron cloud consists then of detecting and counting the number of discharges per unit time. This counting can be achieved by means of a scalar ratemeter similar to that used in the gamma probe studies (3.2.2).

(4.4.2) Equipment and Experimental Design.

In the present study, soil water status was measured using a neutron probe (Wallingford model 225, Pitman Instruments, Surrey), as this provided a reliable, accurate and rapid means of measuring soil moisture content throughout the 1982 growing season. Aluminium access tubes, 0.8 m long, were sunk into the soil in each experimental sub-block, as shown in figure 2.1. The soil water content was measured at weekly intervals, at depths of 0.2, 0.4, and 0.6 m at two positions in each sub-block, and the results expressed as a percentage change in water content relative to field capacity, as measured on the 22/4/1982, where field capacity is the amount of water retained in a soil after drainage towards the water table has resulted in a near-constant soil moisture content (Meidner and Sheriff 1976).

Calibration of the probe was achieved by correlating measured soil moisture contents against the same measurements made with a similar probe, whose calibration had been determined by correlating probe moisture content values with gravimetrically determined soil water contents. A check for calibration drift was made at the beginning and end of each sampling period by taking a neutron scatter count in an access tube mounted within a full water tank.

The results were subjected to an analysis of variance using the computer statistical package GENSTAT, which indicated any variation in soil water content between the experimental sub-blocks, and the experimental irrigation treatments. For later comparison with other measured parameters, the soil water content was averaged over the three depths at each sampling point, and the mean values converted to soil water potentials using the soil moisture release curve (figure 4.4.1) which had been produced at East Malling using tensiometers and the pressure plate technique.

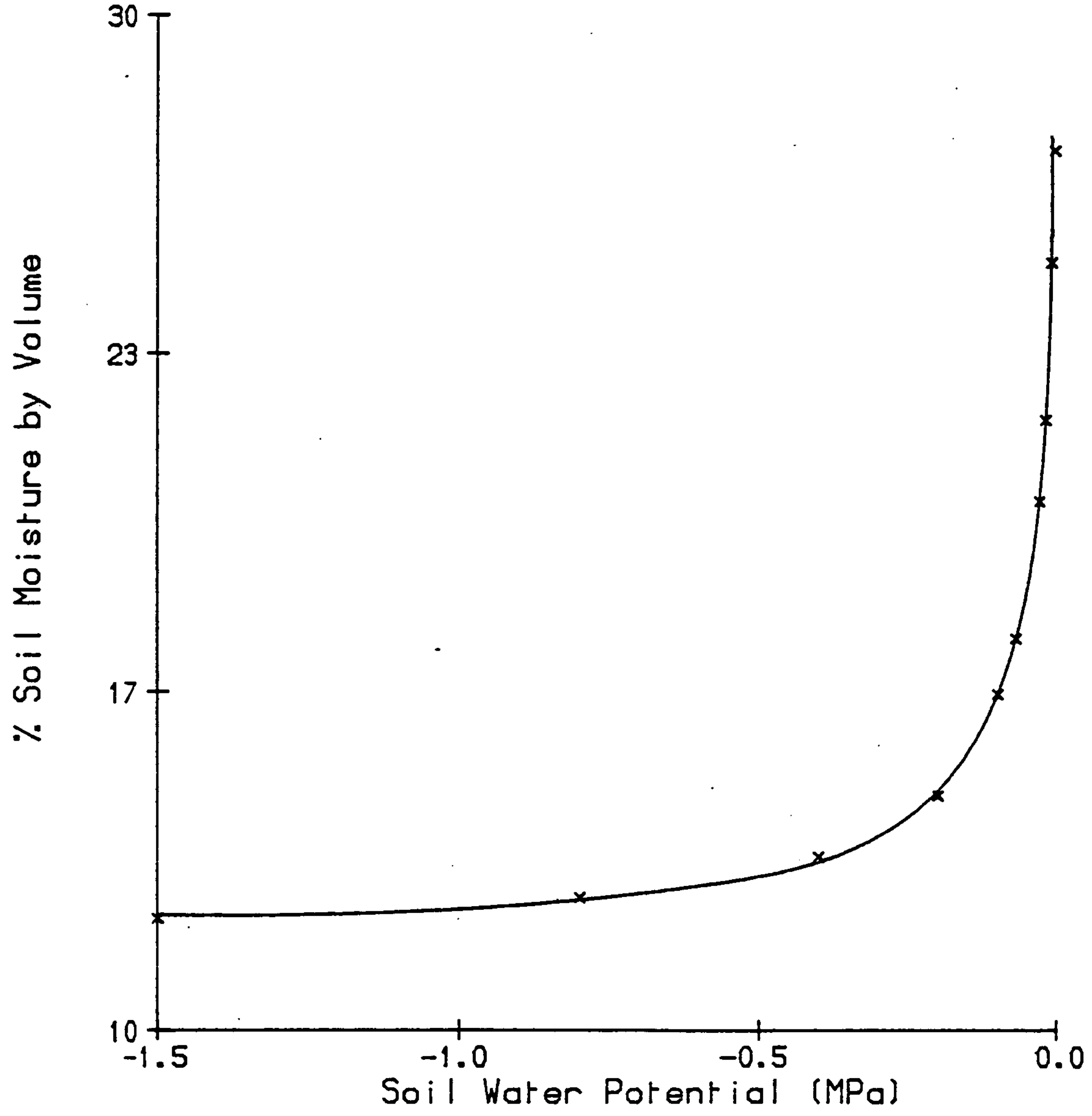

Figure 4.4.1.
Soil Moisture Release Curve obtained using Pressure Plate Apparatus and Soil Tensiometers on Orchard Soil from East Malling Research Station.

(from data obtained by J.E.Goode)

Chapter (5) The Effects of Water Stress on Plant Growth and Productivity.

(5.1) Background Theory and Literature Review.

Plant responses to water stress have been extensivly reviewed in the literature by several authors eg. Kramer (1969), Kozlowski (1968a; 1968b; 1972; 1976; 1978; 1981) and Hsiao (1973). It is noted, that although most land plants can withstand a degree of water stress in their normal life cycle, every process within the plant can be detrimentally ~~e~~affected if that stress is severe or prolonged. The degree of response to this stress will depend upon the sensitivity of the process and the age or condition of the plant (Landsberg and Jones 1981).

As the water content of a soil declines and a water deficit develops within the tissues, the plant closes its stomata and transpiration decreases, preventing further desiccation. Although the plant has now reduced the possibility of a further internal water deficit occuring, the closing of the stomata prevents gas exchange and therefore photosynthesis. Thus if water stress is prolonged, the net assimilation and therefore growth of a plant will be curtailed.

The effects of water stress on the growth of trees is reviewed by Zahner (1968), and on apple trees in particular by Landsberg and Jones (1981). Considering vegetative growth, and in particular that of the extension growth of shoots, Chapman (1973) found that a reduction in plant water status caused significant differences in shoot growth between stressed and unstressed trees. It was noted that the observed reduction in the final length of the stressed shoots was due to decrease in growth rate, and that the shoots ceased to grow long before the unstressed controls.

Although growth is dependant upon cell turgor, as loss of turgidity prevents cell enlargement, and cell division, which may be reduced by up to 50% during a decrease in water potential from 0 - 0.1 MPa. (Meidner and Sheriff 1976), the underlying long term effects of water stress on growth are on the partioning of available resources within the system (Milthorpe and Moorby 1974). Wardlaw (1968) concluded that mild water stress could effect assimilate partioning by altering source - sink relationships within the plant system. If leaf expansion within a canopy was reduced by stress, then the leaf canopy "sink strength" would be reduced, and so lessen competion for other sinks eg. the fruit.

In considering the effects of stress upon reproductive growth and the apple tree's productivity, defined in terms of fruit size and total crop weight, the stress levels at several stages of fruit development must be taken into account. A water deficit in one season can effect productivity in the next, by reducing (Salter and Goode 1967), or increasing (Powell 1976) flower bud morphogenesis. The number of fruit which develop within a tree canopy depends initially upon the number of flowers present in the canopy and the degree of successful pollination. Not all fruits which are initially formed develop to maturity, as a proportion of the fruitlets are naturally "thinned" during a period known as the "June drop".

The extent to which a canopy is thinned would seem to be affected by tree water status. Studies on Cox's Orange Pippin which had been irrigated by overhead spraying, in an attempt to reduce stress levels, lost less fruit than non-irrigated trees. It was also found that in the irrigated trees, the final fruit size was smaller than in the stressed controls (Goode et al 1978). Thus stress can be seen to effect final fruit size by reducing fruit load in a canopy, thus lowering the competition for assimilate resources. If stress continues after this initial drop, then further effects on growth and productivity can be found.

Extensive investigations eg. Assaf, Levin and Bravdo (1974), have indicated a close correlation between fruit growth and plant water status. In a tree with an "adequate" water supply, it was found that fruit volume growth was linear throughout the growing season, and that fruit size at harvest could therefore be determined by measurements during the growing season (Forshey 1971). If however, a tree undergoes a water deficit, the fruit growth curve deviates from this linear relationship, and the final fruit size is reduced (Assaf, Levin and Bravdo 1974).

In the present study it was impossible to investigate the effects of stress on flower bud production, as experimental fieldwork was limited to the 1982 growing season. It was therefore decided to investigate the effects of stress on extension shoot growth and the fruit crop, and determine any possible benefits which might arise from overhead mist irrigation and ground sprinkler irrigation in an attempt to reduce water stress.

(5.2) Equipment and Experimental design.

Variation in shoot growth between stressed and unstressed trees was determined by placing a marker behind the fifth fully expanded leaf on five extension shoots within each canopy on 17/6/1982. The central tree in row 2 (figure 2.1), of each experimental sub-block was chosen for shoot measurement. There were four such trees, resulting in twenty shoot measurements per irrigation treatment. The number of fully expanded leaves in front of the marker was counted on a weekly basis and the mean increase in growth per irrigation treatment determined.

The effects of stress on fruit growth were measured in a similar manner, by tagging twenty five fruit in groups of five, on five separate branches throughout the canopy, on the same trees as above, thus giving a total of 100 fruit per sample per experimental treatment. The maximum diameters of the tagged fruit were measured on a weekly basis, commencing 17/6/1982, using a micrometer, between 9.30 am and 11.00 am on each occasion, so as to avoid errors due to diurnal fluctuations in fruit diameter. The fruit diameter data were subjected to an analysis of variance and the mean fruit diameter per irrigation treatment determined for each sampling period.

The seasonal variation in total fruit volume for each of the experimental treatments was determined by assuming that an apple was a perfect sphere, and converting the diameter measurements to fruit volumes. The total number of fruit within each canopy was counted at harvest, so that the volume of the 100 sampled fruit could, by proportion, be used to calculate the total fruit volume of the canopy at each sampling period. The mean total fruit volume per canopy was calculated for each irrigation treatment, the results being graphed and subjected to analysis, to test for any significant differences in fruit volume which may exist between the treatments.

Chapter 6 Water Relations in Orchard Conditions.

(6.1) Experimental Technique Efficiency.

(6.1.1) Mist Irrigation.

To be effective, mist irrigation should maintain a water film on the majority of leaves. If the adaxial surface is wet, the leaf will be substantially cooled by evaporation, thus reducing transpiration from the stomata on the abaxial surfaces, and hence alleviating water stress. If both surfaces are wet, as was often found to be the case in field trials, there will be no transpiration at all and perhaps some water uptake through the leaf surface. Dry leaves may undergo reductions in transpiration if the humidity of the surrounding air is increased by evaporation of water films and water droplets. No significant increase in humidity within the canopy was detected during the investigations, possibly due to wind blowing through the canopy. Thus the efficiency of the mist spray in wetting the canopy is of prime importance.

The efficiency of the mist system was tested on three days under a variety of environmental conditions as described in chapter 2. Fifteen groups of five leaves were tagged at various positions within the canopy, three groups within a central circle of radius 0.2 m, four at a distance of 0.4 m from the center, and a further four at 0.8 m from the center of the tree, all at an approximate height of 1.0 m from ground level. An additional four groups were tagged at a height of approximately 2.0 m from ground level, at a distance of 0.8 m from the center, so that they lay above the mist nozzles. The canopy was irrigated under normal operating conditions and the wetness of the leaf groups determined on a scale from 0 i.e. dry to 3, totally wet. The mean wetness of leaf groups were displayed on "target" diagrams (figures 6.1.1, 6.1.2, 6.1.3).

The results indicate that the central area of the canopy was totally wet in 90% of the trials that were carried out. It appeared that the ability of of the mist system to wet the rest of the canopy depended upon the prevailing windspeed and wind direction. In the absence of wind, the canopy was normally well wetted at the 1.0 m level except for the very peripheral areas. It was noted that the leaves above the mist nozzles remained dry in the majority of cases in low windspeeds. It was found that the canopies which were mist irrigated were substantially cooled, by up to 7 °C on one occasion, compared with the ground irrigated or control trees. The leaf temperature data used to calculate transpiration rates (chapter 6.3) is presented in appendix B.

In windy conditions it was observed that, in the main, the mist cloud only wetted the leeward areas of the canopy, except within the central zone, as previously stated. The majority of the canopy leaf area in the experimental trees was generally contained within a circle of diameter 2.0 m, and it is noted that in all the trials in this investigation a large proportion of this area was always wetted by the mist system.

(6.1.2) Ground Sprinkler Irrigation.

The ground sprinkler irrigation system was designed to keep the soil at full field capacity, so that the transpirational demands of

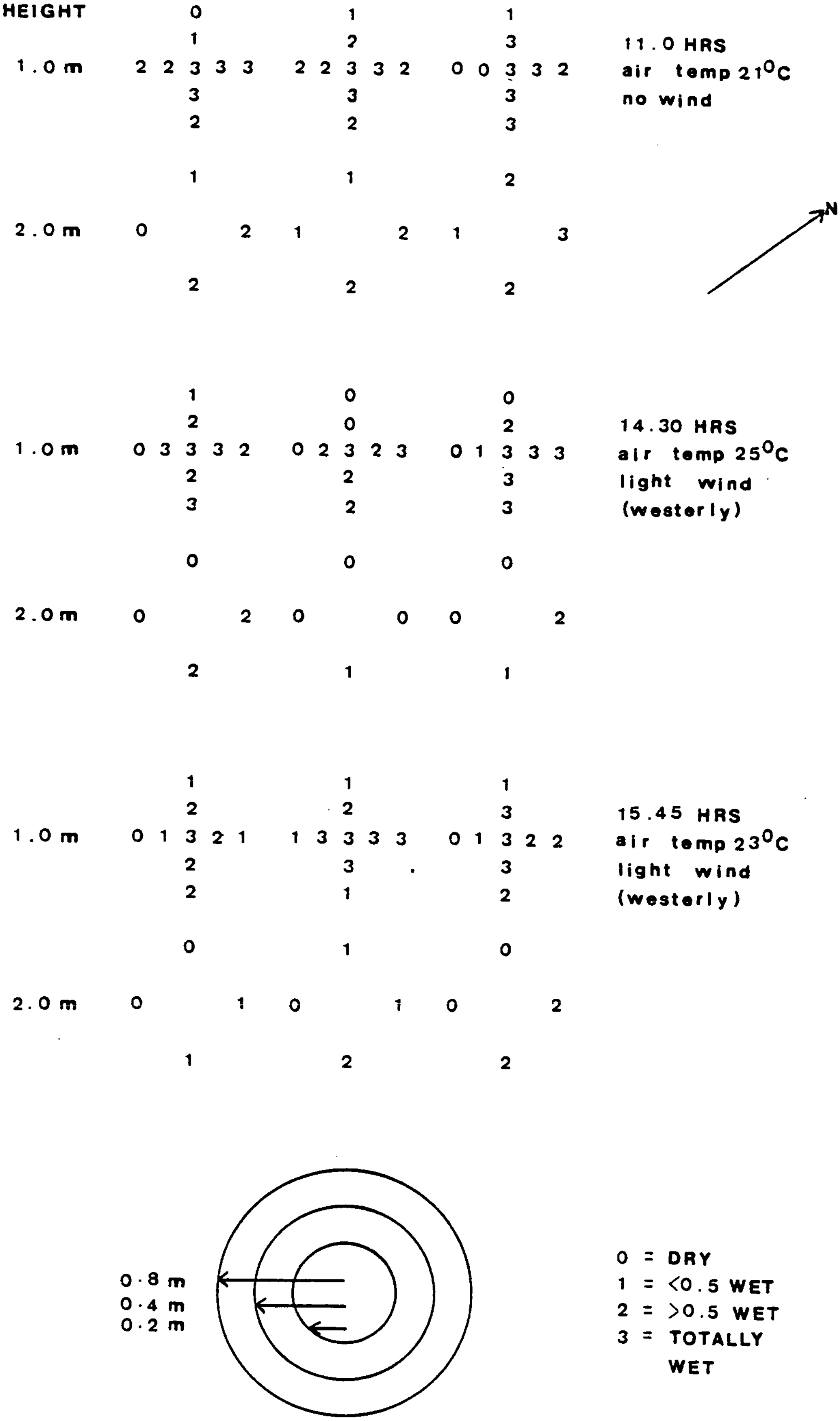

Figure 6.1.3.
Mist Efficiency Experiment 11.8.82.
The efficiency of the overhead mist irrigation system in wetting the canopies of Golden Delicious apple trees under a variety of environmental conditions, as described in the text.

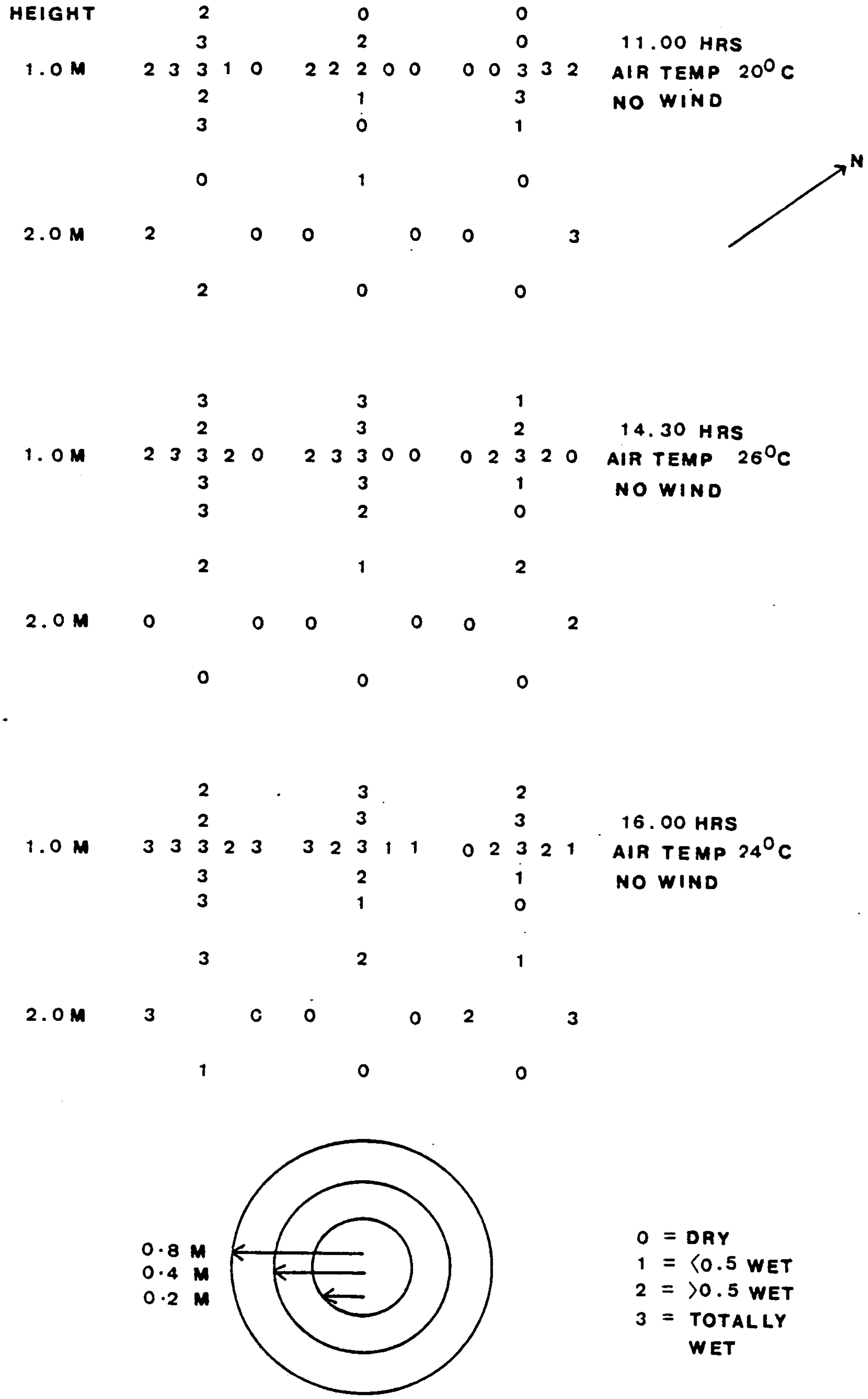

Figure 6.1.2.
Mist Efficiency Experiment 9.8.82.
The efficiency of the overhead mist irrigation system in wetting the canopies of Golden Delicious apple trees under a variety of environmental conditions, as described in the text.

