AN ABSTRACT OF THE THESIS OF

Richard C. Beeson Jr. for the degree of Doctor of Philosophy

in Horticulture presented on May 30, 1986

Title: The Physiology of Picea Grafts

Abstract approved: [redacted]
William M. Proebsting

Grafting is the only economical method of propagation for many ornamental conifer species, but grafting success may often be variable. The objectives of this study were to characterize the roles of water relations and carbon metabolism of the scion in graft success, and evaluate techniques that might improve success. The study was conducted on Picea pungens 'Hoopsi' scions, side-grafted onto potted Picea abies rootstocks which were actively growing in the greenhouse or dormant in an unheated, polyethylene-covered lath house. Scions were grafted in mid-winter and evaluated in mid-summer.

In greenhouse grafts, the water potential (Ψ_T) of the scion declined rapidly the first 2 weeks, thereafter, water movement through callus bridge resulted in gradual increases in Ψ_T, RWC and transpiration rates. Scion bud break occurred 1 to 2 weeks after tracheid connections matured. Grafts overwintered in the covered lath house had lower scion water stress and higher success, but required more time for union development. Imprecise cambial alignment slowed callus bridge formation, increasing scion water stress. In unsuccessful grafts, when Ψ_T declined below a critical point, transpiration increased while turgor pressure remained high due to

osmotic adjustment.

Scion photosynthesis during union development was not required, nor did it affected graft success or scion growth. Needle and bark sugars declined gradually during union development, but needle starch accumulated. There was little movement of scion photosynthates until maturation of the connecting tracheids. Rootstock photosynthates were not translocated into the scion prior to bud elongation.

Overwintering grafts on dormant rootstocks in the covered lath house was the most reliable and economical practice. Applications of plant growth substances improved success but tended to lower scion quality. Repeated debudding of the rootstock improved success and scion quality, but was laborious. Several other treatments were tested, but were either ineffective or detrimental.

Cambial alignment, the post-grafting environment and the physiological state of the rootstock all interact to govern the water status of the scion and therefore graft success.

THE PHYSIOLOGY OF *PICEA* GRAFTS

By

Richard C. Beeson Jr.

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Doctor of Philosophy

Completed May 30, 1986

Commencement June 1987

APPROVED:

Associate Professor of Horticulture in charge of the major

Head of the Department of Horticulture

Dean of the Graduate School

Date thesis presented: May 30, 1986

Acknowledgements

I would like to express my gratitude to Dr. W. M. Proebsting for his confidence, guidance and the demanding, yet unconstraining manner inwhich he conducted my apprenticeship. I also thank my committee memebers, Drs. L. Boersma, D. Copes, J. Potter, T. Allen and D. Heatherbell for their support and criticism over the years.

To the faculty and staff who have made the past years bearable and even enjoyable most of the time, goes my appreciation and thanks; especially Dr. Bud Weiser, the office staff, and Willie Moller.

In my 5 years here, I have had too many close friends who have help ride-out the rough times to name individually. To these, and the rest who shared this time and space with me, words cannot describe the bond the shared hardships have forged between us, nor convey the sorrow at losing the close contact we have had.

I want to extend thanks to Verl Holden of Holden's Nursery and Kerwin Daughton of Tyree Tree Nursery. Without their cooperation and encouragement, this study could never have been accomplished. I also want to thank Bob Lowery and the Agricultural Chemistry Department for the use of their equipment and expertises.

Finally, I want to dedicate this to the one who was always there and happy to greet me after a hard day in the lab, or a long night; and who demanded some flexibility in workaholic crusade - Whiskey.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

THE PHYSIOLOGY OF *PICEA* GRAFTS

Chapter 1

INTRODUCTION

Grafting of ornamental conifers is the only means of propagation for several cultivars and the most economical method for many more. Yet graft success is highly variable, both between years and between cultivars of the same species. Conifer grafts are different from angiosperm grafts in several ways. Conifer scions retain their needles during union development. Yet, callus development is much slower in rate and quantity produced. Thus, scion water relations should be a major factor in graft success. However, scion water stress and related physiological processes, have never been studied in relation to union development and graft success. The objectives of this study were to characterize and quantitate the water relations and carbon metabolism of conifer scions and relate these to union development and graft success. In addition, devise and evaluate techniques that might improve success and/or reduce the variability.