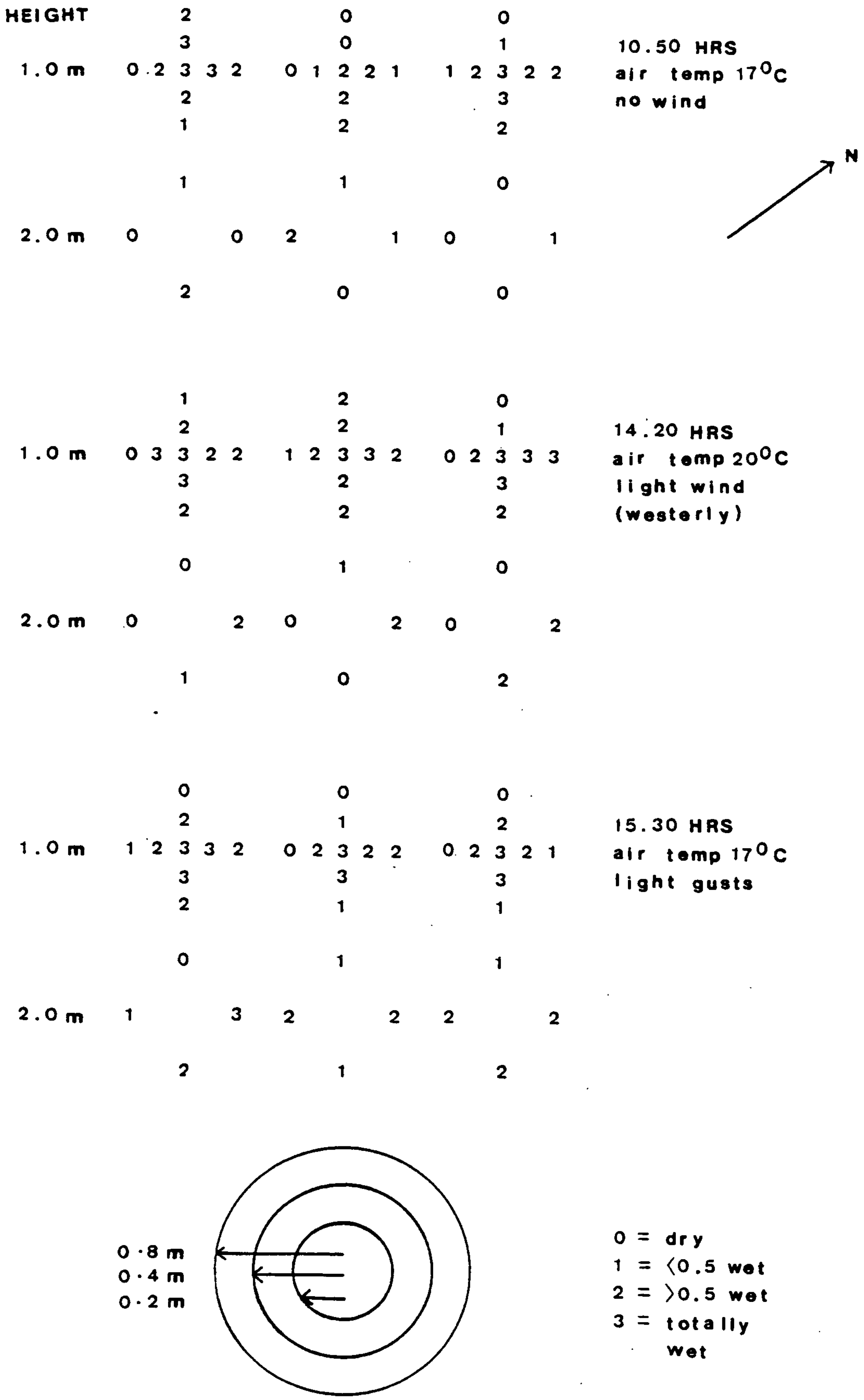

Figure 6.1.1.
Mist Efficiency Experiment 28.7.82.
The efficiency of the overhead mist irrigation system in wetting the canopies of Golden Delicious apple trees under a variety of environmental conditions, as described in the text.

the trees would not exceed the available water supply and so result in water stress. Water was applied to the soil daily to replace that lost by evapotranspiration and the soil water content monitored using a neutron probe. The data from the probe measurements was converted to values of soil water potential by means of a soil moisture release curve (figure 4.4.1) and the seasonal variation in soil water potential for each of the irrigation treatments (figure 6.1.4).

It is noted that the soil water potential of the irrigated blocks remained relativly constant throughout the experimental period with a mean potential of -0.016 +/- 0.002 MPa. In contrast the soil potential of the control blocks remained constant till the third week in July when a steady decline in potential was detected. By the end of the experimental period the soil water potential of the control blocks was approximately five times lower than that of the irrigated blocks i.e. a difference of 0.067 MPa.

(6.1.3) Statistical Analysis Techniques.

The experimental results discussed in this chapter were subjected to an analysis of variance, using the statistical analysis facilities contained in the computing package "Presto" (R. Muetzelfeldt, Forestry and Natural resources, The University of Edinburgh). In the present study the experiments were designed to investigate any sample variation that might exist, due to the effects of the three experimental treatments i.e. mist irrigation, ground sprinkler irrigation and non-irrigated controls. The study was not really concerned with any variation that might exist in the

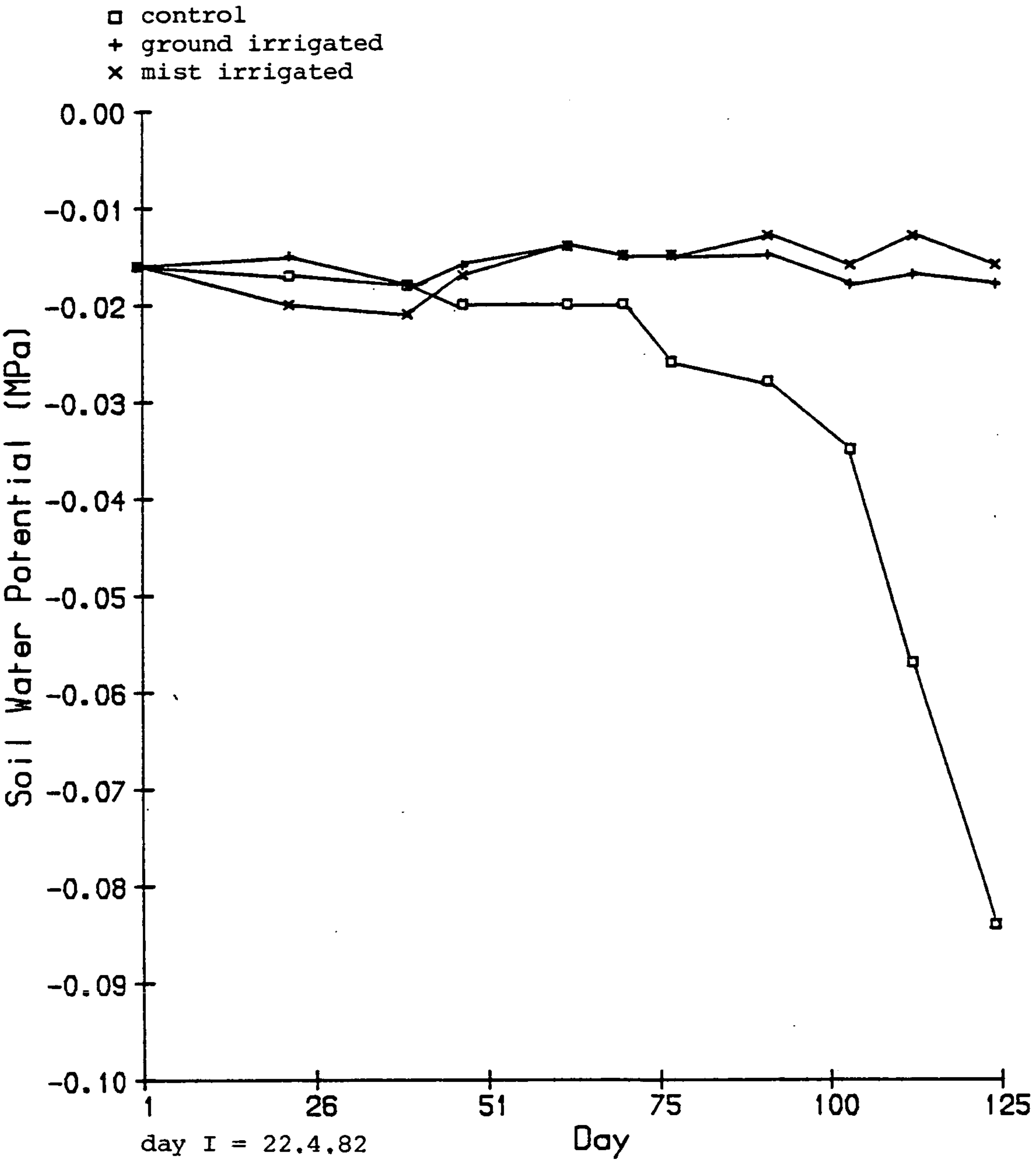

Figure 6.1.4.
Seasonal variation in Soil Water Potential of the irrigated and non-irrigated plots during the 1982 growing season.
(statistical analysis in appendix A)

experimental material that was not due to the treatments nor in variation due to factors such as small differences in timing when sampling. For this reason the experimental plot was divided using a randomised-block design. In this design a number of blocks are defined, each of which contains at least one replicate for every treatment. Uncontrolled variation i.e. variation between blocks that is not due to treatment effects can be removed from the analysis of the results in this system as it can be distinguished from both variation due to treatments and the error variation.

In the present study the experimental plot was divided into four randomised blocks each containing nine replicates for each of the three treatments. Only the Golden Delicious trees were used in the experiments, as described in chapter (2), so the maximum number of replicates available for each experiment was six per treatment per block. As the present study was concerned with the treatment effects on several aspects of tissue water status, such as leaf water potential, relative water content and xylem density, over the growing season, the experiments were designed so that all the various aspects of water status could be measured on each tree within a matter of minutes of each other, so reducing variation due to time. Unfortunately the time required to measure each of the replicates in each block made such a proposition impossible, so measurement was restricted to one tree per treatment per block. This reduced the sensitivity of the analysis but was unavoidable if the study was to be carried out in the time available.

The experiments concerning diurnal variation in tissue water status were carried out on only three trees i.e. one per treatment, all from the same block, thus avoiding variation due to the "block effect". As the experiments in the present study were carried out on only four blocks, or in some cases three individual trees, any results that were obtained may only have been indicative of real effects, as the statistical analysis might not show significance for small effects.

(6.2) Seasonal Variation in Tissue Water Status.

(6.2.1) Seasonal Variation in Leaf Water Potential.

Measurement of leaf water potential indicated a gradual lowering of potential in each of the treatment groups during the experimental period. It was found that the ground irrigated blocks had a higher mean leaf potential i.e. less negative than the other two groups at the beginning of the experiment , but during the first week of July, the mean water potential of all three treatments was approximately the same (figure 6.2.1).

Leaf water potential continued to decline in all three treatments until day 55, when the mean water potential of the misted trees remained relatively constant, while that of the other treatments continued to decline. Statistical analysis of the data indicated, that over the experimental period, there was no overall significant difference at the 5% significance level between the two irrigation treatments. A statistical difference was found to exist between the irrigated treatments and the control at the 5% level.

(6.2.2) Seasonal Variation in Relative Water Content.

It was observed that the relative water content of leaves from all the trees studied gradually declined during the experimental period until the beginning August (figure 6.2.2). At this point the RWC of the misted trees remained constant while that of the ground irrigated and control trees continued to decrease. At the end of the experimental period the mean RWC of the misted trees was higher than that of the ground irrigated trees, which in turn was

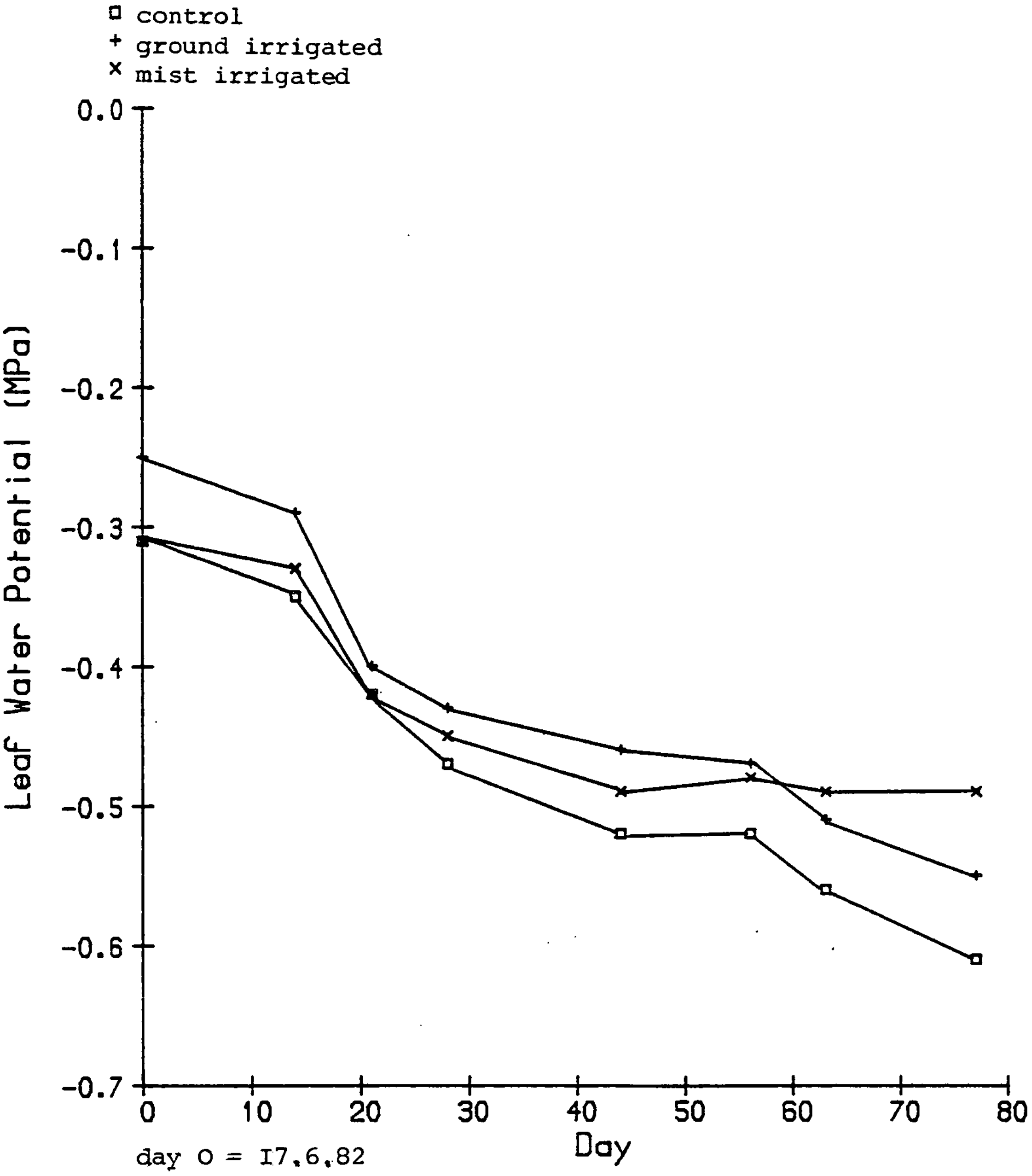

Figure 6.2.1.
Seasonal variation in Leaf Water Potential, measured at dusk, over the 1982 growing season in the three experimental treatments.
(statistical analysis in appendix A)

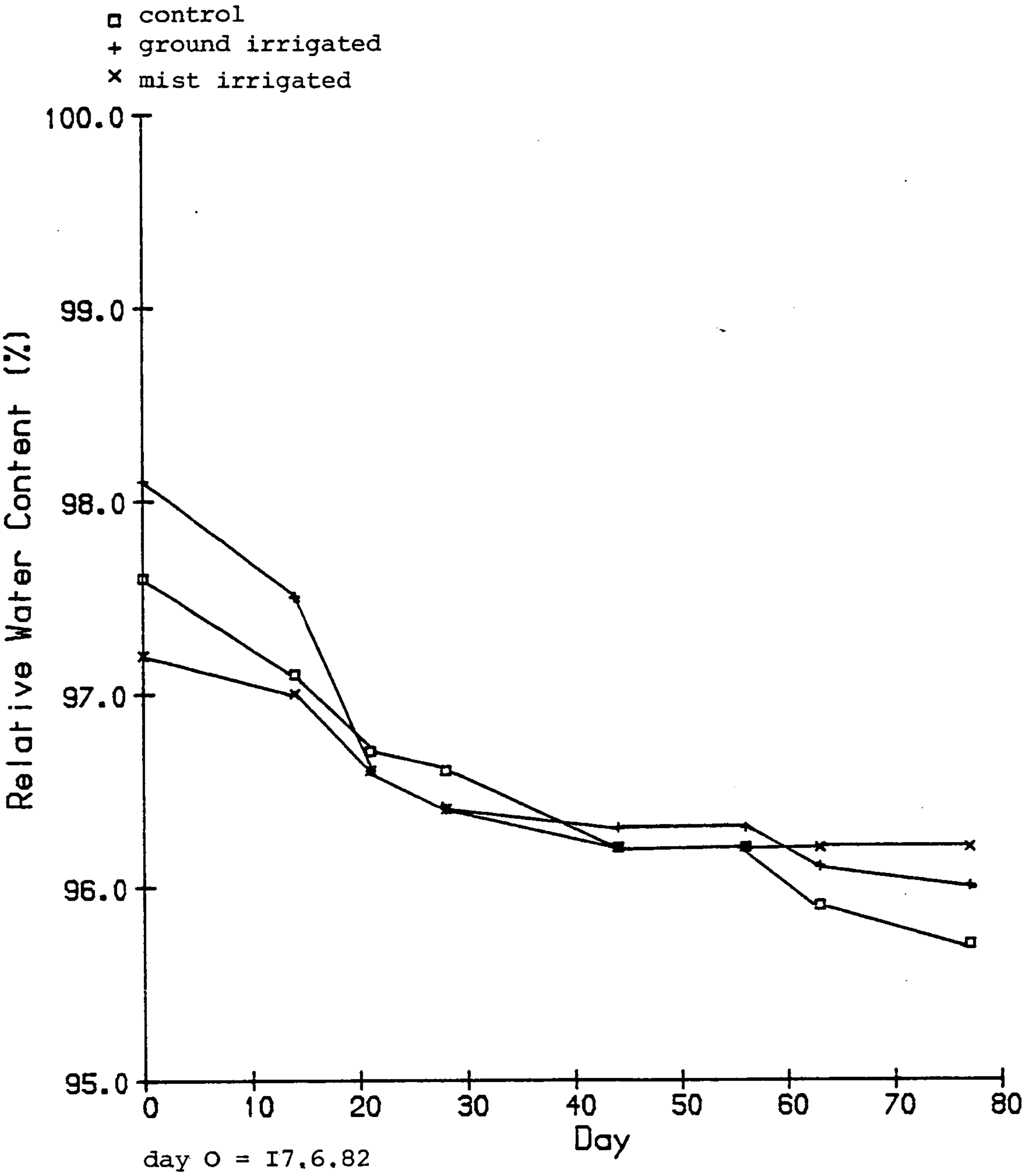

Figure 6.2.2.
Seasonal variation in Relative Water Content, measured at dusk, over the 1982 growing season, in the three experimental treatments.
(statistical analysis in appendix A)

higher than that of the controls. Statistical analysis of these observed differences indicated that these differences were not however significant at the 5% significance level.

(6.2.3) Seasonal Variation in Xylem Density.

Changes in xylem density were investigated throughout the experimental period using the gamma probe, as described in chapter (3.2). The measurements indicated an initial sharp decline in xylem density in all three experimental treatments. By the end of August i.e. approximately 45 days into the experiment , the mean xylem density of the control trees had decreased by approximately 40%, that of the ground irrigated trees by 30% and that of the misted trees by 22% (figure 6.2.3). This initial decline in density was followed by a further more gradual decline in the control trees of approximately 2%, while in the ground irrigated trees, the sharp decline continued, but gradually the rate of decline fell so that the final density measurement was within 2% of the value measured for the control group.

The misted trees showed much more variation in density within the group, by a factor of around two, than either of the other two groups. It was found that, over the growing season, an overall difference in xylem density could be detected between the mist and control treatments at the 1% significance level. A difference at the 5% level was found between the two irrigation treatments.

(6.2.4) Relationships with Soil Water Potential.

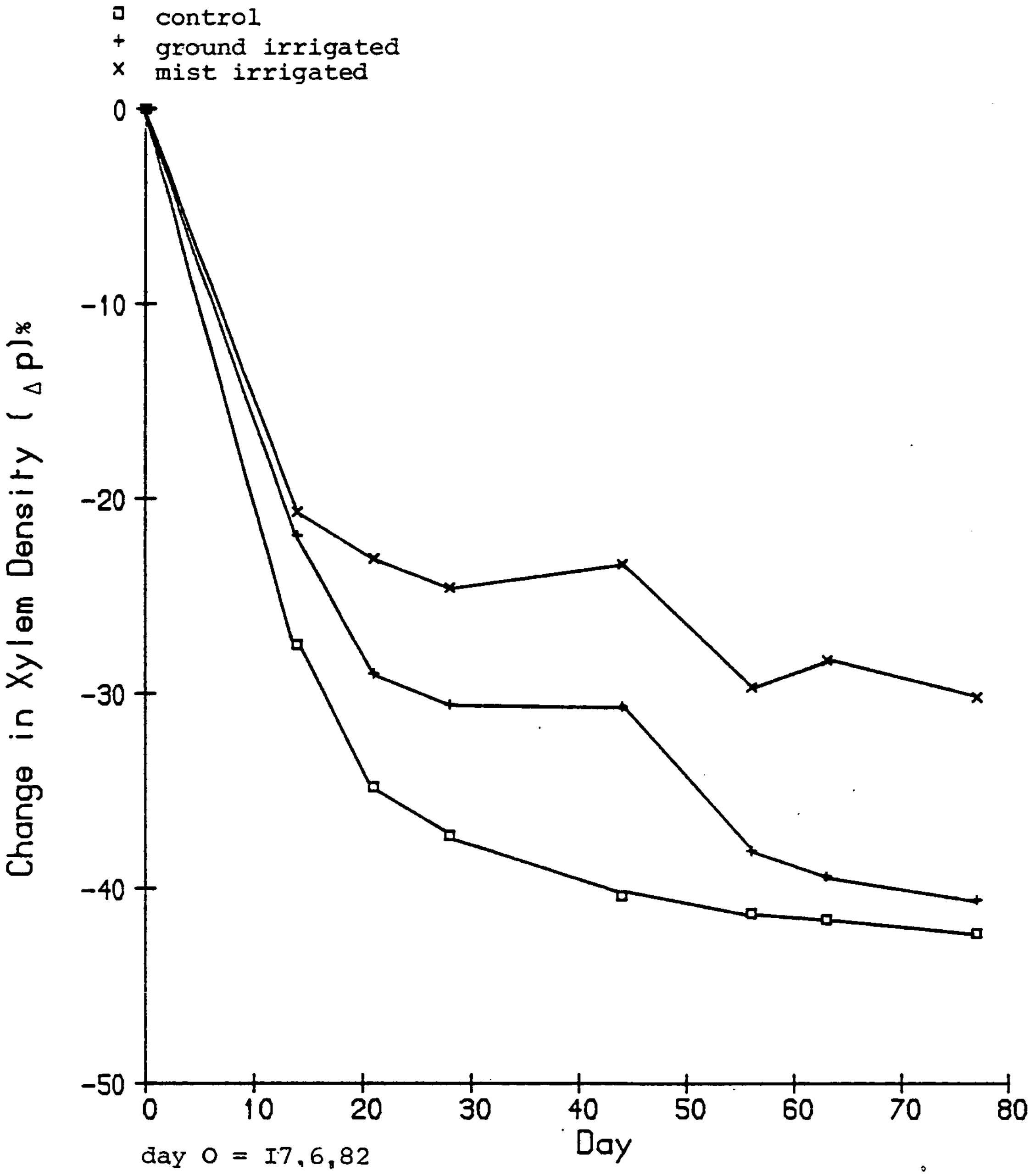

Figure 6.2.3.
Seasonal variation in Xylem Density, measured at dusk over the 1982 growing season, in the three experimental treatments. Δ p is defined as percentage change in density.
(statistical analysis in appendix A)

It was proposed in chapter (4.4.1), that long term water stress was due to a decreasing availability of water. It was found that, in the irrigated plots, the available water, as determined by soil water potential, was relatively constant throughout the growing season, while a significant decline in potential occurred in the non-irrigated control plots. If as proposed, long term plant water status is determined by soil water potential operating either directly or indirectly by affecting soil hydraulic conductivity, then a decrease in soil potential should be accompanied by a decrease in plant water status. To test this hypothesis, the seasonal measurements of tissue water status were correlated with the variations in soil water potential already described.

It was observed that the expected relationship was found in the control tree group for each of the three variables investigated, i.e. decreases in leaf water potential, relative water content and xylem density were associated with a decrease in soil water potential (figures 6.2.4, 6.2.5 and 6.2.6). It was noted that a similar decrease in tissue water status occurred in the ground and mist irrigated trees though the soil water potential remained virtually constant (figures 6.2.4, 6.2.5 and 6.2.6), but the level of reduction in water status in the mist irrigated group was less than in the control.