Chapter 2

REVIEW OF LITERATURE

Introduction

Grafting is the art of joining plant parts together such that they continue to grow as one plant (Hartmann & Kester, 1975). Grafting has been a part of man's history for over 2300 years. The ancient Greek philosopher Aristotle described in detail grafting operations that have changed little subsequently (Roberts, 1949). Until recently, grafting was almost the only means of perpetuating desirable traits of many woody species. Grafting is one of the most expensive methods of plant propagation due to the time, skill, and post-grafting care required (Fordham & Spraker, 1975), but grafting is still used to propagate clones when other asexual methods are more difficult or less successful (Hartmann & Kester, 1975). For the production of many ornamental conifer cultivars, grafting is the only viable method of propagation.

Cultural Aspects

Grafting consists of joining part of a stem of the desired plant (scion), to another plant (rootstock). This is done by making long sloping cuts on both the scion and the rootstock that expose the xylem, phloem and cambium tissues of each. The cambia of stock and scion are aligned on one or, preferably, both sides of the stem and bound

tightly, whereupon post-grafting care is implemented.

There are four main types of grafts used for conifers: the side graft, the veneer graft, tip-cleft, and the whip-and-tongue graft. Only the side graft and the veneer graft, which differs from the side graft by the removal of most of the rootstock flap, are used for blue spruce (Fordham & Spraker, 1977). The side graft is preferred by ornamental nurserymen in the Pacific Northwest. Dorsman (1966) suggested that the rootstock flap of the side graft increases the area of contact, thus increasing the probability of union formation (Copes, 1967). Second, the flap may function as a nutrient sink regulating sap flow to the union zone (Dorsman, 1966).

Scion collection for conifers normally occurs in the winter while the buds are dormant and have received sufficient chilling. Scions are taken only from healthy wood; one year old terminals are preferred (Fordham & Spraker, 1977; Holst et al, 1956; Orr-Ewing & Prideaux, 1959). Ortet age did not have a significant effect on success for Pinus radiata grafts, but success tended to be higher with scions from younger trees which also had significantly more post-graft growth (Pawsey, 1970). But for white pine, scion tree age significantly influenced graft success (Ahlgren, 1962).

Propagators prefer to graft freshly cut scions, but field grafting normally occurs in mid to late spring, therefore storage of dormant scions is essential. Fall collected scions of white spruce, black spruce and white pine were not viable if stored tightly bundled at -3° C due to freezing injury, or at 2° - 4° if loosely bundled because of the development of mold (Ontario Forest Research Center, 1979). However successful storage of Douglas-fir occurred at 34° F when

extra-long scions were packed in polyethylene bags with wet moss (Orr-Ewing & Prideaux, 1959). Red pine also was successfully stored when packed in a mixture of snow and sawdust, wrapped by a polyethylene film, and stored at 0° C (Holst, 1956a).

Most rootstocks used for ornamental conifers grafts are seedlings. Seedlings are used for three reasons: production is relatively simple and economical, seedlings are usually virus-free, and they develop better root systems than cuttings (Hartmann & Kester, 1975). Normally 2/0, 3/0, or 2/2 trees, depending on rootstock availability and the size of the scions are used in greenhouse grafting (Holst et al, 1956). Rootstocks perform best if they are potted one growing season before grafting (Fordham & Spraker, 1977). In Pinus, the species of rootstock influences scion growth and cone production (Schmidtling, 1973). Norway spruce (Picea abies) is the most commonly used rootstock for blue spruce grafting (Holst et al, 1956), but white spruce (Picea glauca) and blue spruce (Picea pungens) are also used (Hanover, 1975). With these species there is no incompatibility, a problem in some conifer grafts (Hanover, 1975).

Ornamental conifers are generally grafted in the greenhouse during the winter. In the Pacific Northwest, rootstock growth is usually induced prior to grafting. Grafting commences with the first elongation of roots or at bud swell of the rootstock for Norway and white spruce (Holst, 1956a) and Douglas-fir (Orr-Ewing & Prideaux, 1959). Later, vigorous rootstock growth interferes with union formation requiring that the rootstock activity be slowed by imposing a mild water stress (Dorsman, 1966). While actively growing rootstocks were preferred for Norway and white spruce (Holst, 1956a; Holst et al,

1956) and Douglas-fir (Orr-Ewing & Prideaux, 1959), Nienstaedt (1966) concluded that the rootstocks physiological state of spruce at grafting did not affect success. Spruce can also be successfully grafted in the greenhouse from August through November (Holst, 1956b). September and early October grafts are more successful if kept in the greenhouse six weeks and then moved to a cold frame. Success was improved if chilled scions were grafted onto unchilled rootstocks (Holst, 1956b).

Water loss from conifer scions is an important cause of graft failure (Grigsby, 1957; Nienstaedt, 1959; Smith et al, 1972); especially in field grafting. Several techniques have been developed to reduce scion water loss. Webb (1961) used opened polyethylene bags, covered by aluminum foil or a paper bag, on field grafted loblolly pines. Both treatments produced approximately 90% success but scions covered with the aluminum foil had significantly more post-union scion growth. Covering the polyethylene bag/paper bag combination with 50% shade cloth had no additional effect on success of loblolly or slash pine (Smith et al, 1972). Success was nearly 100%, even for difficult to graft pine species when grafts were placed under intermittent mist (15 seconds/10 minutes) in addition to polyethylene/paper bag enclosure of the scion (Smith et al, 1972).

The shoot of the rootstock of conifer grafts is generally removed in two stages. For actively growing rootstocks of Scots pine (Holst, 1956a) or fall-grafted spruce (Holst et al, 1956), the lateral branches of the rootstocks were cut back at grafting to reduce stem diameter growth and callus development which prevented the "walling-off" of weak scions (Orr-Ewing & Prideaux, 1959). However, rootstock shoots of Norway and white spruce were not pruned until six or seven weeks after

grafting (Nienstaedt, 1966). Generally the final portion of the rootstock shoot is removed after one growing season (Holst, 1956a; Holst et al, 1956; Orr-Ewing & Prideaux, 1959; Nienstaedt, 1966). But dormant shoots of Norway spruce, grafted with Picea pungens 'Kosteriana' scions in Janurary, were reduced to half 6 weeks after grafting, and removed completely when both the rootstock and scion showed signs of active growth (Willard, 1968).

Graft Development

The few chronological studies of graft development in conifers indicate that while there are some developmental variations among species, the time required for each stage is generally the major difference. Anatomical studies of graft unions of Pinus contorta 'latifolia', Pinus peuce and Pinus sibirica scions on Pinus sylvestris rootstock (Nenjuhin, 1966), Juniperus sp. (Evans and Rasmussen, 1972), Douglas-fir (Copes, 1969), Pinus sylvestris and Picea abies (Dormling, 1964) have been reported and will be described here.

Within two days after grafting, a contact layer is formed between the scion and the rootstock (Nienstaedt et al, 1958; Copes, 1969; Dormling, 1964). The contact layer consists of dead cells, cell fragments, dried cytoplasm and often resin (Nenjuhin, 1966; Dormling, 1964; Copes, 1969). By the fourth day, enlargement of epithelial cells plugged the resin canals in Douglas-fir (Copes, 1969), Norway spruce and Scots pine (Dormling, 1964).

Ten days after grafting, several changes had occurred at the graft

union. In Douglas-fir, suberization of the contact layer was nearly complete (Copes, 1969). Extensive areas of callus formation were observed. The callus tissue arose from cortical and phloem parenchyma and ray cells (Evans & Rasmussen, 1972; Copes, 1969; Dormling, 1964). Callus also arose around the cambium (Nejuhin, 1966; Dormling, 1964) and xylem ray cells (Dormling, 1964) in Pinus. Dormling (1964) reported that the xylem ray cells in spruce were too lignified for de-differentiation and, therefore, callus development. Until the tenth day, the contact layer remained intact (Copes, 1969; Dormling, 1964). Callus formation, several cells deep, occurred on both sides of the contact layer (Copes, 1969; Dormling, 1964). In Douglas-fir the majority of the callus formed during the first ten days was produced by the scion (Copes, 1969). After ten days, callus bridges spanning the contact layer were observed between the scion and rootstock (Copes, 1969; Nenjuhin, 1966; Dormling, 1964); thereafter the majority of the callus was produced by the rootstock (Kostoff, 1928; Copes, 1969). The formation of the callus bridges was hypothesized to result in the translocation of plant growth regulators from the scion to the rootstock producing the increase in callus formation by the rootstock (Copes, 1969). Development of these parenchymal unions after ten to fifteen days was deemed essential by Dormling (1964) if the grafts were to be successful.

Twenty days after grafting, callus tissue filled most of the union zone of Douglas-fir (Copes, 1969) and all of the zone in Scots pine (Dormling, 1964). Callus cells exposed to the environment were suberized and lignification of some cells across the entire union was observed (Copes, 1969). Dormling (1964) found phellogen unions between

fifteen to twenty days after grafting. Some contact layer fragments were encircled by continuous sheaths of cells in the Douglas-fir and some cambial formation could be seen (Copes, 1969).