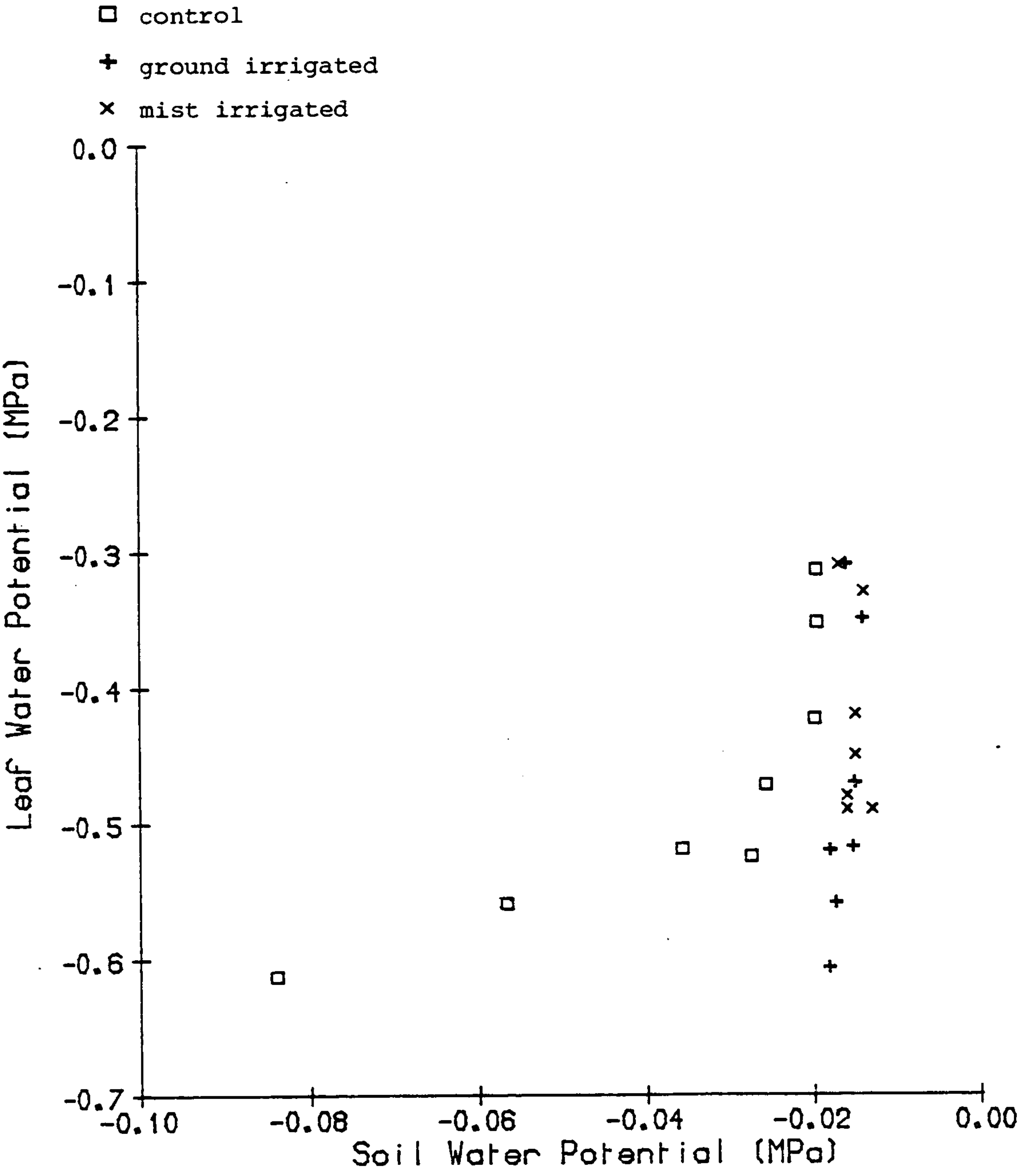

Figure 6.2.4.
The relationship between Leaf Water Potential and Soil Water Potential over the 1982 growing season in three experimental irrigation treatments on Golden Delicious apple trees.

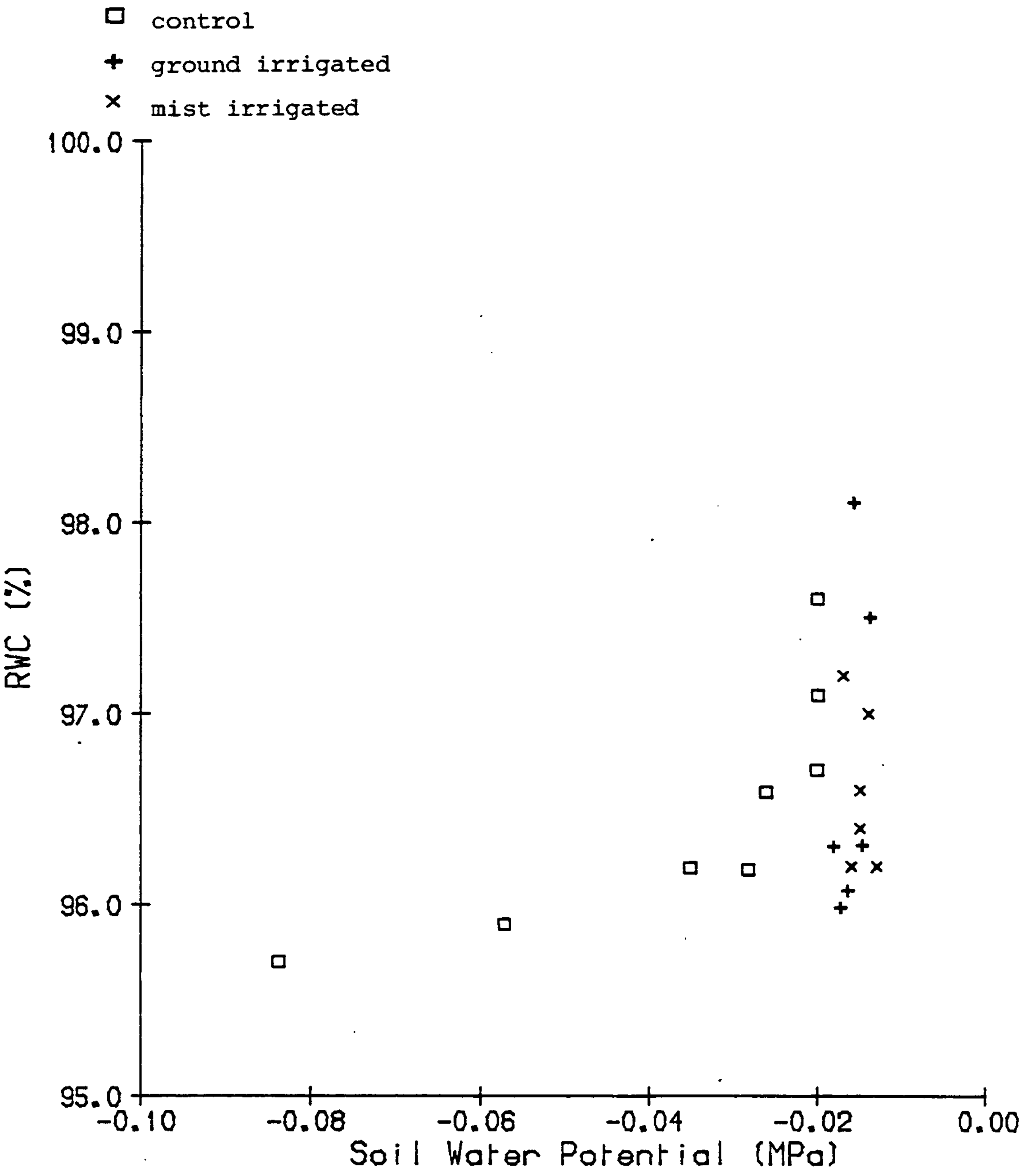

Figure 6.2.5.
The relationship between Leaf Relative Water Content and Soil Water Potential over the 1982 growing season in three experimental irrigation treatments on Golden Delicious apple trees.

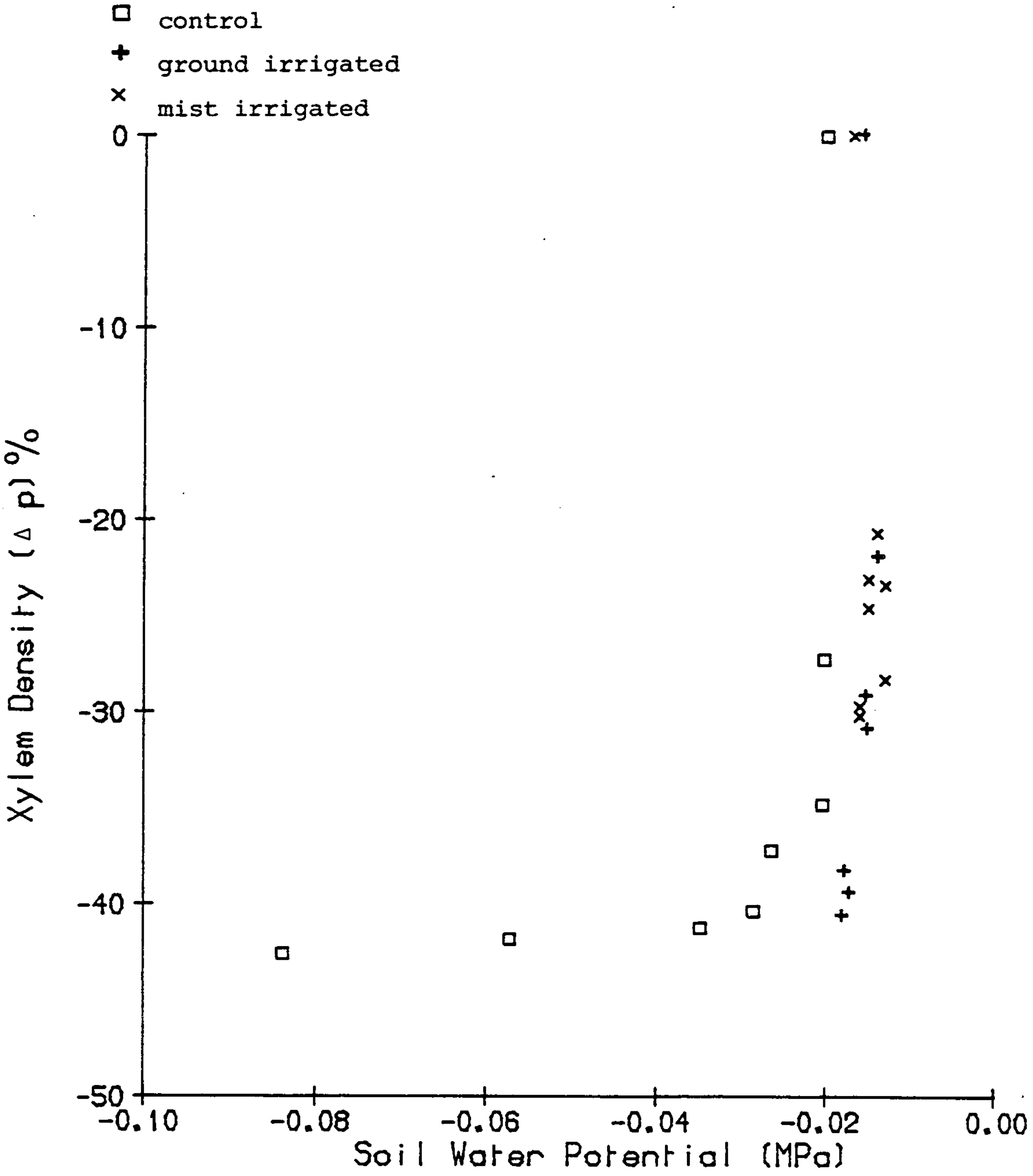

Figure 6.2.6.
The relationship between xylem density and Soil Water Potential over the 1982 growing season, in three experimental irrigation treatments on Golden Delicious apple trees.

(6.2.5) The Relationship Between Relative Water Content and Leaf Water Potential.

It was observed that similar diurnal variation patterns existed in leaf water potential and relative water content. An approximately linear relationship between these variables has been reported for apple leaves (Jones and Higgs 1979), and a signifiant difference found to exist between irrigated and non-irrigated trees. In the present study, diurnal and seasonal variations in relative water content were regressed against leaf water potential (figures 6.2.7 and 6.2.8).

It was found that an approximately linear relationship existed between RWC and water potential (cf. Ehlig and Gardner 1964, Macklon and Weatherley 1965 and Weatherley 1965), in the diurnal data, though in the seasonal data a curved relationship was detected (cf. Slatyer 1960, Jarvis and Jarvis 1963 and 1965). A small but significant treatment variation was observed in the seasonal data (figure 6.2.8), but in the diurnal data (figure 6.2.7), it was found that the differences between the three treatments were not significant at the 5% level, and so the data were combined to calculate the linear regression.

(6.2.6) Discussion of the Seasonal Variation.

The reduction in soil water potential in the control plots, over the growing season was less than 0.1 MPa, but even this small reduction should cause a significant reduction in soil hydraulic conductivity. Although the soil water potential in the irrigated

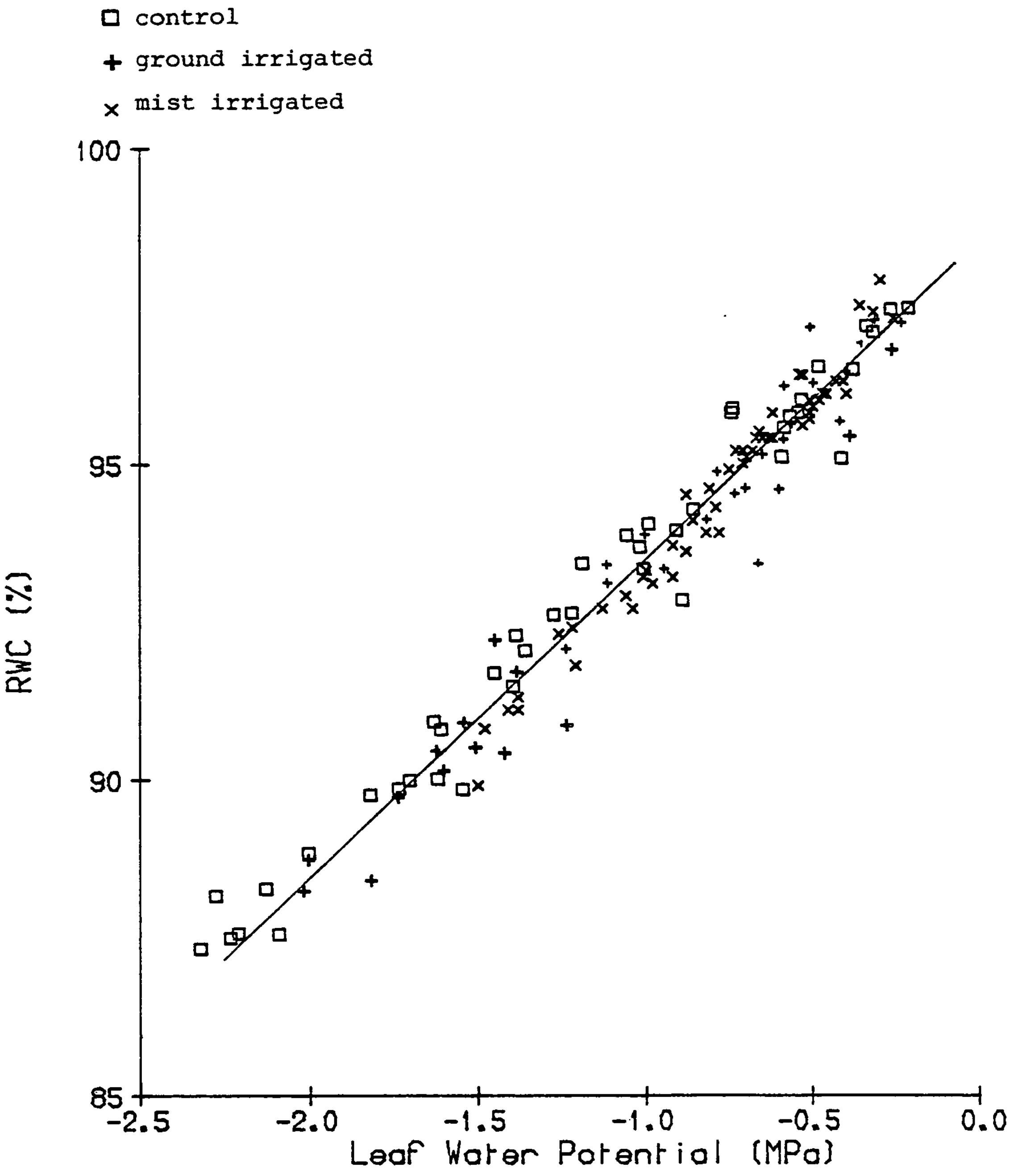

Figure 6.2.7.
The relationship between Leaf Relative Water Content and Leaf Water Potential, measured over four diurnal cycles during the 1982 growing season on Golden Delicious apple trees, under three experimental irrigation treatments.

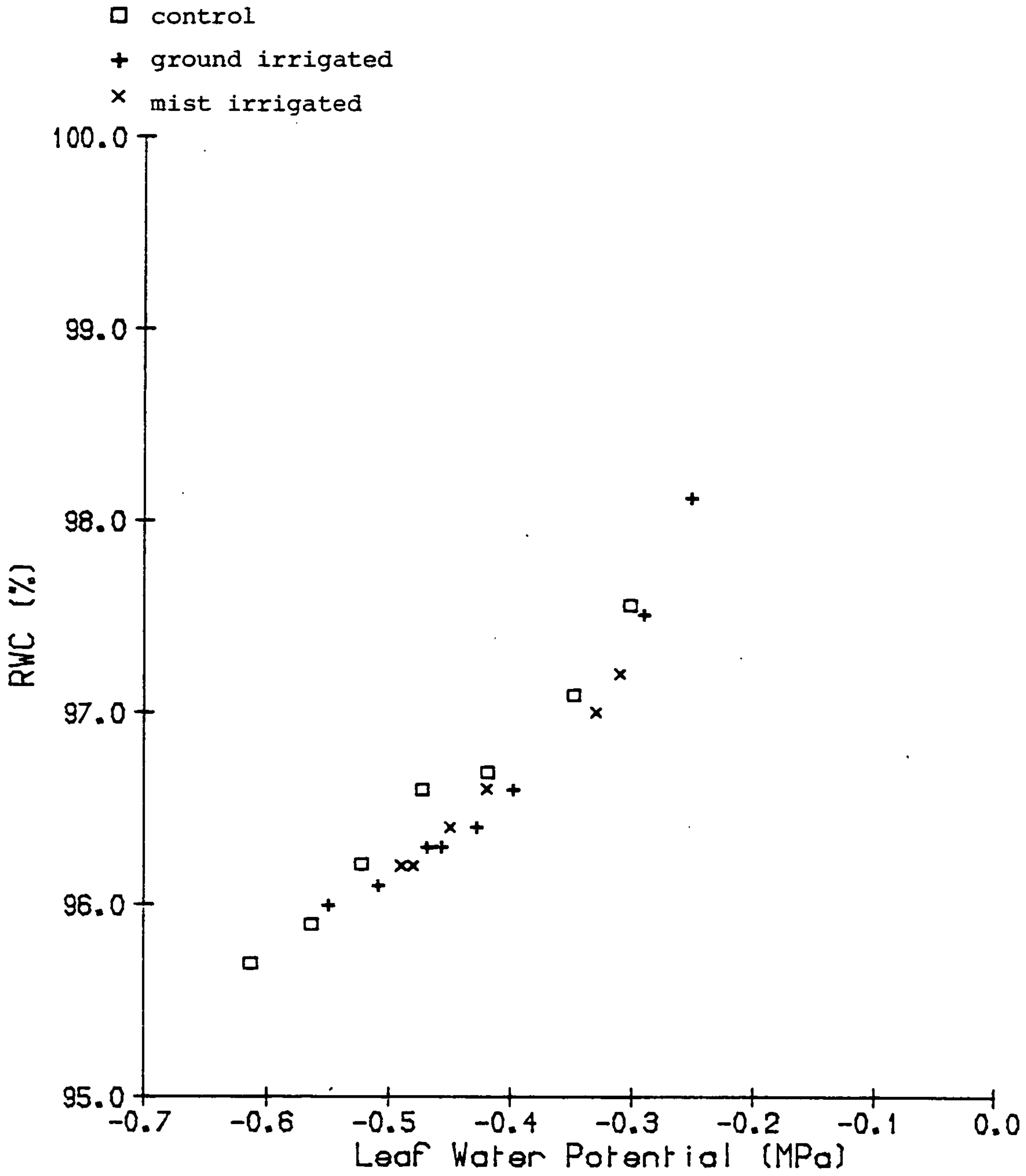

Figure 6.2.8.
The relationship between Leaf Relative Water Content and Leaf Water Potential over the 1982 growing season, on Golden Delicious apple trees, under three experimental irrigation treatments.

plots remained relatively constant, the plot area covered by the ground irrigation system was an area of approximately one meter square, centered round the base of the tree. Outside this area the soil water status was probably similar to that found in the control plots, so that the part of the trees root system that lies in this region will experience the same degree of water stress as the control trees. This could possibly explain the decline in tissue water status that was observed in the irrigated trees during the course of the growing season.

If the gamma probe measurements are taken as a measure of xylem water content, then the reduction in xylem density of around 40%, experienced by all three treatment groups concurs with the work of Gibbs (1935), who detected xylem water content changes of around 40%, in various species of Birch and Poplar, using an incremental boring technique. Similar large changes in stem water content in numerous conifers are discussed by Whitehead and Jarvis (1981). In all the studies of seasonal variation in sapwood water content, the minimum water content was found in late summer with a gradual recovery starting in late autumn, the maximum occuring in early spring. The cavitation hypothesis would suggest that this reduction in water content is due to massive cavitation within the xylem, with decavitation in the autumn as water stress levels are reduced. This interpretation will be discussed more fully at a later point.

The experiments indicated a gradual increase in plant water stress over the growing season, as determined by leaf water potential and leaf relative water content. There is considerable benefit in simultaneous measurement of RWC and leaf water potential, if the range of RWC measured extends into the wilting range i.e. below the point of incipient plasmolysis. If this situation occurs, the relationship between the two variables can be used to construct a classic Hoefler diagram or pressure-volume curve, and the turgor and osmotic potentials at any RWC determined. The net water potential of plant tissue is the sum of hydrostatic or turgor potential Ψ_p, and solute or osmotic potential Ψ_s. As a plant wilts the turgor pressure is reduced until the point of incipient plasmolysis, when turgor pressure falls to zero and the total water potential is equal to the osmotic potential (Meidner and Sheriff 1976). If the reciprocal of water potential is plotted against RWC, the relationship becomes linear after the point of incipient plasmolysis has been reached. Extrapolation of this linear portion of the curve towards higher values of $1/\Psi$ and lower relative water contents allows the solute potential to be determined at any water content or potential (Hellkvist, Richards and Jarvis 1974). The turgor potential Ψ_p, can be estimated by the difference between the total potential and the solute potential and a Hoefler diagram constructed showing the change in turgor with changing water content and water potential.