Callus development of Juniperus was slower. After twenty days callus had only partially filled the union zone but new radial xylem production from uninjured cambium was observed in both the scion and rootstock (Evans & Rasmussen, 1972).

Thirty days after grafting, callus tissue development was complete in the union zone in Douglas-fir (Copes, 1969) and Juniperus (Evans & Ramussen, 1972). Cambial initials originating mainly from secondary phloem and cortex, joined the scion and rootstock through the callus bridges (Copes, 1969; Dormling, 1964). In well-fitted grafts, only a small number of irregular cells formed prior to normal tracheid production initated at this time (Dormling, 1964; Copes, 1969). Phloem connections consisted initially of parenchyma cells; sieve cell connections occurred more slowly (Dormling, 1964; Copes, 1969). In Douglas-fir unions, the cambial and phloem cells were initially larger than normal; normal sized cells being formed after 80 days. By 80 days, the amount of vertically disoriented tissue was reduced or eliminated (Copes, 1969). Grafts made with good cambial alignment had few disoriented vertical tissues; while poor graft alignment resulted in three or more months delay in cambial union (Copes, 1969).

After thirty-five days, callus bridges formed in previously unbridged areas and developed cambia in Douglas-fir (Copes, 1969). Further union development consisted mainly of expanding existing tissue systems including the differentiation of normal phloem and xylem tissues (Copes, 1969).

In Juniperus sp., both the scion and rootstock continued to form xylem tissue separated by the callus until sixty days after grafting (Evans & Ramussen, 1972). During this time increasing xylem production of the scion and rootstock compressed and killed existing callus cells. At sixty days, the new xylem tissues formed bridges spanning the remaining callus layer. Cambial initials formed within the bridges and began producing normal tracheids (Evans & Ramussen, 1972).

Water Relations

Water stress in conifer scions is one of the major factors governing the success of grafting (Grigsby, 1957; Nienstaedt, 1959; Smith et al, 1972). Nonetheless, quantitative studies of water stress in conifer scions have not been published. Thus, inferences of scion water relations and their effect on scion physiology are based on studies of stress in seedlings and detached branches.

Water potential is expressed as the difference between chemical potential of tissue water and free water, divided by the partial molar volume of water. This energy per unit volume is termed total water potential (Ψ_T) and has units of bars or megapascals (MPa) (Nobel, 1983). The two main components of plant water studies are solute or osmotic potential (Ψ_π) and turgor pressure (P) (reviewed by Nobel, 1983; Slatyer, 1964). Osmotic potential is determined by the concentration of solutes in the system, while turgor pressure is the hydrostatic pressure. These are related as: $\Psi_T = \Psi_\pi + P$. Bulk water movement in plants is driven by a gradient of negative hydrostatic

pressure (Sinclair & Ludlow, 1985) established by transpiration from the stomata and extending through the xylem to the roots and into the soil water (reviewed by Fiscus et al, 1983; Nobel, 1983; Boyer, 1985). With increasing water stress, Ψ_T declines and water becomes less available for cell function resulting in numerous metabolic and physiological changes in the plant (reviewed by Hsiao, 1973; Hsiao et al, 1976; Aspinall, 1980; Kozlowski, 1982). However, the use of Ψ_T to relate water status to stress has been questioned (Sinclair & Ludlow, 1985; Hsiao, 1973).

Plants mitigate water stress by two major mechanisms - osmoregulation and stomatal aperture regulation. Osmoregulation is a limited reduction of Ψ_π (reviewed by Turner & Jones, 1980; Morgan, 1984; Hsiao et al, 1976) which extends the range of Ψ_T at which stomata remain open and is essential for the protection of the photosynthetic system during water stress (Downton, 1983). With water stress, stomata close, and cellular metabolism changes such that the osmotic concentration increases (Tyree & Jarvis, 1982). The osmoticum is generally considered to include free sugars, organic acids and potassium ions (K^+) (Tyree & Jarvis, 1982). Other factors alter Ψ_π in addition to accumulation of osmoticum. Starch accumulation associated with water stress decreases the osmotic volume of cells thereby increasing the existing osmotic concentrations (Ackerson, 1981). Tissue elasticity can also modify cellular volume in response to water stress (Davies & Lakso, 1979). Demonstration of osmotic adjustment in grapes (Downton, 1983), apples (Davies & Lakso, 1979) and western hemlock seedlings (Kandike et al, 1980) have been reported. However, Hinckley et al (1980) found no diurnal osmotic adjustment and only low

levels of seasonal adjustment in several European drought-tolerant shrubs .