In the present study however, the range of RWC that was measured did not fall below the wilting point in either the seasonal data (figure 6.2.9), nor in the diurnal data (6.2.10). Although the relationship appeared to be becoming linear in the diurnal data insufficient data points were available to precisely determine the wilting point. The relationship between leaf water potential and RWC depends upon wall elasticity which can be affected by growing conditions (Meidner and Sheriff 1976), but without a Hoefler diagram

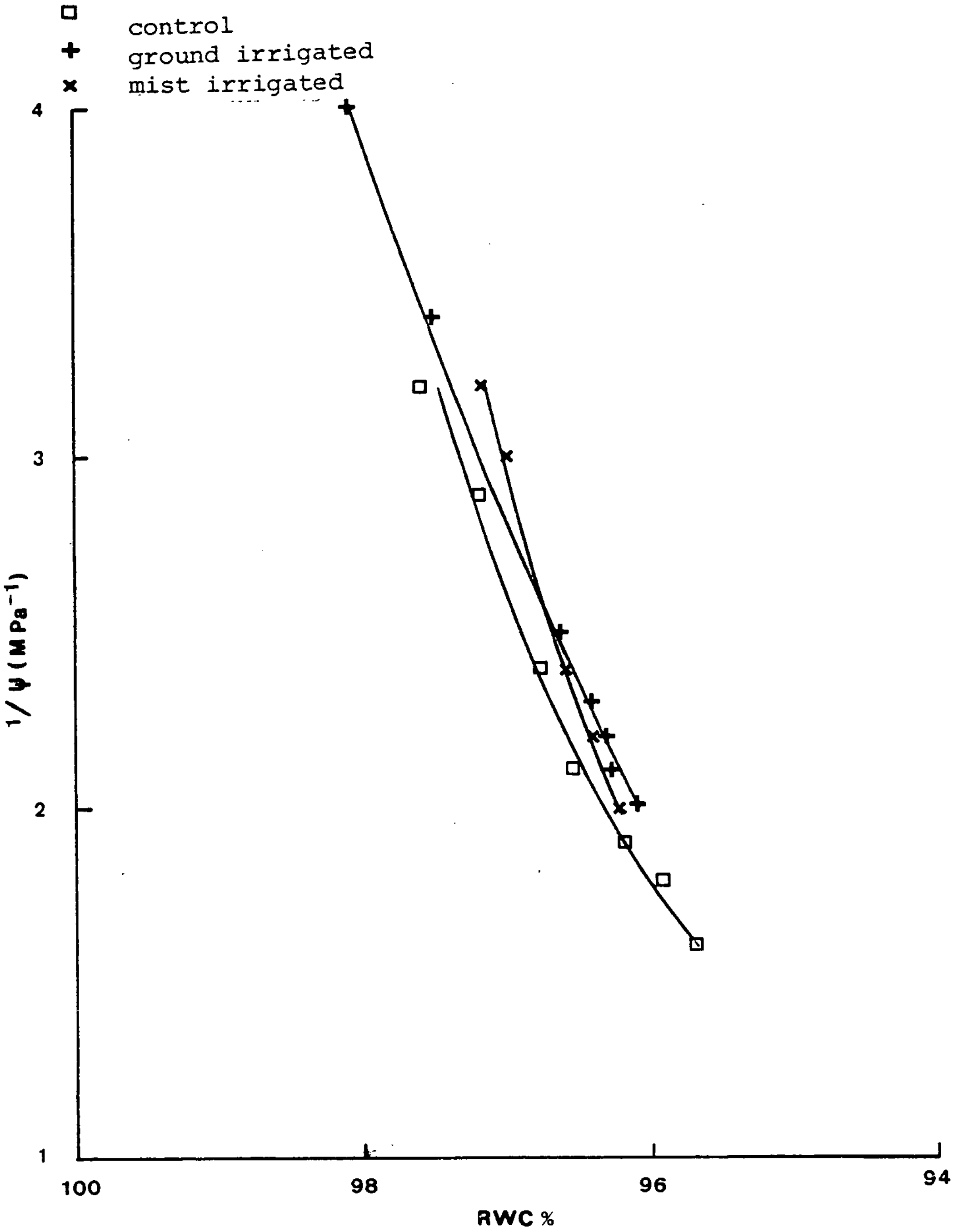

Figure (6.2.9)
The relationship between the reciprocal of leaf water potential and leaf relative water content over the 1982 growing season in Golden Delicious apple trees under three experimental irrigation treatments

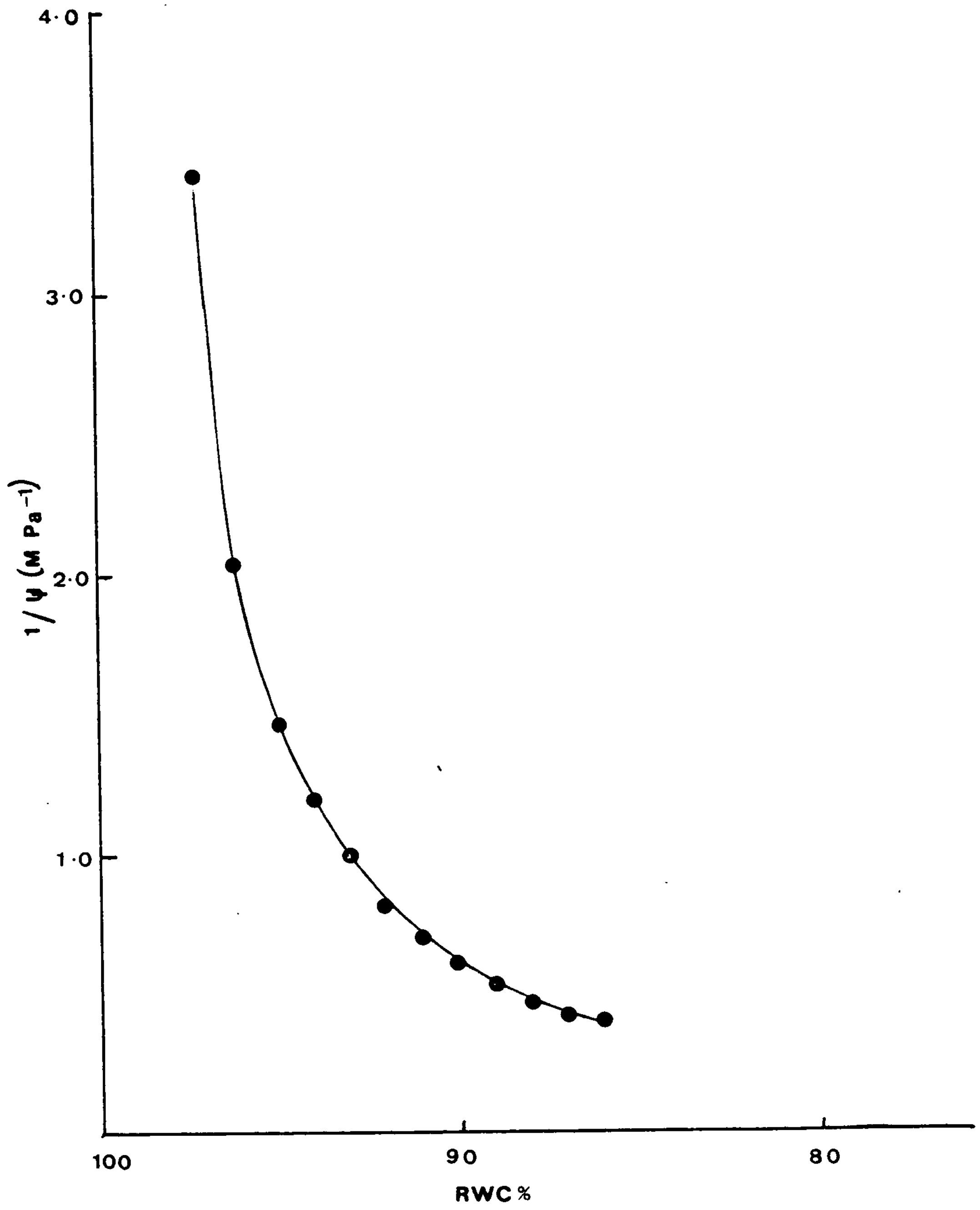

Figure (6.2.10)

The relationship between the reciprocal of leaf water potential and leaf relative water content over four diurnal cycles based on the linear regression indicated in figure (6.2.7)

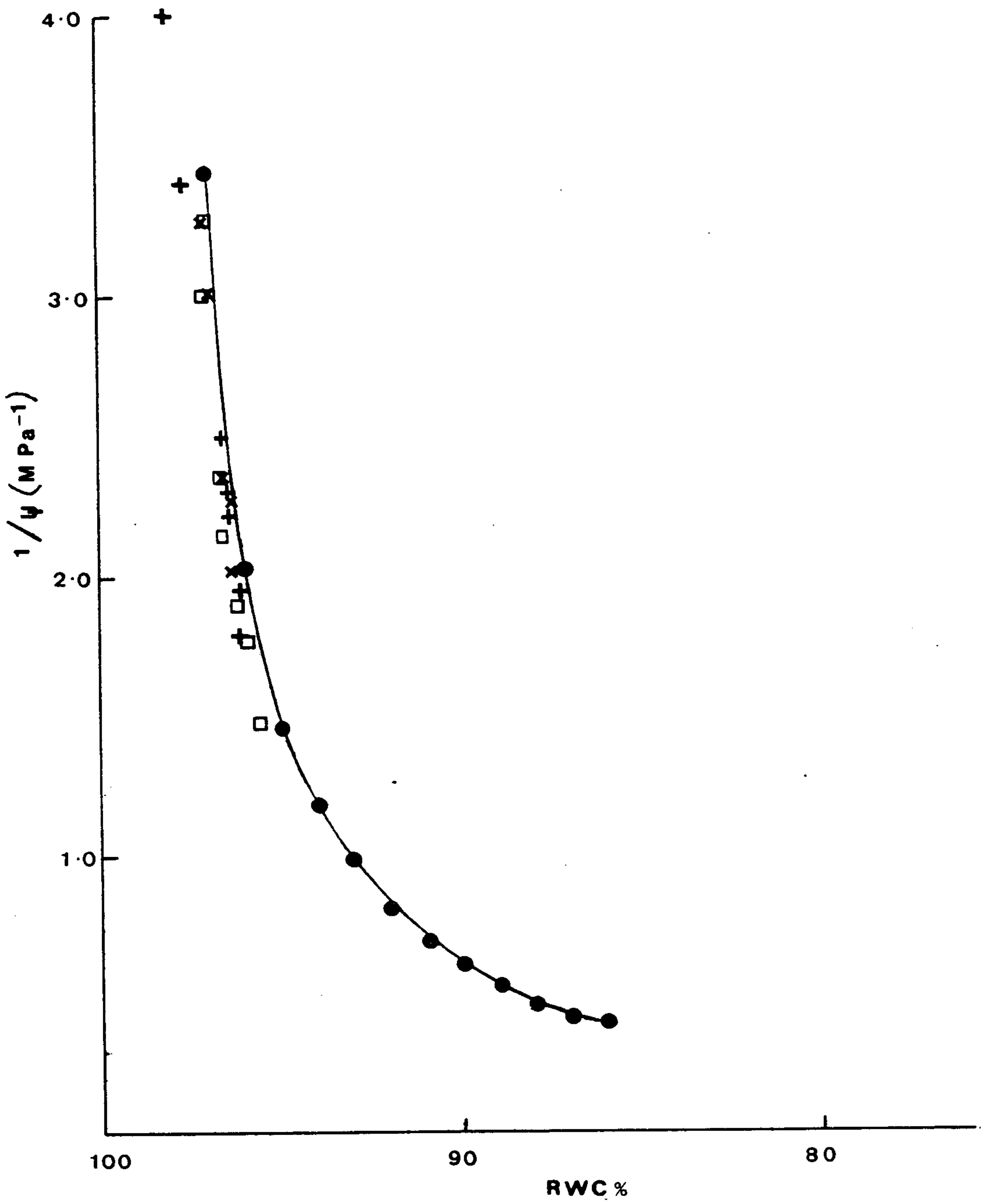

Figure (6.2.11)
The relationship between the reciprocal of leaf water potential and leaf relative water content. (figures 6.2.9 and 6.2.10 combined)

it is impossible to determine whether the treatment differences in the seasonal data were due to wall effects and turgor pressure or to differences in osmotic adjustment as stress increased during the growing season.

If the seasonal data ~~is~~ are superimposed upon the diurnal data (figure 6.2.11), it is noted that the relationships between the variables are in fact the same and it therefore suggested that the results of the present study show the relationship between RWC and leaf water potential above the point of incipient plasmolysis, where the relationship can be approximated, in this instance, by a straight line.

(6.3) Diurnal Variation in Tissue Water Status.

Four days were selected to study diurnal variation in tissue water status i.e. 7/7/82, 21/7/82, 28/7/82 and 1/8/82, as examples of the environmental conditions that existed during the 1982 growing season. For the purposes of discussion each days investigations will be treated separately.

(6.3.1) Day One - 7/7/82.

The 7th. of July was a fairly cloudless sunny day with a maximum air temperature of 21.2 °C, measured at a height of two meters above ground level, which occurred at 1300 hours BST. Estimates of evapotranspiration, leaf water potential and relative water content were made at hourly intervals for each of the experimental treatments, as described in chapter 4.

It was found that over the course of the experiment, xylem density declined in a manner directly proportional to leaf water potential and relative water content, and inversely proportional to evapotranspiration (figures 6.3.1 and 6.3.2). The estimated evapotranspiration rate was relatively constant throughout the morning and afternoon, though water stress, as determined by leaf water potential and RWC "peaked" around 1400 hours and then fell. A decrease in xylem density of over 7% was noted at the point of maximum stress in the control and a smaller reduction in the other two treatments,and it was observed that xylem density began to increase when stress levels were reduced.

Statistical analysis of the data, using an analysis of variance,

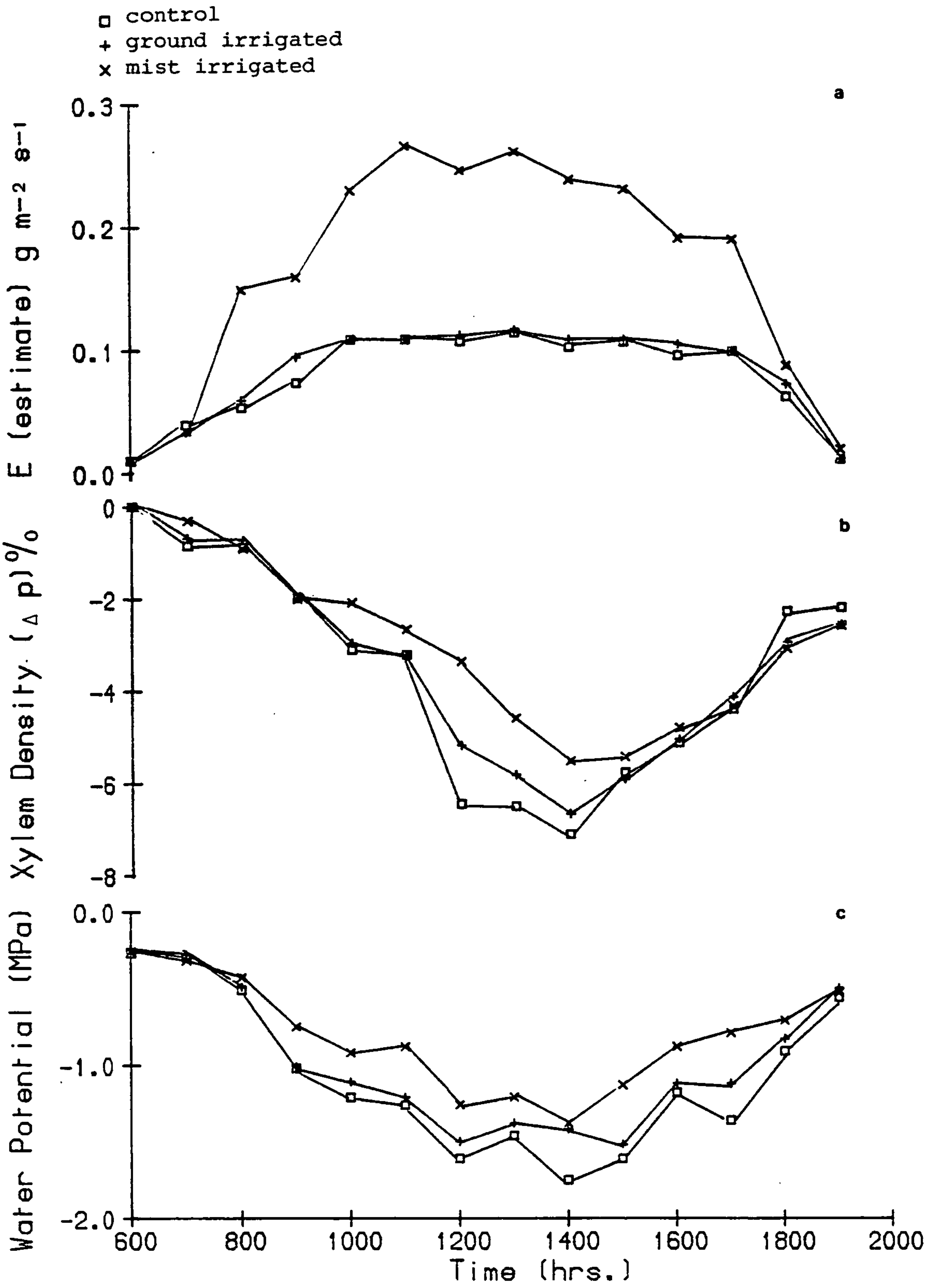

Figure 6.3.1.
Diurnal variation in tissue water status, 7.7.82, of Golden Delicious apple trees, under three experimental irrigation treatments.
(statistical analysis in appendix A)

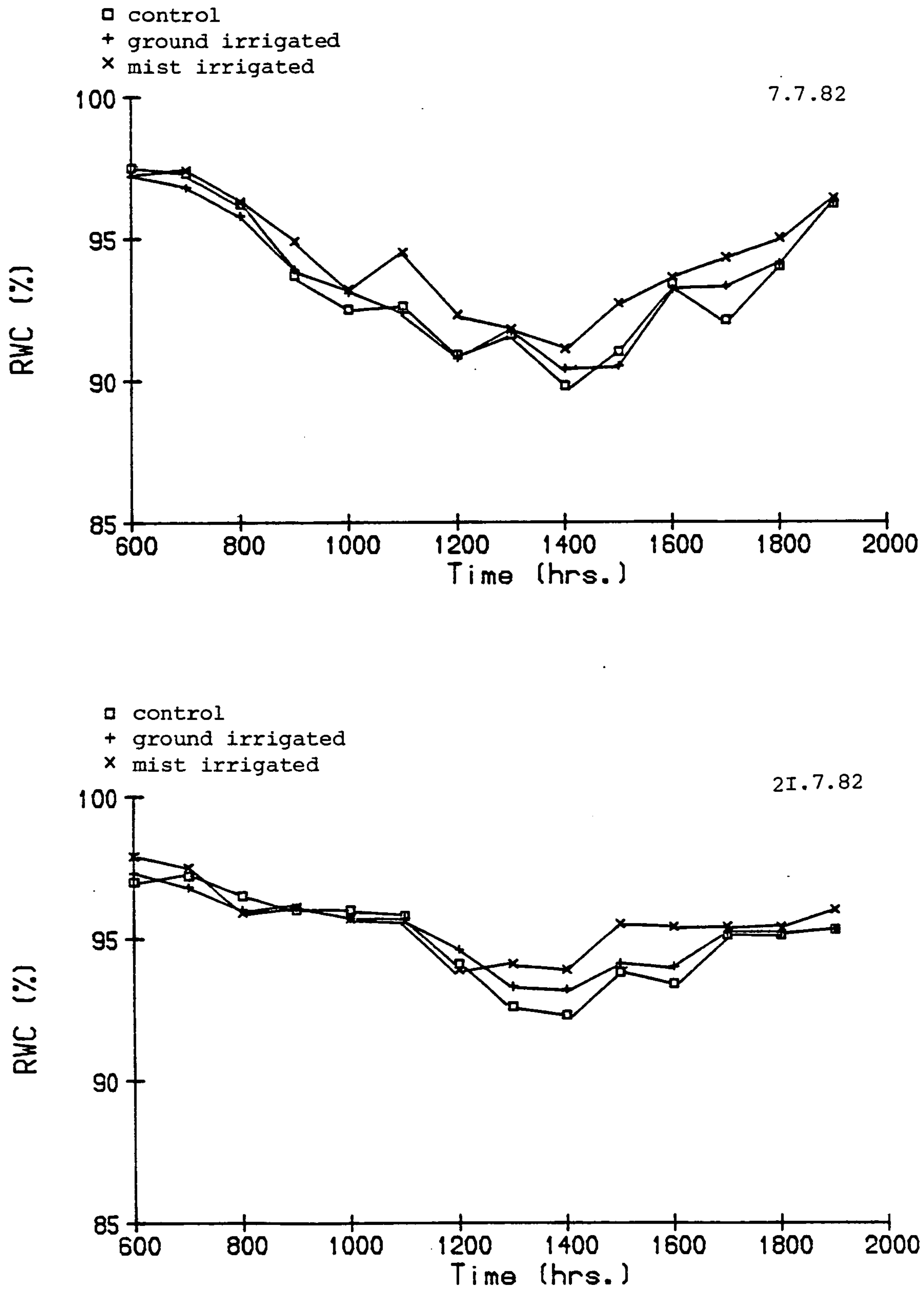

Figure 6.3.2.
Diurnal variation in Leaf Relative Water Content, 7.7.82 and 21.7.82, of Golden Delicious apple trees under three experimental irrigation treatments.
(statistical analysis in appendix A)

as previously described, indicated a significant difference in evapotranspiration rate between the mist and other two treatments at the 0.1% level. No difference could be detected between the ground irrigation treatment and the control at the 5% level. The mean evapotranspiration rate in the misted trees was at least twice that of the other treatments due to the evaporation of the mist from the leaf surfaces.

Similar analysis of leaf water potential and relative water content indicated an overall significant difference at the 5% level between the mist and control trees, though no difference was found at this level between the ground irrigated and control trees. The only significant difference in the xylem density measurements was at the 5% level between the irrigation methods.

(6.3.2) Day Two - 21/7/82.

The 21st. of July was a relatively warm, sunny day with a maximum air temperature of 17.6 °C occurring at 1500 hours BST. The sky was rather overcast until mid-day when the cloud cover broke; the sky remaining clear for the rest of the day. As in Day 1, it was found that xylem density varied in direct proportion to leaf water potential and relative water content, and inversely with evapotranspiration (figures 6.3.2 and 6.3.3).

No significant difference could be detected between the ground and control treatments in estimated evapotranspiration, xylem density, leaf water potential or relative water content. A difference between treatments, significant at the 5% level was detected between the mist and control treatments in leaf water

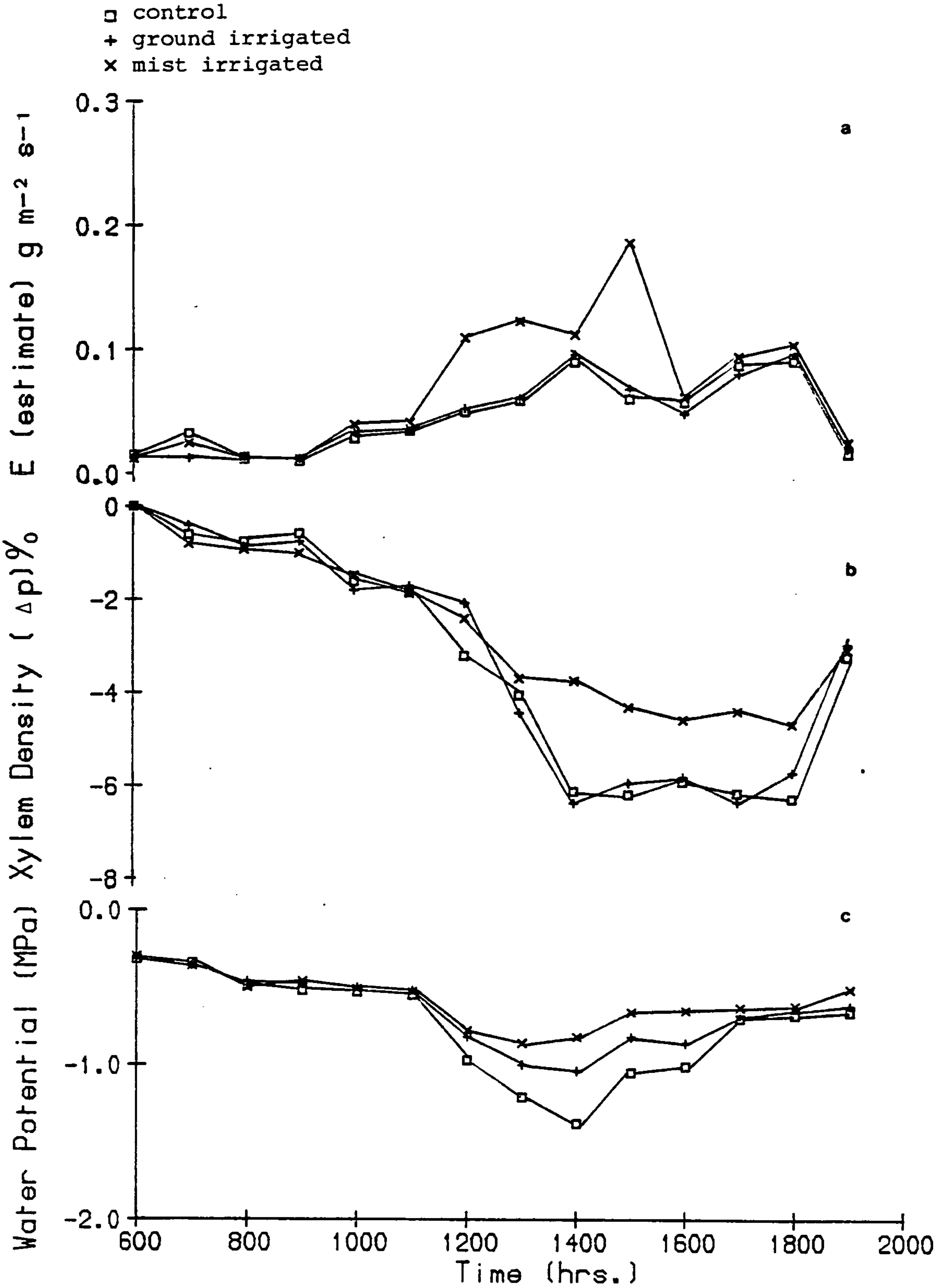

Figure 6.3.3.
Diurnal variation in tissue water status, 21.7.82, of Golden Delicious apple trees under three experimental irrigation treatments.
(statistical analysis in appendix A)

potential, relative water content and xylem density.

It is noted that on this occasion xylem density did not start to increase when the stress levels were reduced, but remained relatively constant for four hours when a sharp increase was observed.

(6.3.3) Day Three - 28/7/82.

Day Three was similar to Day Two, in that the morning was rather overcast but by mid-day the sun broke through. Air temperature reached a maximum of 19.0 ^{o}C at 1400 hours BST. Diurnal variation in tissue water status followed similar patterns to that of days One and Two (figure 6.3.4). No significant difference, at the 5% level, could be detected between the estimated evapotranspiration rates of the ground and control trees. Significant differences in water potential at the 5% level were found to exist between the two irrigation techniques and a difference at the 1% level was found between the mist irrigated and control treatments.

Although no significant differences were found in the RWC measurements, at the 5% level, between the ground irrigation and control treatments, a 5% difference was detected when these two groups were compared with the misted trees (figure 6.3.5). No significant difference at the 5% level in xylem density could be detected in any of the three treatments.

(6.3.4) Day Four - 1/8/82.

The 1st. of August was a warm sunny day with a maximum air

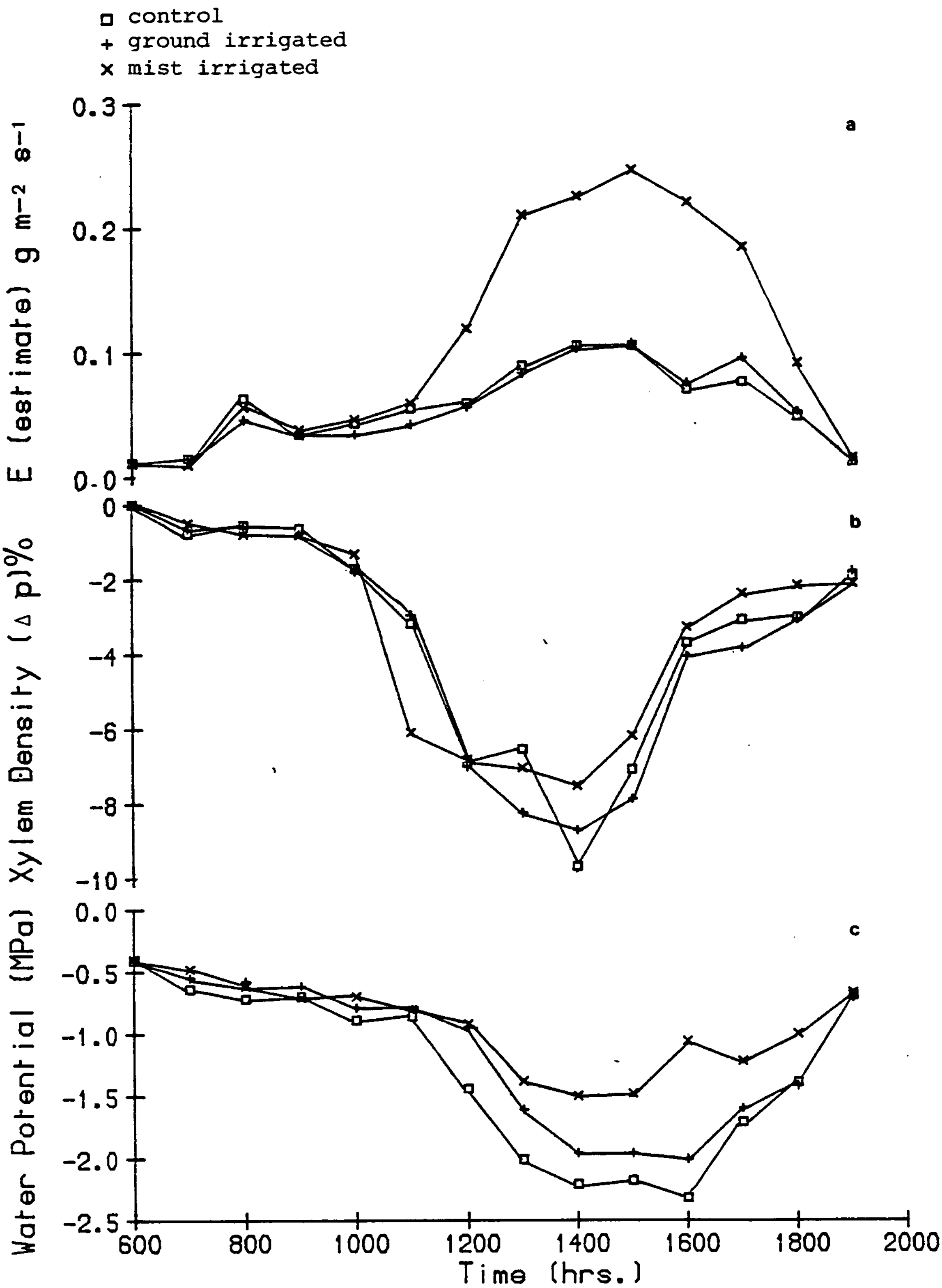

Figure 6.3.4.
Diurnal variation in tissue water status, 28.7.82, of Golden Delicious apple trees under three experimental irrigation treatments.
(statistical analysis in appendix A)

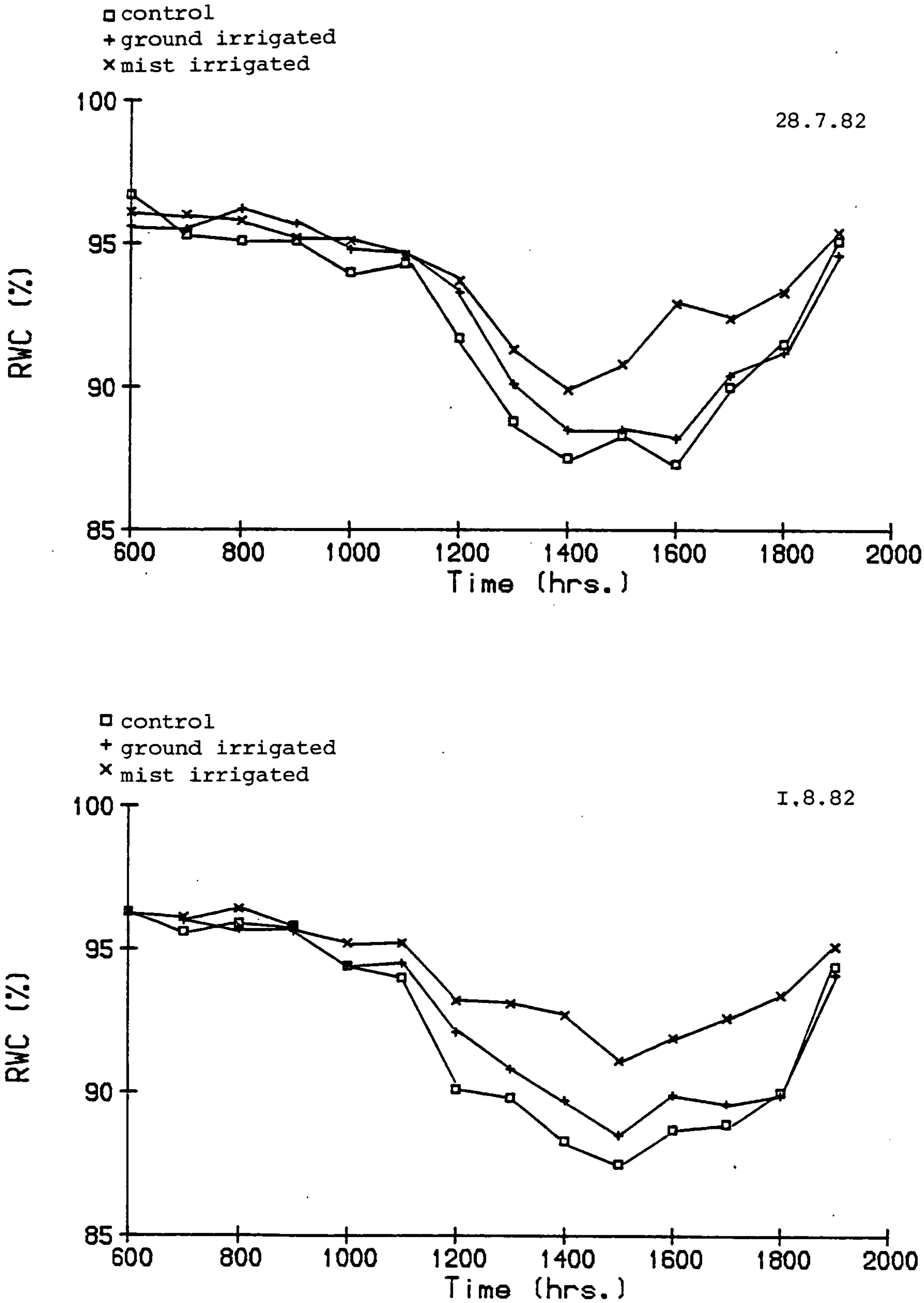

Figure 6.3.5.
Diurnal variation in Leaf Relative Water Content, 28.7.82 and 1.8.82, of Golden Delicious apple trees under three experimental irrigation treatments.
(statistical analysis in appendix A)

temperature of 22.3 °C which occurred at 1500 hours BST. As in the previous experimental days, a certain amount of cloud cover was present during the morning till around 1100 hours when the cloud broke leaving the sky completely clear until dusk. A high radiation load and elevated air temperature resulted in the highest estimated evapotranspiration rates, by a factor of two, compared with the other experimental days.

The patterns of diurnal variation in tissue water status were similar to those found on the previous three experimental days (figure 6.3.6). The levels of water stress experienced by the trees, as indicated by RWC and leaf water potential, were the highest of the four days, and it is noted that a significant statistical difference was found between the treatments in almost every aspect of tissue water status studied. The only exception was in evapotranspiration rate, where no difference was found between the ground irrigated and control treatments.

(6.3.5) Stem Tissue Shrinkage.

Measurement of diurnal variation in stem xylem thickness was required for accurate interpretation of the gamma probe studies, and so the thickness of the xylem and the phloem plus cambial tissues was measured as described in chapter (3.3), on the four sample days. The results obtained on the 7/7/82, are shown in figure (6.3.7).

It was observed that the thickness of both the xylem and the phloem plus cambial tissues varied diurnally, with the largest degree of shrinkage occurring at the time of maximum water stress.

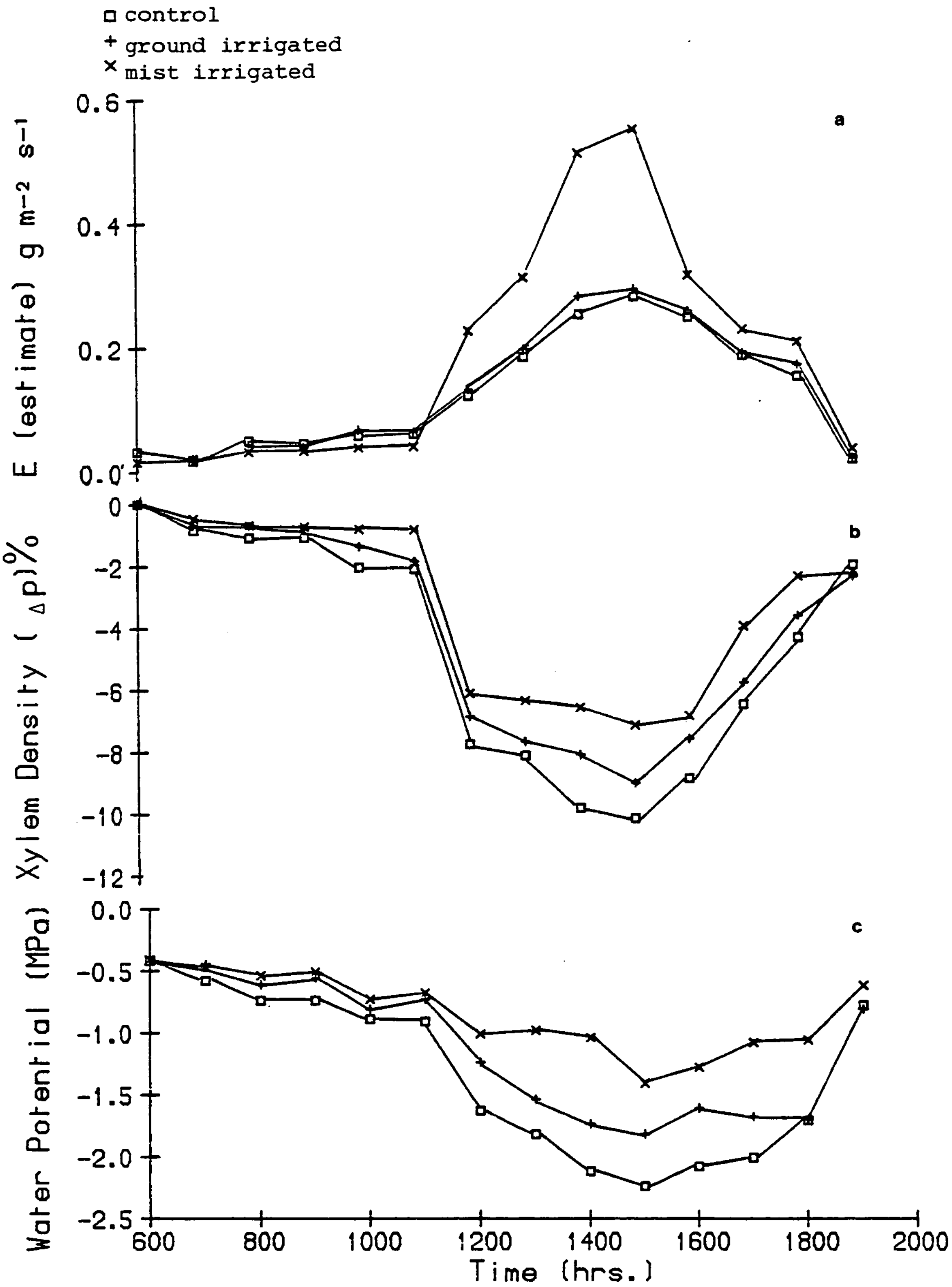

Figure 6.3.6.
Diurnal variation in tissue water status, 1.8.82, of Golden Delicious apple trees under three experimental irrigation treatments.
(statistical analysis in appendix A)

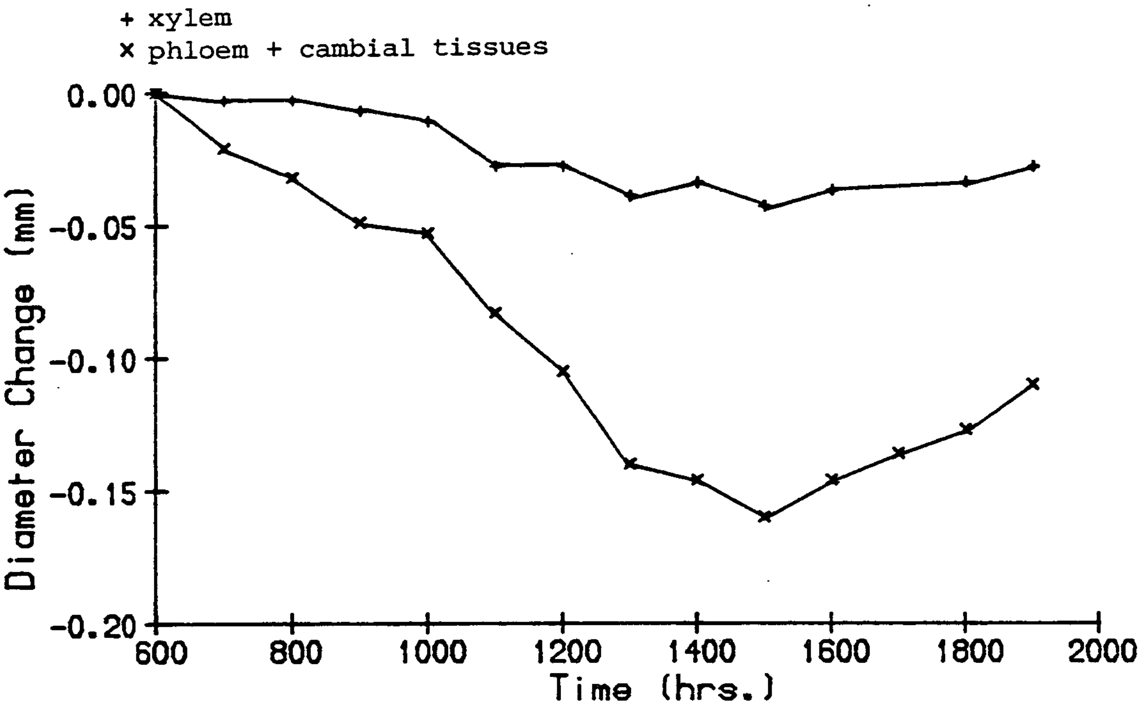

Figure 6.3.7.
Diurnal variation in stem tissue dimensions of a "control" Golden Delicious apple tree. (7.7.82)

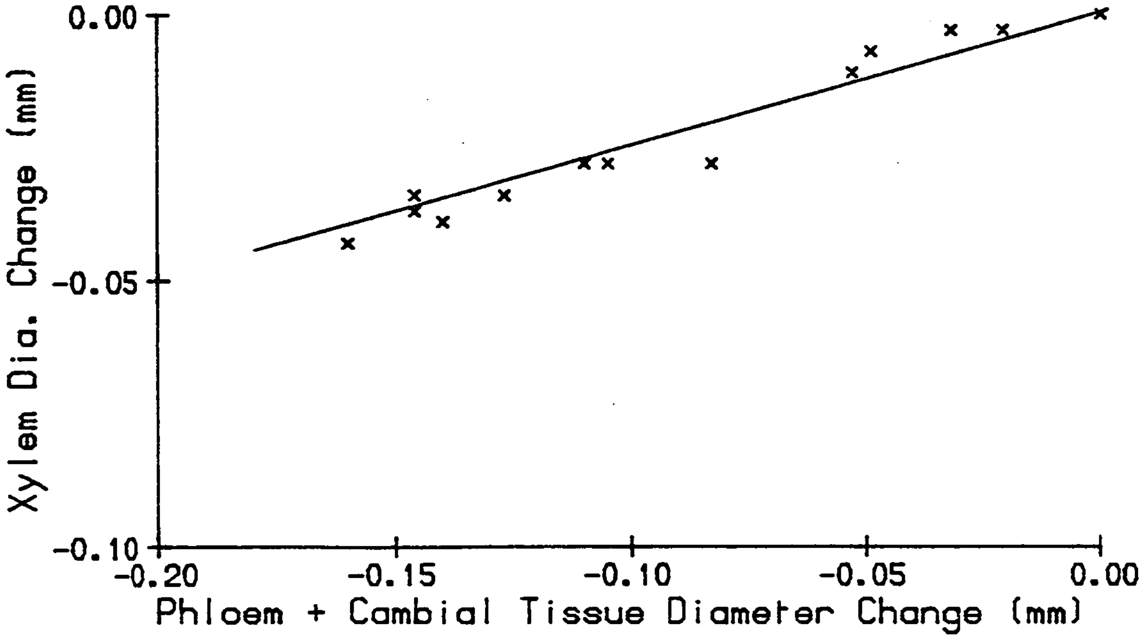

Figure 6.3.8.
The relationship between xylem and phloem plus cambial tissue diameter changes over a diurnal cycle. (7.7.82)

It is noted that the shrinkage exterior to the xylem is over three times greater than that of the xylem tissues and that both the xylem and the phloem appear to vary in unison. If the change in the xylem is plotted against the change in the phloem no significant hysteresis is found (figure 6.3.8), thus confirming this observation.

As described in chapter (3.2), the gamma probe can detect changes in xylem water content with a resolution of approximately 0.8%. The maximum shrinkage found in the xylem was a diameter change of 0.044 mm, which, in a 60 mm diameter stem, is a change of 0.073%, an order of magnitude outside the resolution of the probe. It is therefore concluded that xylem shrinkage cannot influence the measured xylem density.

(6.3.6) The Relationship Between Water Potential and Transpiration Rate.