The primary regulation of plant internal water is through the control of stomatal aperture. Cuticular transpiration generally accounts for less than 10% of the total transpiration of most conifer needles (Hinckley et al, 1978; Beadle et al, 1979). Conifer scions are essentially water-stressed twigs for 4 to 6 weeks after grafting until the development of functional xylem (Dormling, 1964; Copes, 1969). The only access to water of the scions during this time is by diffusion across callus bridges, thus stomatal control of transpiration should be critical to graft success. Various factors and interactions that would influence scion stomatal apertures will be considered here.

Stomatal aperture is controlled primarily by regulation of the osmotic concentration, primarily K^+, of the guard cells (reviewed by Outlaw, 1983; Robsinson & Preiss, 1985). The regulation of K^+ transport depends on the complex effects of several factors: light, Ψ_T, leaf temperature, abscisic acid (ABA) concentration, internal CO_2 concentration and vapor pressure deficits (vpd) (Hinckley et al, 1978). However it appears that some factors, such as vpd may cause stomatal closure independent of the K^+ efflux, but this is currently under debate (Zeiger, personal communigue'). Reviews of the effects of these factors and their interactions on stomatal aperture can be found (Cowan, 1977; Jarvis, 1980; Ludlow, 1980; Sheriff, 1979). Due to interaction of these components, stomatal closure very often occurs at mid-day in conifers (Gates, 1966; Nobel, 1983).

The stomata of many plants respond to a threshold Ψ_T by closing rapidly. Depending on the growing conditions, the threshold level for

stomatal closure of Sitka spruce has been measured between -1.05 to -2.6 MPa (Beadle et al, 1979; Coutts, 1980; Coutts, 1981; Watts & Neilson, 1978), -1.6 MPa for Engelmann spruce (Lopushinsky, 1969) and -2.06 to -2.3 MPa for Douglas-fir (Drew & Ferrell, 1979). However Lopushinsky (1969) did not find a threshold value for Douglas-fir or grand fir.

Transpiration rates change seasonally, declining in late fall and early winter. Kozlowski (1943) attributed this to a decline in soil temperatures, however, other factors may play a role as well. The transpiration rates of dormant, cold-stored white spruce were 10 to 33% those of the freshly lifted trees upon outplanting. The cold-stored trees had low Ψ_T and a high degree of dormancy, conditions associated with higher ABA concentrations (Blake, 1983). Similar effects of dormancy on transpiration were measured in spruce (Christersson, 1972), Abies amabilis (Teskey et al, 1984), Pinus sylvestris (Smit-Spinks et al, 1984) and rhododendron (Parker, 1963). Exdogenous application of ABA further decreased transpiration in dormant plants (Little & Edit, 1968).

Light, internal CO_2 concentration and temperature affect stomatal aperture independent of the other components. Maximum stomatal conductance (k_s) of forest trees occurs when radiation levels exceed 10% of full sun, approximately 200 uE/m^2-sec (Hinckley et al, 1978). Two independent photosystems in guard cells respond to light quality. Additional blue light increases stomatal aperture above the response to photosynthetically active light (Zeiger & Field, 1982; Zeiger et al, 1985). Conifer stomata are sensitive to changes in air CO_2 levels (Levering & Jarvis, 1979), but in Sitka spruce Ψ_T overrode any effects

of changes in CO_2 concentration (Beadle et al, 1979). Generally, increases in leaf temperature increase k_s (Beardsell et al, 1972). However during the winter and early spring, increases above 5^o C sharply decrease the k_s of high elevation Sitka spruce (Beardsell et al, 1972). Thus temperature affects appear seasonal.

In plants under long and severe drought, stomata opened with increased water deficit and closed when the deficit declined (Slatyer, 1964). Slatyer (1964) thought the opening could be due to low internal CO_2 concentration. This response occurred in cut Thuja occidentalis saplings. Stomata closed from -2.0 to -2.5 MPa then k_s increased as Ψ_T declined from -2.5 to -3.0 MPa to close permanently below -3.0 MPa (Dixon et al, 1984). Kaufman (1976) also recorded abnormally large stomatal apertures under high water stress in Engelmann spruce and correlated this with low light levels and a difference in absolute humidity between the leaf and the air of 2.7 ug/cm^2. A decline in leaf resistance in Douglas-fir seedlings at low Ψ_T was associated with decreasing ABA concentrations (Blake & Ferrell, 1977).