The permeability of stem tissue to water flow can be described by the Darcy equation i.e.

$$K = \frac{q \, l \, \eta}{A \, \Delta P}$$

K = permeability of the conducting tissue (m^2)

A = cross sectional area of the conducting tissue (m^2)

l = length of conducting tissue (m)

q = flow rate of the conducting liquid ($m^3 \ s^{-1}$)

η = viscosity of the liquid ($N \ s \ m^{-2}$)

ΔP = pressure difference (Pa)

If the Darcy equation is an adequate model for xylem water flux, then the flow rate will be linearly proportional to the xylem cross-sectional area, the pressure difference and permeability. Cavitation within the xylem will cause a proportion of the xylem vessels to become non-functional i.e. non-conducting, and if the cross-sectional area remains the same, the permeability of the stem will decrease.

The change in permeability of a stem over a period of time can thus be estimated by measuring the change in flow rate relative to the change in pressure difference, where the slope of the relationship is a measure of the permeability. The flow rate in a stem is determined by the cross sectional area of the stem, but the driving force for that flow i.e. the pressure difference is dependent upon transpiration which is dependent upon leaf area. The slope of the transpiration / water potential difference, i.e. the differential between the leaves and the soil, relationship can, over a period of time, be used as a measure of the change in permeability of a stem. As the variables are dependent upon stem cross sectional area and canopy leaf area, any change in these factors will obviously alter the relationship. It was observed that by the middle of July, extension shoot growth had almost ceased in the experimental trees, and although leaf fall begins in july, the canopy leaf area must have remained practically constant during the period of this experiment. If the levels of cavitation within a stem are sufficient to cause a reduction in permeability, then an increase in cavitation due to an increase in water stress might be observed using this technique. The relationship between transpiration and water potential has been used by several authors for modelling diurnal variation in leaf water potential and transpiration rate (Jones 1978) and stem permeability (Edwards and Jarvis 1982).

Jarvis (1976) proposed that hysteresis found in plots of water potential in relation to transpiration rate might be due to diurnal variation in resistance of the liquid flow pathway, coupled with changes in the amount of water stored in the stem. Thus the effects of capacitance should be considered when interpreting such relationships.

Estimated evapotranspiration was correlated with leaf - soil water potential in a control tree i.e. $\Psi_l - \Psi_s$, using the data obtained on the four sample days. Little hysteresis was found in each of the relationships and it was therefore felt that a linear regression of the data points would give reasonable estimates of the relationships (figure 6.3.9). These regressions (table 6.3.1), indicated an increase in permeability between the 7th and the 21st of July followed by a decrease till the 28th A further increase was noted from the 28th to the 1st of August. As the actual values of canopy leaf area and xylem cross-sectional area were not known, then the slope obtained by this method only gives an approximate value for permeability.

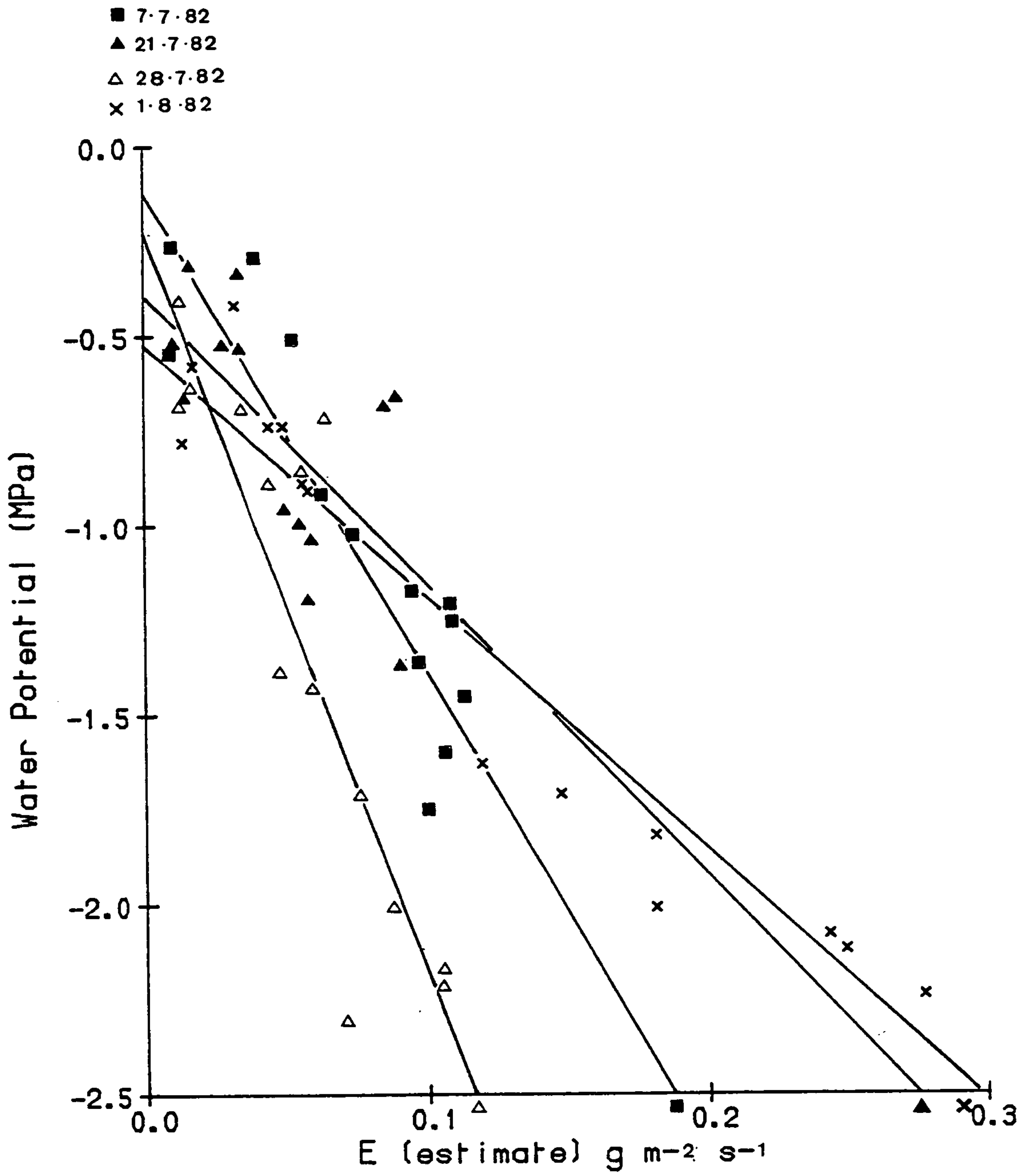

Figure 6.3.9.
The relationship between transpiration rate and water potential in a "control" Golden Delicious apple tree, on four days during the 1982 growing season.

Date	Regression	Standard Error of Slope	Correlation Coefficient
7/7/82	$\Psi = -12.14E - 0.14$	1.68	0.90
21/7/82	$\Psi = -7.39E - 0.41$	2.50	0.65
28/7/82	$\Psi = -18.83E - 0.24$	3.10	0.87
1/8/82	$\Psi = -6.85E - 0.52$	0.54	0.96

Table (6.3.1) Regression Coefficients of the Relationship Between Evapotranspiration and $\Delta\Psi$.

(6.3.7) Discussion of Diurnal Variation Experiments.

Observation of tissue water status over several diurnal cycles indicated that plant water deficits, as determined by leaf water potential or relative water content, vary in inverse proportion to an estimate of evapotranspiration, so that maximum stress occurs at the point of maximum evapotranspiration.

It was noted that gamma probe measurements of xylem density also varied in inverse proportion to evapotranspiration and in direct proportion to plant water stress, so that as stress increased, xylem density was reduced. Minimum density was observed at the point of maximum stress and as stress levels reduced xylem density increased. Only on one occasion was a significant lag period observed between the reduction in stress and the increase in density. Following the cavitation hypothesis, it can be suggested that cavitation of xylem vessels was brought about by increases in plant water stress, resulting in reduced xylem density, and that decavitation occurred as stress was reduced. It was noted that xylem density underwent significant reductions at tensions of less than 0.5 MPa in three of the four diurnal cycles examined. If the gamma probe does in fact measure cavitation events then, these results do not concur with those of West and Gaff (1976), where acoustic detection of cavitation indicated that caviation in excised apple leaves did not occur until the leaf was subjected to tensions around 1.2 MPa.

Xylem density was reduced by an average of around 8 to 10% on the four days measured, which agrees with the findings of Gibbs (1935) who detected similar reductions in water content using incremental boring. Edwards (1983), using a gamma probe technique based on Caesium 137, measured a reduction in sapwood relative water content of up to 10% just below the canopy in *Pinus contorta* (Dougl.), which in some instances appeared to be closely coupled to transpiration.

It could be argued that changes within the xylem, other than embolism formation, could account for the observed changes in gamma beam attenuation. Such changes include

a) the proportions of living ray tissue to xylem, which might increase over the growing season,

b) the formation or removal of storage products such as starch granules within the ray tissue and

c) the removal of water from ray cells.

The proportion of ray tissue within the xylem of an apple tree lies between 5 to 10% of the total xylem tissue (Doley 1974).

Therefore taking the maximum value, if all the ray tissue underwent a 50% reduction in water status, which seems unlikely, it might account for around 70% of the observed density changes. It is possible that a change in tissue water status of the ray tissue might account for the small density reductions that were observed in the diurnal cycles at low tension levels. These reductions, at around -0.5 MPa, do not concur with the acoustic studies of West and Gaff (1976), but if it is proposed that these reductions are not due to cavitation, but simply the removal of water from "storage" ray tissues, there is still a remaining reduction of around 5% which could be attributed to embolism formation within the tissues. No data were ~~was~~ available on other parenchyma tissue which might exist within the xylem and so could not be taken into account in these calculations.

It is noted that, as in the previous seasonal investigations, the mist irrigated trees experienced sigificantly lower levels of water stress, as determined by leaf water potential and relative water content, than the control trees, which resulted in lower reductions in xylem density. It could therefore be concluded that mist irrigation might be important in preventing cavitation and perhaps in assisting decavitation. This point will be more fully dicussed later.

The relationship between leaf water potential and transpiration has been well documented by various authors eg. in wheat (Jones 1978) and in apple (Landsberg et al 1975, Powell and Thorpe 1977). As previously explained (chapter 6.3.6), this relationship can be used as an estimate of the permeability of the stem. If the slope of this relationship changes over a period of time, and no stem area or canopy growth occurs, then that change will indicate either an increase or decrease in stem permeability, a decrease in slope i.e. becoming more negative, indicating a reduction in permeability.

The results of the present study were rather inconclusive. Following the cavitation hypothesis, if cavitation occurs on a diurnal basis then it would seem reasonable to assume that some of the cavitated vessels might not be restored. As the season progresses, if stress levels are maintained, a progressivly larger proportion of the xylem vessels in the stem might become non-functional in this manner, so that stem permeability could be affected. Therefore as stress levels increase, it might be expected that the slope of the transpiration/water potential relationship would decrease, thus indicating a decrease in permeability due to an increase in cavitation. In the present study, regressions on the relationship indicated an increase in permeability followed by a decrease, followed by an increase, over the period of one month, near the end of the growing season. This variation in permeability might have ben due in part to the effects of cavitation, as gamma probe studies indicated a reduction in density of around 4% over this period. The apparent increases in permeability might have been due to canopy or stem cross sectional area growth which would obviously affect the relationship but as canopy growth had ceased by the time of the experiment it is debateable whether stem growth effects alone could have accounted for the observed variation.

Work by Edwards and Jarvis (1982) indicated that a reduction in sapwood relative water content from 100 to 90% caused the permeability of a stem to fall to about 10% of the saturated value, which was thought to be the result of cavitation. Crombie (1983), using a similar technique, found that the permeability of shoots and stem segments was reduced by 60% as the sap tensions within the system exceeded 1 MPa. Sap almost ceased to flow in samples which were subjected to tensions around 4 MPa. From these results it might have been expected that some decrease in permeability would have been detected in the present study, but it is assumed that the combined effects of growth and cavitation caused the permeability of the trees to fluctuate in the observed manner.

(6.4) Growth Relations.

(6.4.1) Fruit Growth.

Measurement of fruit diameter over the experimental period indicated an initial similarity in growth rate for both the irrigated and non-irrigated trees (figure 6.4.1). By the last week in July, around day 40, the mean fruit diameter of the ground irrigated trees was significantly lower, at the 5% significance level, than that of the mist or control trees. No difference was detected between the latter two groups. The mean diameter of the misted fruits did become significantly higher than that of the controls by the second week in August, and by the end of the experiment, it was found that a difference at the 5% significance level was present between those groups. No significant difference could be detected between the ground irrigated trees and the controls.

When the fruit diameter data was converted to fruit volume per canopy, as previously described (chapter 5.2), it was clearly

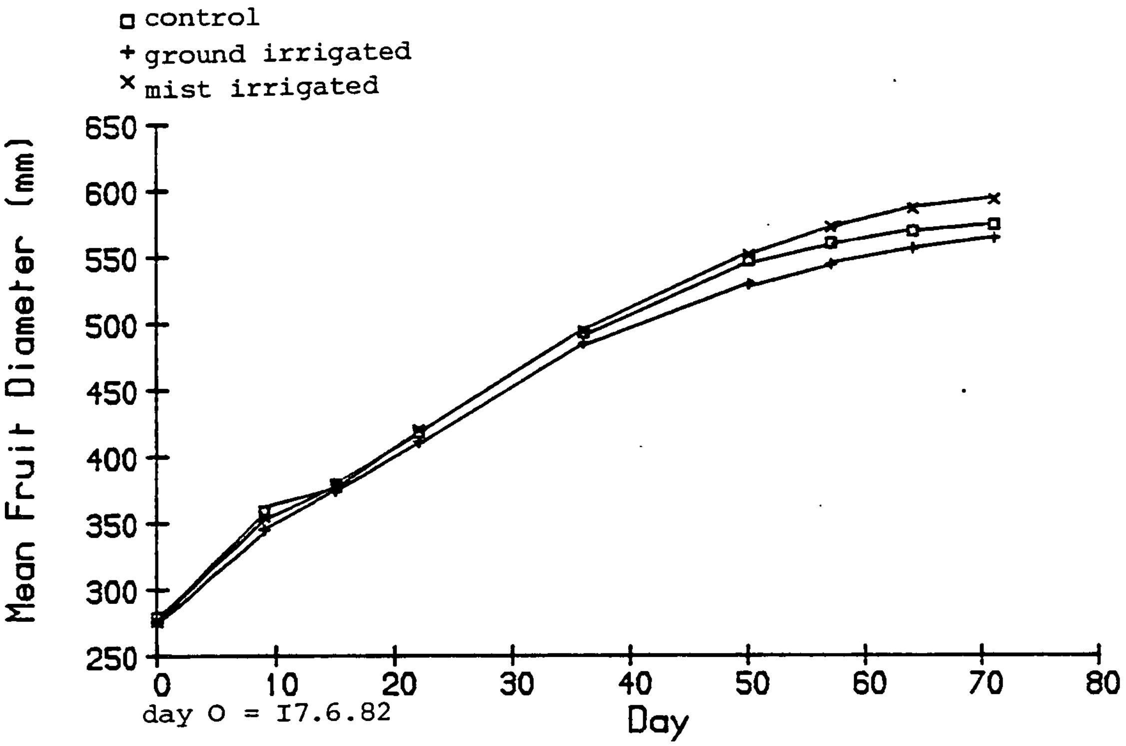

Figure 6.4.1.
Seasonal variation in fruit diameter over the 1982 growing season, in Golden Delicious apple trees under three experimental irrigation treatments.
(statistical analysis in appendix A)

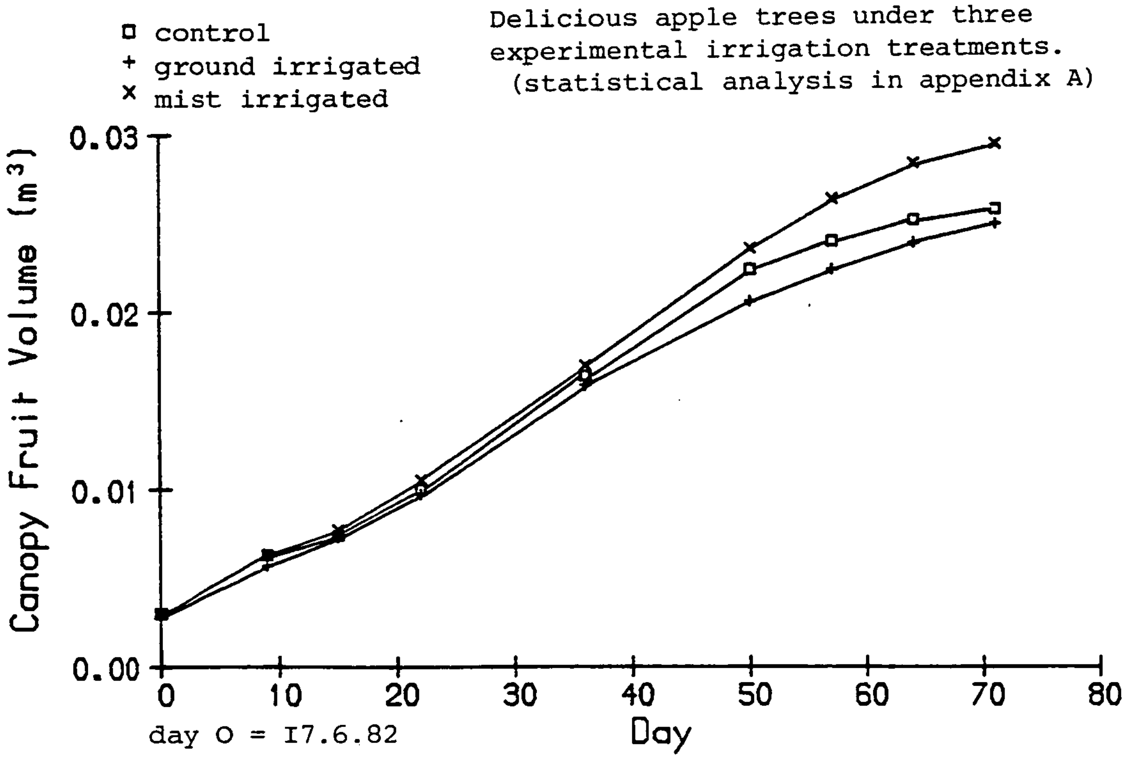

Figure 6.4.2.
Seasonal variation in total canopy fruit volume over the 1982 growing season, in Golden Delicious apple trees under three experimental irrigation treatments.
(statistical analysis in appendix A)

demonstrated that, by the first week of August, the mean volume of fruit per canopy was highest in the misted trees, and lowest in the ground irrigated trees (figure 6.4.2),the differences being significant at the 5% significance level. Although canopy fuit volume and fruit diameter are not independent of each other, fruit volume was felt to be a better measure of tree productivity as it includes data on fruit number, counted at harvest. By the end of the experiment no significant difference was found between the ground irrigated treatment and the control though the mean volume of the mist treatment was significantly higher at the 5% level. The actual crop yields of each tree measured are presented in appendix C.

(6.4.2) Shoot Growth.

Measurement of shoot growth, as described in chapter (5.2), indicated significantly different growth patterns between the treatments until mid July (figure 6.4.3), with the highest growth rate occurring in the ground irrigated trees and the lowest in the misted trees. All shoot growth appeared to cease around the first week in August, though the only significant growth since day 35 had been in the misted trees. At the end of the experimental period no statistically significant differences in shoot length could be detected between the three treatments at the 5% significance level.

(6.4.3) Discussion of the Growth Study Results.

Studies by Chapman (1973) indicated that shoot growth in apple

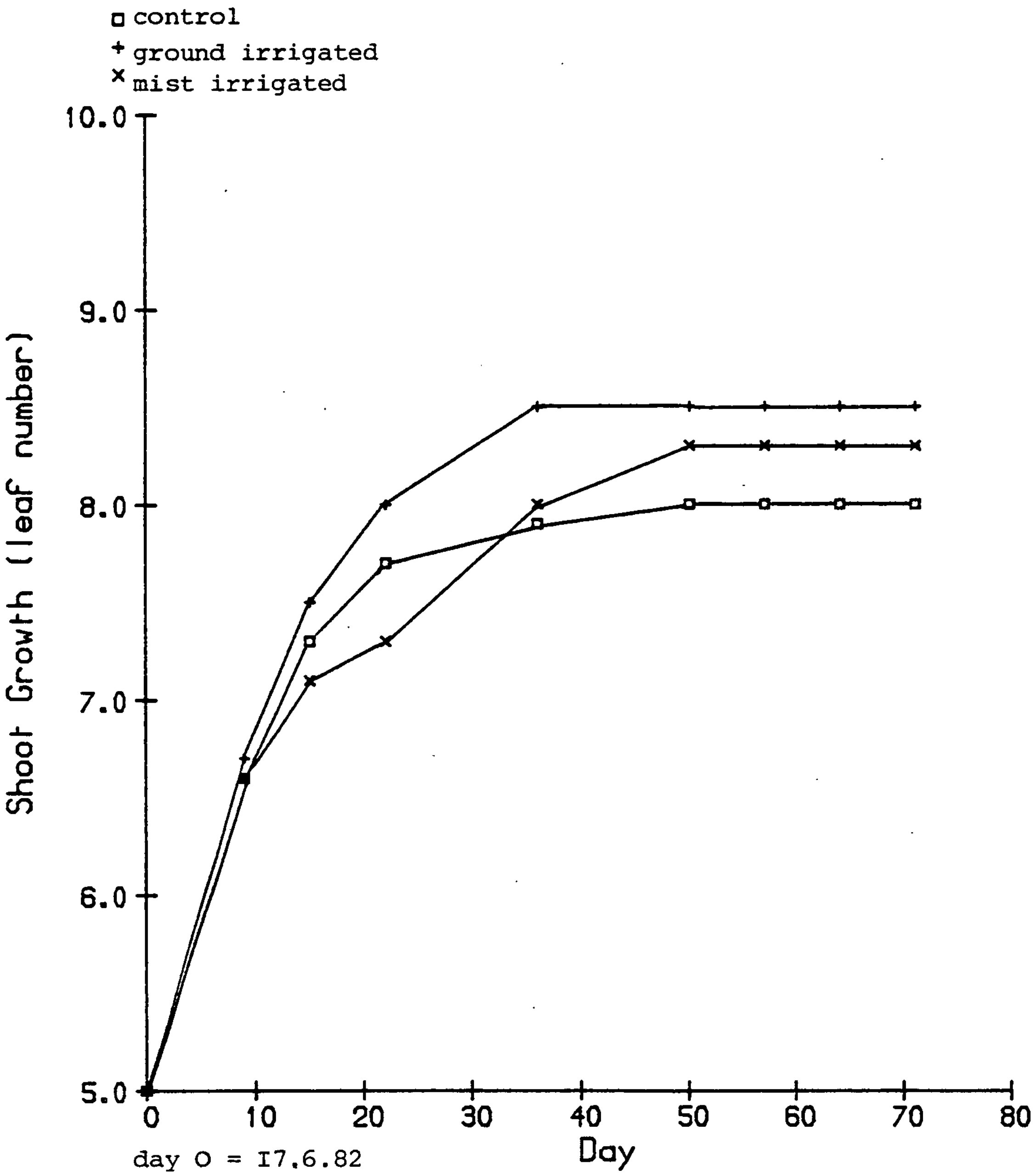

Figure 6.4.3.
Seaonal variation in extension shoot growth over the 1982 growing season, in Golden Delicious apple trees under three experimental irrigation treatments.
(statistical analysis in appendix A)

trees was retarded if the trees underwent water stress, but in the present study it was found that while the initial growth rate of the ground irrigated trees was faster than that of the controls, the controls grew faster than the misted trees. No significant difference in final shoot length could be detected between any of the treatments. It is possible that the low levels of water stress i.e. minimum soil water potentials around 0.09 MPa, did not in fact affect shoot growth, but it is more likely that the small sample size i.e. four trees per treatment, did not allow sufficient sensitivity in the analysis to give statistical weight to the observed growth differences.

It was observed that significant differences in fruit diameter existed between the mist irrigated trees and the ground irrigated and controls. The larger "misted fruit" do not concur with the results of Goode, Higgs and Hyrycz (1979), who found that trees which were ground irrigated produced larger fruit than those which were mist irrigated both of which were larger than in the controls. It was found that, although the mist fruit were smaller, the number of fruit per canopy was higher, due to a reduction in the number of fruit which were thinned during the "June drop", and thus the reduced fruit size was due to resource partitioning effects.

As previously discussed, it has been proposed that mist irrigation will increase fruit number by increasing flower bud morphogenesis. In the present study no significant levels of mist irrigation had been applied to the plots during the 1981 season, so it would be expected that flower production would be the same in all three treatments in the 1982 season. When fruit number was counted for the three treatments at harvest it was found that no significant difference could be detected between the treatments, so it is proposed that the increase in fruit size in the misted trees is due to its efficiency in relieving water stress.

(6.5) Dye Uptake Studies.

Two trees, one which had been mist irrigated and the other a non-irrigated control, were severed at the base of their stems and allowed to take up a dye as described in chapter (3.4). After drying the stems were sectioned every 100 mm and the area of dyed xylem vessels determined as a percentage of the total xylem cross sectional area.

It was found that in the control tree there was a central core, approximately 40% of the total area which had not stained. This would tend to suggest that this region of the stem was either not conducting or that the flow rate in this region was too slow to be detected during the time course of this experiment. The stem sections were cut longitudinally and it was noted that this "non-conducting" region possessed rather distinct boundaries, with the stained region commencing in the spring wood of the fourth year of growth.

A similar pattern was found in the stem sections of the misted tree with a non-stained central core, once again approximately 40% of the total area. From these results it would suggest that at the time of felling, water transport was occurring predominantly in the previous three years of xylem growth.

Studies by Edwards (1980) indicated that sap flow, at varying rates, could be detected over almost the total area of a stem, so it could be concluded that, in the present study, the unstained region might, in the main, have just been areas of very slow sap flow. It has been shown that xylem density underwent a large reduction, around 40%, over the first few weeks of the experiment, which has been interpreted as a 40% reduction in xylem water content. The gamma probe cannot distinguish between water in conducting tissue and water in storage tissue, and it is proposed that this large density reduction is, in the main, due to the removal of water from "store" to meet transpirational demand as the availability of water in the soil decreases. Such a store might exist in this region of slow conducting tissue which could become fully hydrated over the autumn and spring months when stress was at a minimum which might explain the autumnal increase in stem water content found by Gibbs (1935), after the large declines of the summer period. After this store had been depleted, further demands might cause the main conducting tissues to cavitate, so accounting for the 2 to 3% reduction in density which occurred over the rest of the season.

The level of water stress experienced by the apple trees during the 1982 growing season, as indicated by a maximum soil water tension of less than 0.01 MPa, did not appear to have a large effect upon the growth or productivity of the trees, though effects on tissue water relations were detected which might become important in more extreme seasons.

Chapter (7) The Cavitation Hypothesis - a Critical Experiment.

It was proposed in chapter (3.2), that cavitation within the xylem could be detected by passing a beam of gamma radiation through the xylem and measuring changes in the attenuation of the beam, where a decrease in attenuation indicated a decrease in xylem density brought about by the cavitation of the xylem vessels. Experiments on orchard grown trees indicated a diurnal variation in density in direct proportion to plant water stress as determined by leaf water potential and leaf relative water content, and inversely proportional to an estimate of evapotranspiration. A decline in density was also noted, over the growing season, which appeared to be related to soil water potential.

Although these results tend to confirm the hypothesis that cavitation and decavitation are normal events in the "life" of a plant, it is necessary to confirm that the gamma probe is in fact measuring cavitation and not some artifact or other phenomenon. The main technique for studying cavitation in previous investigations was that of acoustic detection (chapter 3.1). If the acoustic probe and gamma probe, used in conjunction with each other on the same tree, indicate that declines in density are associated with acoustic signals, then this would support the cavitation hypothesis.

To this end an experiment was designed, as outlined in chapter (3.1.2), to investigate the relationship between the rate of click production and plant water stress, and that between click rate and xylem density. Measurements of click rate, xylem density and leaf water potential were made at intervals over a thirty hour period on trees which were stressed by cutting through the stem at ground level and allowing the trees to transpire. It was decided that as most of the previous studies on cavitation had been done on *Ricinus*, this species should be used in the present study to test the experimental technique. Cavitation was measured acoustically by placing the pickup detector on a petiole and monitoring the plant for periods of three minutes at intervals over the course of the experiment. Measurements of xylem density changes, relative to the density measured before severing the stem, and leaf water potential were made at the same intervals.

It is noted that "cavitation" clicks could not be detected for the first three hours of the experiment, but from this point the click rate was observed to rise very quickly and peak approximately eight hours later (figure 7.1). The click rate then slowly decreased to approximately one per minute some twenty one hours into the experiment and remained relatively constant until the end of the experiment nine hours later. Xylem density also remained relatively constant for the first three hours of the experiment (figure 7.2), but was then found to decline in a roughly linear manner for the next six hours. Approximately nine hours from the start of the experiment the decline in density began to slow down and by the twenty one hour point the density had fallen by 28% relative to the initial xylem density. At this point the gamma source was removed and replaced seven hours later, when the density was found to have fallen to around 62% and the experiment terminated. Leaf water potential was found to decrease from -0.56 MPa at the beginning of the experiment to just under -3.0 MPa some nine hours later (figure 7.3). It was not possible to measure

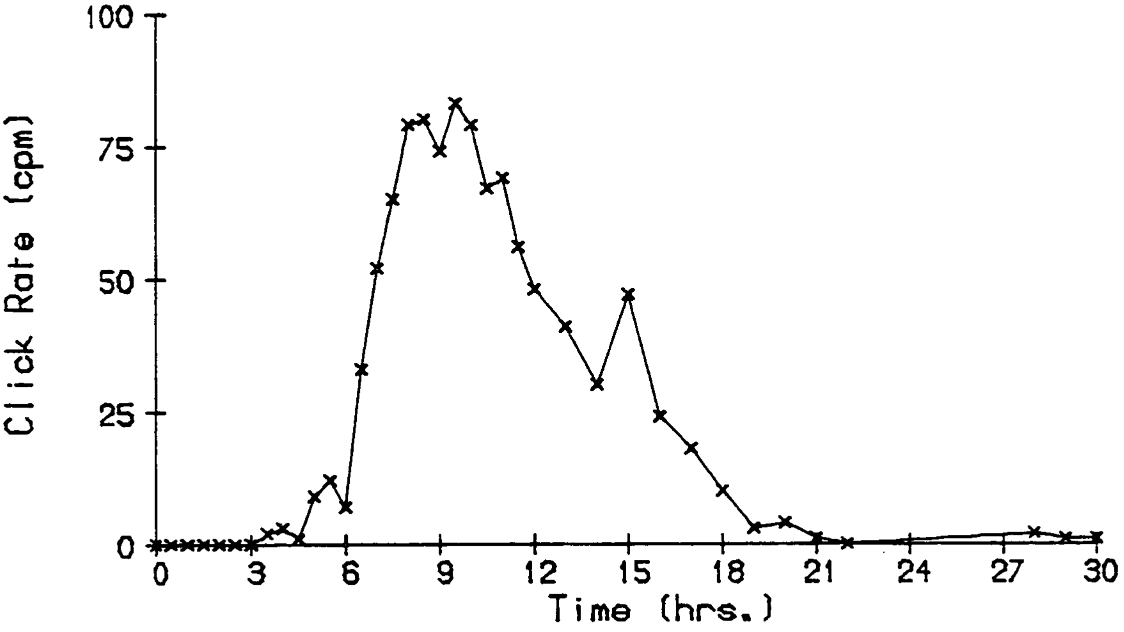

Figure 7.1.
The time course for click production during a 30 hr. period of water loss in an excised *Ricinus* stem.

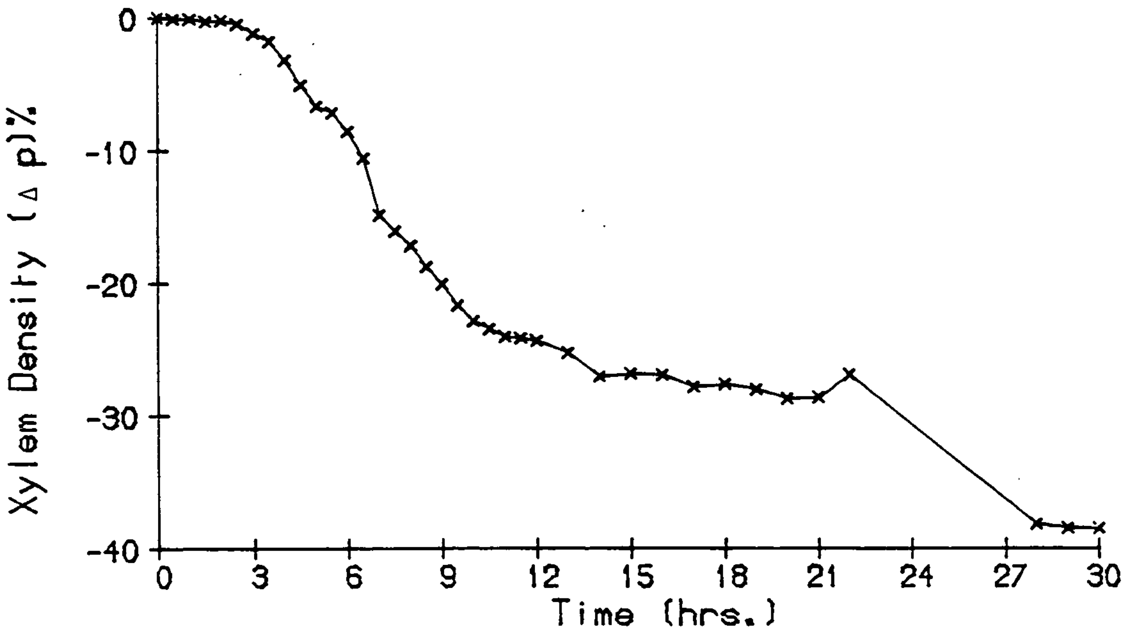

Figure 7.2.
Variation in xylem density over a 30 hr. period of water loss in an excised *Ricinus* stem.

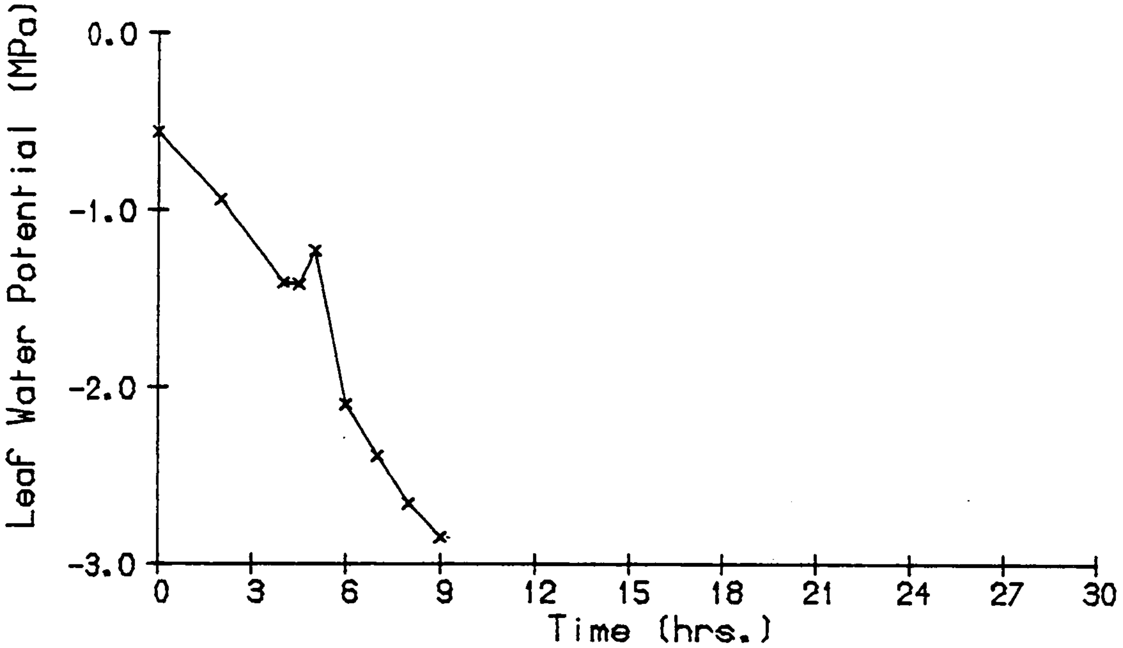

Figure 7.3.
Variation in leaf water potential over a 30 hr. period of water loss in an excised *Ricinus* stem.

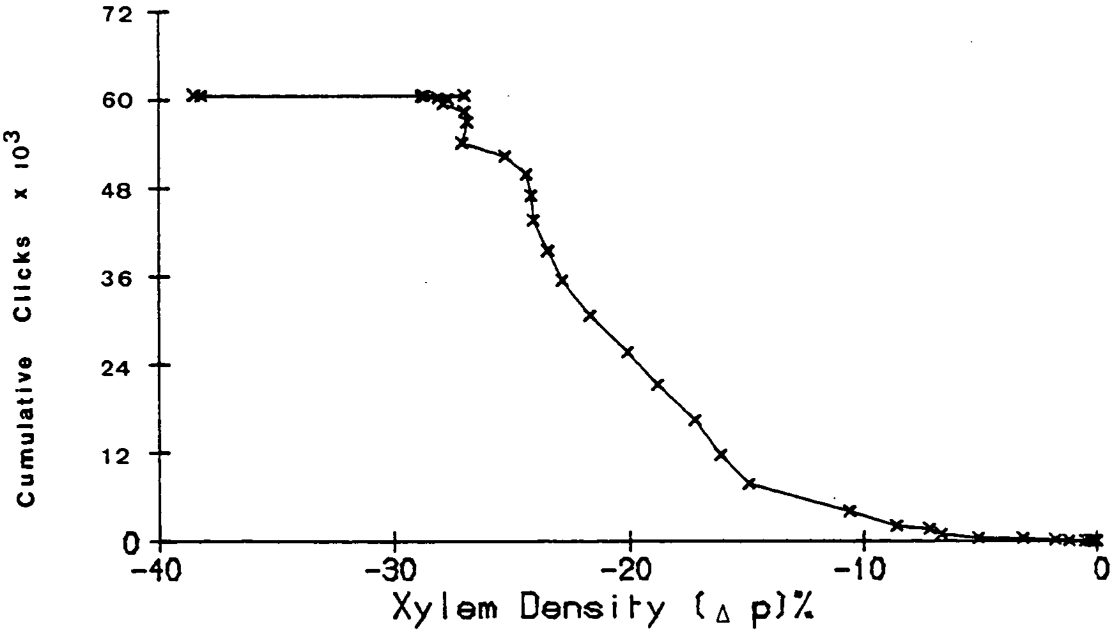

Figure 7.4.
The relationship between xylem density and click production during a 30 hr. period of water loss in an excised *Ricinus* stem.

water potential after this reading as the next measurement exceeded 3.0 MPa, and was therefore outside the range of the pressure bomb.

It was noted that xylem density varied in direct proportion to leaf water potential, as was found in the orchard experiments. When the relationship between xylem density and cumulative click rate was investigated (figure 7.4), it was found that, at the beginning of the experiment relatively few clicks were produced for a quite large decrease in xylem density of around 8%. As the experiment progressed click rate and density appeared to vary in direct proportion to each other but with a gradual decrease in click rate despite further reductions in density. When xylem density had decreased by approximately 28%, the click rate remained relatively constant although density had fallen by a further 10% by the end of the experiment.

When the experiment was repeated, this time with a five year old apple tree (Arthur Turner), it was found that the relationships between leaf water potential, click rate and xylem density were similar to those found in Ricinus. The tree was found to have a click rate of around three per minute before the tree was severed, and this rate remained approxiately constant for the first six hours of the experiment (figure 7.5). The click rate was then found to rise very rapidly and peak at 143 clicks per minute some fifteen hours into the experimental period, before lowering to around 20 clicks per minute at the end of the experiment. Xylem density was found to fall by just under 18% (figure 7.6) over the thirty hour period, in direct proportion to leaf water potential (figure 7.7). Unfortunately, as in the previous experiment, the water potential of the tree exceeded the range of the pressure bomb

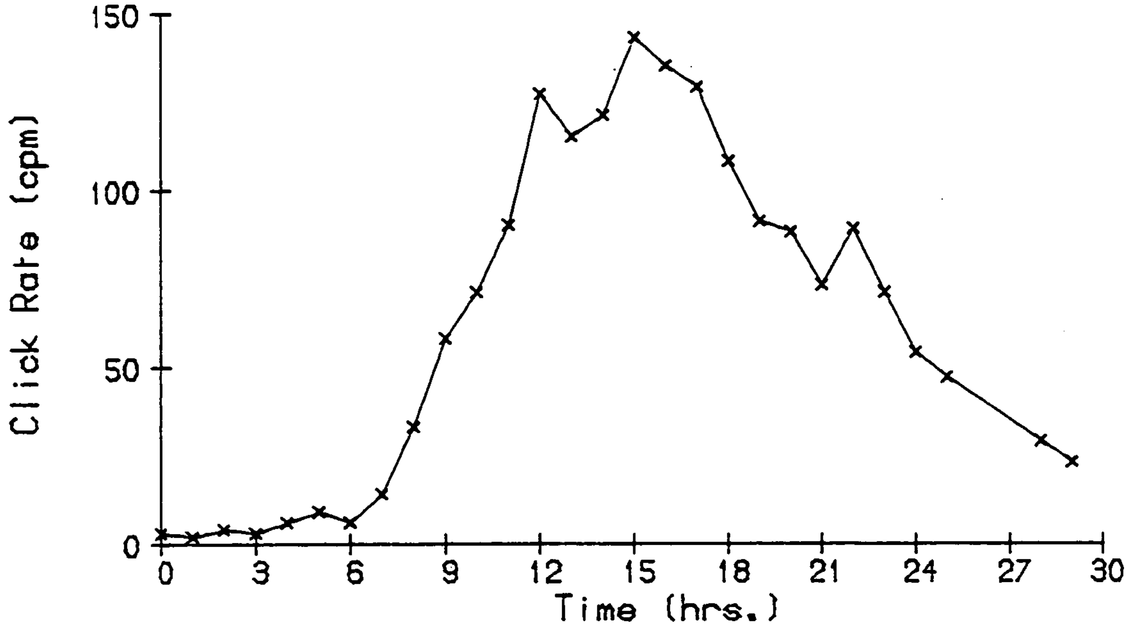

Figure 7.5.
The time course for click production during a 30 hr. period of water loss in an excised apple stem (Arthur Turner).

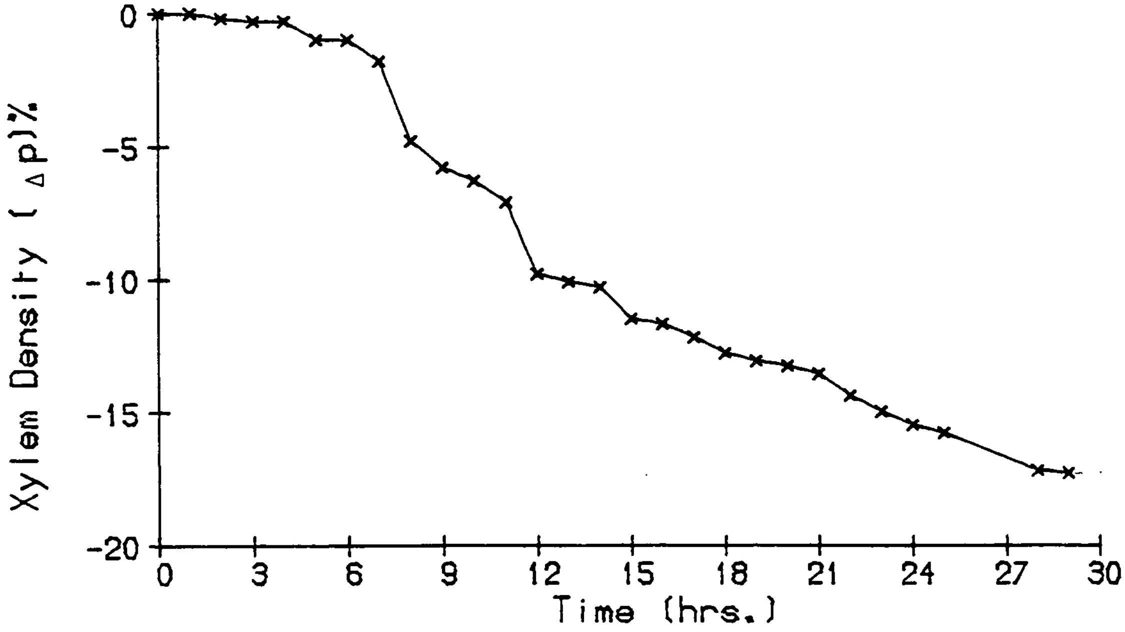

Figure 7.6.
Variation in xylem density over a 30 hr. period of water loss in an excised apple stem (Arthur Turner).

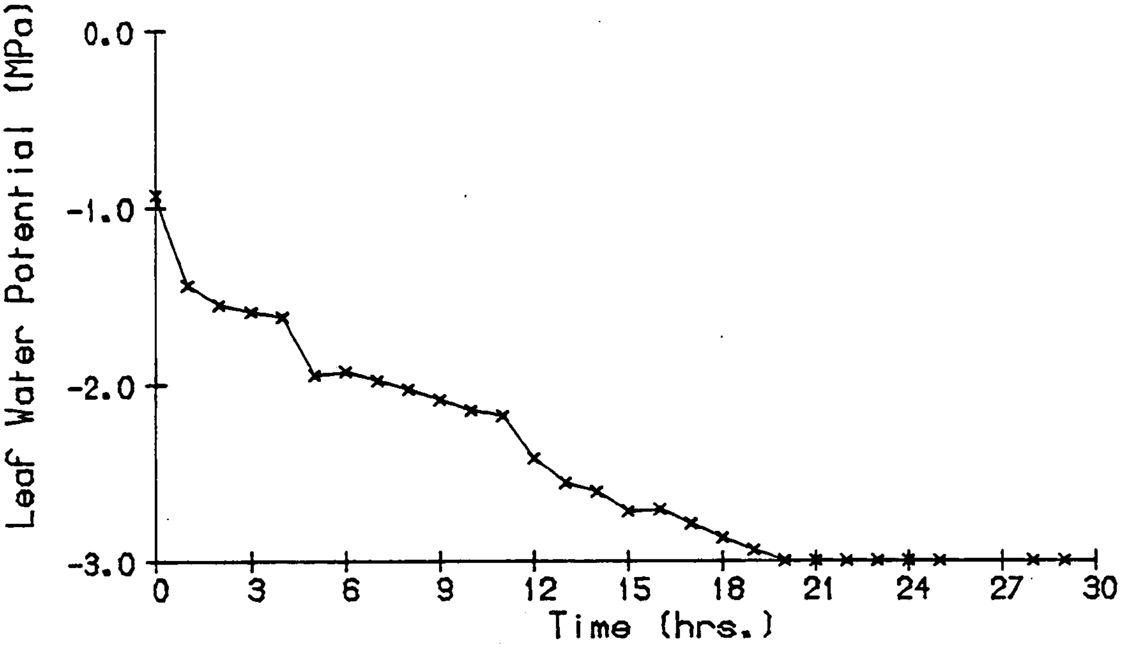

Figure 7.7.
Variation in leaf water potential over a 30 hr. period of water loss in an excised apple stem (Arthur Turner).

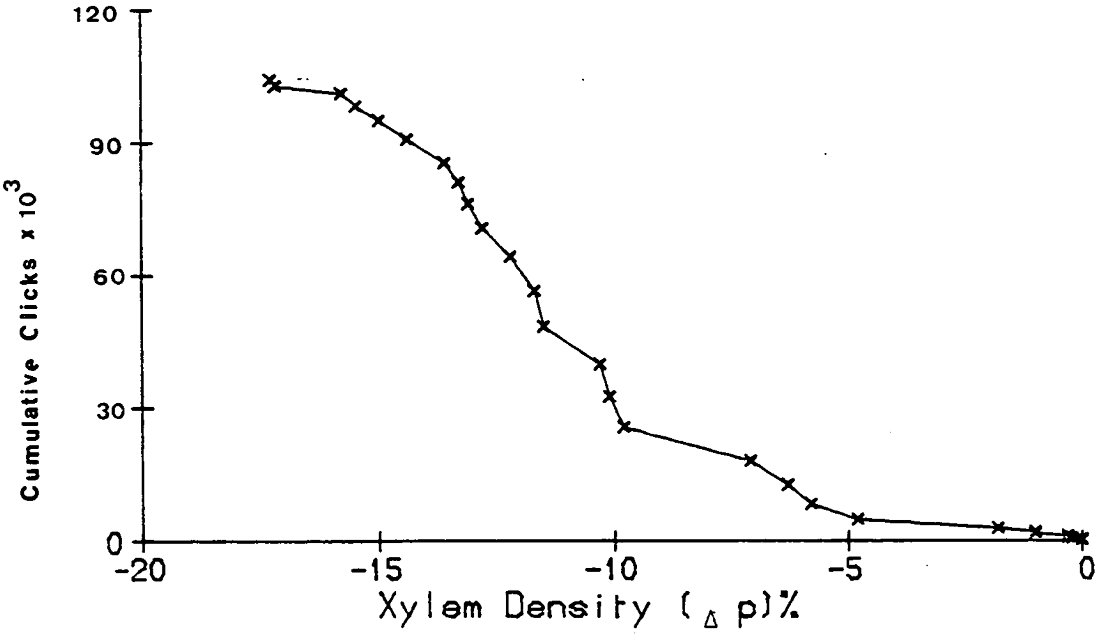

Figure 7.8.
The relationship between Xylem Density and click production during a 30 hr. period of water loss in an excised apple stem (Arthur Turner).

before the end of the experiment.

When the relationship between cumulative click rate and xylem density was investigated (figure 7.8), it was found that it was very similar to that found in Ricinus, with large density reductions being associated with low click production at the beginning of the experiment. Click production was then found to increase as density declined but then slowed down despite further reductions in density.

The aim of this experiment was to investigate a possible relationship between acoustically detected "cavitation" clicks, xylem density, as measured by gamma attenuation and leaf water potential. A relationship between click rate and leaf water potential had been demonstrated by Milburn and Johnson (1966), and later confirmed by Milburn (1973a, 1973b, 1975), Milburn and Davis (1973), Milburn and McLauchlin (1974), West and Gaff (1976) and Crombie (1983). It was proposed that this relationship, in conjunction with other lines of evidence, indicated that the occurence of these clicks was due to water stress causing cavitation of xylem vessels, the clicks being vibrations of the vessel walls, set up as they cavitated (chapter 3.1). Milburn and Johnson (1966) and Milburn and McLauchlin (1974) indicated that although mechanical distortion of the tissue did not produce clicks, some "tissue noise" resembling clicks could be detected in their experiments so care was needed to distinguish between cavitation events and background noise. Crombie (1983) demonstrated that the appearance of "gas" in xylem conduits of Acer was associated with clicks, and that the production of clicks in

Rhododendron was dependent upon sap tension and not cell turgor. On the basis of these results and the preceeding reports in the literature, he concluded that clicks were almost certainly caused by cavitation.

In the present study it has been proposed that decreases in xylem density, measured using a technique based on the attenuation of a beam of gamma radiation, were caused by the cavitation of xylem vessels. These decreases were shown to be closely related, in the field, to changes in leaf water potential which, it was proposed, supported the proposition that the gamma probe was in fact measuring cavitation.

In an attempt to confirm this proposition, the two techniques i.e. acoustic detection and the gamma probe were used in conjunction with each other on the same tree. If similar cavitation patterns were observed, using the two techniques, then this would support the cavitation hypothesis. The results indicated, that as in previous investigations, both click rate and xylem density were related to leaf water potential i.e. an increase in click rate was associated with a decrease in water potential and also a decrease in xylem density.

It is noted that the relationship between click rate and water potential differs between plant species, with herbaceous species having a lower threshold of cavitation, in the main, than trees (Crombie 1983). In the present study the threshold at which cavitation clicks readily occurred was around -1.3 MPa, which concurs with the findings of Crombie (1983),who found high click rates at tensions exceeding 1.0 MPa. West and Gaff (1976) found that the threshold in apple was around -1.2 MPa but in the present study rapid click rates were not observed until the sap tensions approached -2.0 MPa, though clicks were detected at tensions around -1.0 MPa. The acoustic detector works by sensing click vibrations by means of a ceramic "Hi-Fi" pickup. It was found that the sensitivity of the pickup depended upon the "tracking weight" that was applied to the crystal when it was attached to a petiole or stem. It is proposed that the differences in click rate that are observed for the same species under the same conditions of tissue water status might in part be due to differences in sensitivity of the detector system to detect clicks.

It is clearly demonstrated, that in both species investigated, a relationship exists between click rate and xylem density i.e. small reductions in density are associated with low click rates. As density declines, in response to increasing water stress, click rate also increases until a maximum is reached and as the rate of density decline slows, so also does click rate.

As previously described, every xylem vessel will have a cavitation threshold depending upon its size, i.e. large vessels will cavitate at lower tensions than narrower conduits. Large vessels conduct more water than narrower ones, the hydraulic conductance of a capillary being proportional to the fourth power of its radius according to the Hagen-Poiselle equation (Milburn 1979), so their disruption should be detectable as large decreases in xylem density. As it is assumed that the larger vessels will cavitate first then it would be expected that large reductions in xylem density would be observed at the cavitation threshold. As tensions increase the narrower conduits will fracture, but as they conduct less water, their disruption will cause a smaller decrease in density.

Concurrently with the present study, work by Tyree and Dixon (unpub.) at the University of Toronto, suggests that ultrasonic emissions from the sapwood of Thuja occidentalis L. were caused by cavitation. These emissions, in the frequency range of 0.1 to 1 MHz, only occur when the tensions within the xylem exceed a threshold level of around -1.0 MPa, the rate of emission increasing as tension increases. The rate of emission was also found to increase as the net rate of water loss from the tissue increased, but the emissions could be stopped by supplying water to the tissue so reducing sap tension below -1.0 MPa. As previously described in chapter (3.1.2), the frequency response of the detectors used in the present study was tested, and were found to operate up to the limit of the test system i.e. 0.1 MHz. It is entirely possible that the detectors operated at frequencies above this level, but this could not be tested. The results of Tyree and Dixon indicate that their ultra-sonic emissions behave exactly like the clicks detected in the present study, and as the emission and click frequencies overlap, it is proposed that they are caused by the same event i.e. cavitation of xylem vessels.

The results of this study, and those of preceeding investigations, would support the hypothesis that acoustically detected clicks are caused by the cavitation of xylem conduits, and that this cavitation can be measured as xylem density reductions using a gamma probe.

Chapter 8 General Discussion.

The aim of the present study was to assess whether the diurnal and seasonal declines in stem density, reported by Giggs (1935), Clarke and Gibbs (1957) and Markstrom and Hann (1972) could be detected in orchard grown apple trees, and is so whether they could be attributed to embolisms formed by a series of cavitations in the xylem water columns, which might affect the growth and productivity of the trees. A secondary aim was to observe the effect of two irrigation systems on plant water stress, an overhead mist system which deposits water on the leaf canopy and a ground sprinkler system which wets an area approximately one meter square around the base of the tree.

The main technique used in the present study to assess cavitation was of necessity an indirect one, relying upon the attenuation of a beam of gamma radiation (chapter 3.2). The formation of embolisms within the xylem will cause the overall density of the tissue to decrease, so that, if the effects of any increase in path length caused by growth are removed, this change in density will cause the attenuation of the beam to vary accordingly, so giving a measure of the extent of cavitation. Experimental results indicated a seasonal reduction in xylem density of around 40%, in three groups of experimental trees i.e. overhead mist irrigated trees, ground irrigated trees and non-irrigated controls, the reduction in the irrigated trees being found to be less than in the controls. This reduction in density was accompanied by increasing plant water stress, which tends to indicate that the reduction in density may be due to cavitation brought about by the increasing stress.

Stem growth, which might cause misinterpretation of the results, was prevented by removing the phloem and cambial tissues in the path of the beam (chapter 3.2.1). Another source of error was possible changes in the thickness of the xylem under investigation. It is well known that the diameters of stems change in response to water stress (Klepper et al. 1971) so it might be argued that any apparent change in density could be caused by stem diameter changes. However, much of the shrinkage in stems reported in the past arises from elastic shrinkage of cells external to the xylem. When the diurnal variation in xylem diameter was measured on several occasions using linear variable displacement transducers, it was found that the maximum diameter change was an order of magnitude outside the resolution of the gamma probe (chapter 6.3.5), so that xylem shrinkage could not account for the observed diurnal density changes.

The gamma probe cannot differentiate between water in conducting tissues and water held in "storage tissues" eg. in the living ray parenchyma which forms between 5 to 10% of the xylem (Doley 1974). It is therefore possible that the large density reduction, around 40%, that was measured over the growing season, which concurs with the findings of Gibbs (1935), may in part be due to the removal of water from storage tissues. These tissue water reductions will occur when the level of transpirational demand increases and soil water becomes limiting. It is felt however, that fluctuations in storage water cannot account for all of this reduction, nor for the further reductions in density, between 2 to 5%, which occur over the remainder of the season. It has been proposed that around 5%

of the diurnal variation in xylem density could be due to fluctuations in the water content of storage tissue, but that the remainder of the variation, 3 to 5%, would appear to be due to the cavitation of the xylem conduits. The results of these experiments, and those that demonstrated a close relationship between xylem density and acoustically detected cavitation, support the hypothesis that cavitation in intact living tree stems can be assessed using the gamma probe.

The experimental evidence suggests that cavitation and decavitation are normal events in the diurnal cycles of apple trees. Various mechanisms for cavitation were outlined in chapter (1), where it was proposed that some nucleation event occurred at critical thresholds of sap tension. Such nuclei might be produced from the respiration of the living ray tissue within the xylem or in some instances from air entering the transport system as a result of wounding or other tissue damage. As tensions within the sap increase, the probability that a fracture will occur will increase, the threshold of fracture depending upon the size of the individual vessel.

For cavitation to occur in this manner, "gas" nuclei must be able to enter xylem conduits perhaps via the pores in the pit memranes. Crombie (1983) has shown that the tensions required for "gas" to overcome the surface tension of the menisci in the pores of wet pit membranes in Rhododendron are the same as those at which cavitation can be acoustically detected. Thus cavitation in one vessel element could result in cavitation in other elements, or gas entry, caused by tissue damage, into one part of the system might bring about cavitation at sites remote from the damaged area.

Crombie proposed that the variation in threshold level at which cavitation occurs in different species, in xylem of different ages, or even simply in different leaves of the same plant might be due to differences in the size of pores in the pit membranes.

For cavitation to occur as a normal event in the diurnal cycle some system for decavitation must occur. Mechanisms for decavitation were discussed in chapter (1), with the conclusion that some source of external positive pressure, such as root pressure, was required to repair the embolised vessels. Milburn and McLauchlin (1974) proposed that decavitation would occur during the nocturnal part of the diurnal cycle, when the tensions within the sap were at a minimum, probably in most cases, around 0 MPa. For a cavitation bubble to dissolve, the external pressure surrounding it must be greater than its internal pressure. In the xylem, the external pressure surrounding a bubble is a combination of the sap tension and the surface tension at the bubble/sap interface.

In the present study, it was observed that cavitation occurred as sap tensions increased, and that decavitation appeared to take place as soon as the tension reduced. In three of the four cases measured there was no significant lag phase between the reduction in tension and the onset of decavitation. This would appear to be in direct contradiction to previous proposals, though Gibbs (1935) measured similar patterns in stem water content over the diurnal cycle.

It is therefore proposed that the diurnal variation in xylem density measured in the present study, is partly due to a change in the amount of water stored in the ray tissues to meet transpirational demand and partly to the cavitation and decavitation of the xylem conduits. It is impossible to accurately state what proportion of the variation can be attributed to each phenomenon, though it is felt that storage changes may account for approximately 5%, with decavitation by means of root pressure occuring once the tensions in the xylem have lowered to around zero MPa.

Measurement of xylem permeability did not indicate a reduction in permeability over a proportion of the growing season, as might have been expected from the xylem density data. This may have been due to stem or canopy growth which would alter the slope of the transpiration/water potential relationship (chapter 6.3.6), or to errors in estimating transpiration.

In the present study, orchard grown trees were subjected to three different irrigation treatments, overhead misting, ground sprinklers and no irrigation, as a control, to investigate whether irrigation systems could relieve water stress and so prevent cavitation or assist in decavitation. Milburn and McLennan (unpub.) found that the sap pressure of <u>Larix</u> twigs could be reduced from around -1.5 to -0.03 MPa, a point at which it was proposed decavitation could occur by spraying them with water to simulate rain. Using the mist irrigation system (chapter 2), the maximum reduction in leaf water potential that was achieved during a diurnal cycle, was 0.83 MPa, compared with a control tree under the same environmental conditions, which brought the water potential of the mist canopy to around -1.4 MPa at 1500 hrs. Therefore although the mist system was capable of significantly reducing leaf water potential, it could not completely eliminate stress. The results outlined in the preceeding chapters indicate that the mist system did reduce stress levels throughout the growing season, which resulted in smaller reductions in xylem density, compared with the ground irrigated and control trees. This reduction in stress was also exhibited as an increase in fruit growth, though no difference in shoot growth could be detected between the treatments. It was noted that in general, the ground irrigation system was not able to relieve diurnal plant water stress, nor reduce the seasonal increases in stress, as efficiently as the mist system.

In general, the present study has indicated that xylem density in orchard grown apple trees undergoes diurnal variation, which is in direct proportion to plant water stress. A proportion of this density variation, perhaps as much as 70%, can be accounted for by changes in the water content of "storage tissues", but it is proposed that the remainder is due to the cavitation and refilling of the xylem vessels. The seasonal variation in xylem density can be explained in a similar fashion with possibly around 90% of the observed density reductions being due to storage changes.

It was found that the mist irrigation system could reduce plant water stress more efficiently, on a diurnal and seasonal basis, than ground sprinkler irrigation. This reduction in stress resulted in lower reductions in stem water content, presumed to be partly due to a reduction in cavitation within the xylem tissue, and increases in fruit growth. Little difference was observed between the ground irrigated and control trees, though significant differences in soil water potential were observed between the two plots. The study has indicated that gamma ray attenuation can be used to measure changes in the internal water balance of stems in intact living tissue which may prove very useful for future work in this area.

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Appendix A.

The results of the statistical analysis carried out on the experimental results shown in figures (6.1.4) to (6.4.3); the analysis conducted as described in chapter (6.1.3).

		Treatment Differences	
Figure	M : G	M : C	G : C
6.1.4	0	1	1
6.2.1	0	5	5
6.2.2	0	0	0
6.2.3	5	1	5
6.3.1 a	0.1	0.1	0
b	5	0	0
c	0	5	0
6.3.2 7.7.82	5	5	0
21.7.82	0	0	0
6.3.3 a	5	5	0
b	5	5	0
c	5	5	0
6.3.4 a	0.1	0.1	0
b	0	0	0
c	5	1	1
6.3.5 28.7.82	5	5	0
1.8.82	1	1	5

6.3.6 a	0.1	0.1	0
b	5	5	5
c	5	5	5
6.4.1	5	5	0
6.4.2	5	5	0
6.4.3	0	0	0

M = Mist Irrigated

G = Ground Irrigated

C = Control

0 = No Significant Difference

5 = Treatment difference significant at the 5% significance level.

Appendix B. Data used in the calculation of diurnal transpiration rates. 7.7.82

Time (hrs)	Air Temp. (°C)	Mist Canopy Temp. (°C)	Control Canopy Temp. (°C)	Ground Canopy Temp. (°C)	Non-Transpiring Leaf Temperature (°C)	Net Radiation (W m^{-2})
600	12.2	12.1	12.1	12.3	13.0	29.3
700	12.1	12.0	11.9	12.2	13.9	114.7
800	13.7	12.1	15.2	15.0	16.9	325.9
900	14.7	12.5	16.9	15.8	20.6	379.3
1000	18.8	16.3	19.8	19.7	22.9	466.4
1100	18.0	15.8	19.3	19.1	21.7	543.1
1200	21.2	19.3	24.4	24.2	28.3	631.0
1300	22.1	20.5	26.0	25.9	30.2	711.2
1400	21.9	20.3	25.8	25.5	29.9	647.4
1500	21.4	20.0	25.0	24.9	29.2	636.2
1600	21.1	18.1	22.5	22.1	26.9	406.9
1700	20.4	17.6	21.8	21.9	26.3	416.4
1800	19.0	21.1	18.4	18.9	18.7	181.0
1900	18.2	14.4	16.8	16.6	19.4	12.9

21.7.82

Time (hrs)	Air Temp. (oC)	Mist Canopy Temp. (oC)	Control Canopy Temp. (oC)	Ground Canopy Temp. (oC)	Non-Transpiring Leaf Temperature (oC)	Net Radiation (W m-2)
600	14.3	14.0	13.9	14.0	14.4	9.8
700	14.3	14.1	14.0	14.0	14.4	25.8
800	15.8	15.5	15.6	15.5	16.0	17.2
900	15.9	15.6	15.7	15.6	16.2	17.2
1000	16.0	15.6	15.8	15.7	16.3	52.6
1100	16.0	15.7	15.8	15.8	16.3	65.3
1200	17.7	16.2	19.0	18.9	21.3	248.3
1300	17.4	16.1	18.8	18.7	21.2	294.0
1400	17.1	16.0	16.4	16.3	18.0	162.1
1500	17.6	13.7	19.8	19.4	22.6	338.0
1600	16.4	15.8	15.9	16.1	17.2	110.3
1700	16.8	16.0	16.1	16.2	17.3	115.5
1800	17.0	16.0	16.2	16.1	17.5	110.3
1900	16.0	15.7	15.9	15.8	16.2	29.3

28.7.82

Time (hrs)	Air Temp. (°C)	Mist Canopy Temp. (°C)	Control Canopy Temp. (°C)	Ground Canopy Temp. (°C)	Non-Transpiring Leaf Temperature (°C)	Net Radiation (W m^{-2})
600	12.6	12.2	12.3	12.2	12.7	8.9
700	12.8	12.6	12.4	12.4	12.8	16.4
800	13.7	13.2	13.1	13.3	13.8	30.2
900	14.1	13.5	13.6	13.6	14.5	50.0
1000	14.7	13.9	14.0	14.2	15.0	41.4
1100	15.7	15.1	15.2	15.5	16.5	110.3
1200	16.9	15.3	18.2	18.1	20.6	272.4
1300	18.5	17.8	22.0	22.2	25.1	621.6
1400	19.0	17.6	22.4	22.5	26.6	622.4
1500	18.9	16.6	22.4	22.3	26.7	595.7
1600	18.5	16.2	23.2	23.0	26.5	559.5
1700	18.6	15.6	20.8	19.9	24.5	398.3
1800	17.8	13.9	16.4	16.1	19.1	74.1
1900	16.2	15.9	16.1	16.0	16.8	33.6

1.8.82

Time (hrs)	Air Temp. (°C)	Mist Canopy Temp. (°C)	Control Canopy Temp. (°C)	Ground Canopy Temp. (°C)	Non-Transpiring Leaf Temperature (°C)	Net Radiation ($W m^{-2}$)
600	13.0	12.8	12.5	12.6	13.1	17.3
700	16.2	15.1	15.3	15.1	16.4	98.6
800	17.1	17.8	17.2	17.4	18.8	170.7
900	16.5	16.8	16.4	16.6	17.9	134.5
1000	17.3	18.0	17.4	17.1	19.2	192.2
1100	17.4	17.6	17.1	17.0	18.6	150.9
1200	18.8	16.1	18.3	18.1	20.8	312.2
1300	20.3	17.8	21.8	21.4	27.5	562.5
1400	21.9	16.9	21.3	20.8	25.5	697.4
1500	22.3	16.4	20.8	20.6	25.3	603.5
1600	22.3	19.0	20.3	20.1	24.9	449.1
1700	22.1	18.8	19.9	19.8	24.6	314.7
1800	19.6	18.0	18.6	18.4	20.2	180.9
1900	17.7	16.9	17.6	17.5	18.1	33.1

Appendix C.

1982 Golden Delicious Crop Yield.

Tree	Irrigation Treatment	Total Weight Fruit Produced	Total Number Fruit Produced
268		36.5	323
267	M	22.7	189
266		37.1	329
263		33.5	289
262	C	24.8	205
261		23.1	269
258		35.5	270
257	G	34.1	270
256		11.2	97
253		33.6	307
252	C	24.5	177
251		34.0	246
243		30.1	237
241	M	36.0	321
240		24.9	218
235		34.0	317
234	G	22.4	224
233		34.1	323

232		31.6	340
231	G	32.7	300
230		22.7	279
227		30.2	310
226	C	26.9	268
225		25.4	251
220		34.8	290
219	M	34.7	311
218		27.0	231
217		32.1	300
214	M	24.9	264
213		27.2	228
209		33.8	341
208	C	22.8	185
207		30.8	285
204		17.1	211
203	G	46.1	371
202		20.8	189
468		40.9	345
467	M	42.6	357
466		11.4	102
463		30.7	279
462	C	31.2	302
461		28.1	282

458		41.3	311
457	G	22.3	174
456		31.9	233
453		33.2	316
450	C	23.1	223
449		33.5	30.6
442		22.3	213
441	M	33.3	328
440		35.5	343
435		12.9	116
434	G	38.6	390
433		25.6	237
432		18.9	171
431	G	36.2	340
430		37.7	366
427		38.0	383
426	C	45.3	448
424		37.0	330
420		24.6	211
419	M	24.2	222
418		25.6	249
417		30.9	328
414	M	13.0	126
413		25.0	233