Abscisic acid has a role in the regulation of stomatal aperture in response to water stress. Most research has focused on the production of ABA in leaves and needles; but Blake and Ferrell (1977), working with Douglas-fir, proposed that the root system might be the drought-sensing organ and produce ABA for translocation.

Abscisic acid content in tissues increases with short days in larch (Wozdzicski, 1964) and with a decline in Ψ_T below threshold levels for most species (Cornish & Zeevaart, 1985; Johnson & Ferrell, 1982; Blake & Ferrell, 1977). The threshold level was related more to turgor pressure than in herbaceous species; more than 80% of the total

ABA accumulated as turgor declined between 0.1 and 0 MPa (Pierce & Raschke, 1980). Stomatal closure normally occurs within 15 minutes after wilting, but whole leaf ABA does not significantly increase until 15 minutes after stomatal closure (Dorffling & Tietz, 1985; Cornish & Zeevaart, 1985). Applying ABA to Commelina communis epidermis peels Dorffling and Tietz (1985) concluded that ABA levels were not correlated with rapid stomatal closure. However, Cornish and Zeevaart (1985) observed that ABA was intially eluted into the apoplast of the leaf upon wilting. They hypothesized that this apoplastic ABA was carried to the guard cells by the transpiration stream resulting in stomatal closure.

Levels of ABA associated with stomatal closure differ with species and previous conditioning of the plant (Kriedemann et al, 1972). Relief from water stress results in declining ABA levels correlated with increased stomatal conductance (Dorffling et al, 1971; Dorffling et al, 1977). In most plants, stomatal opening was delayed beyond the time required to regain full turgor (Hiron & Wright, 1983; Dorffling et al, 1977). This "after-effect" is dependent on the length of the stress period and is correlated with ABA content. Not all stress-related increases in ABA are by de novo synthesis. Three year-old Douglas-fir contained high levels of an ABA conjugate prior to stress treatment. With stress, increases in free ABA paralleled identical declines in the ABA conjugate. Conversion of the conjugated ABA to free ABA, rather than new synthesis, was postulated (Johnson & Ferrell, 1982).

Applied ABA is transferred throughout the plant, the amount translocated dependent on the application site (Bellandi & Dorffling,

1974). Labelled ABA was translocated more readily when applied to photosynthetically active leaves than to shoot tips (Bellandi & Dorffling, 1974). Stomata of spruce cuttings placed in an ABA solution closed as the ABA was transported through the xylem (Little & Eidt, 1968).

The effect of ABA on stomatal aperture is not absolute. Light partially counteracts the effects of ABA (Kubik and Plunka, 1984). Cytokinins appear to reduced stomatal sensitivity to ABA (Blackman & Davis, 1983). Soil water stress was hypothesized to decrease cytokinin tranlocation to leaves thus increasing the sensitivity of stomata to ABA (Blackman & Davis, 1983). Auxins (IAA) prevent the response of the stomata of Commenina communis to changes in CO_2 content. ABA counteracts the IAA, restoring the sensivity to CO_2 concentration (Snaith & Mansfield, 1982.)

The relationship between stomatal aperture and vpd or evaporative demand has been the focus of many studies. Vapor pressure deficits result from the difference in the absolute humidities between the leaf and surrounding air (Nobel, 1983). The current hypothesis for stomatal response to vpd is the "feed-forward" mechanism. In the feed-forward scheme, changes in the environment result in changes in k_s which then affects the transpiration rate (Farquhar, 1978). In the alternative, the "feed-back" mechanism, a change in the transpiration rate due to some environment factor results in a change in the k_s which then brings about a corresponding change in the transpiration rate (Farquhar, 1978). The key point in the feed-foward hypothesis is guard cell sensitivity to changes in the vpd due to evaporation from guard cells termed "peristomatal transpiration". Farquhar (1978), using

mathematical models, concluded only the feed-forward mechanism explained the decreasing transpiration observed by others with increasing vpd (Neilson & Jarvis, 1975; Kaufman, 1976; Davis & Kozlowski, 1974; Lange et al, 1971). Stomata close rapidly (less than five minutes) when confronted with sudden, large vpds, but require five-fold longer to reopen when the vpd is reversed (Lange et al, 1971). The hysteresis of the stomata is evidence of peristomatal transpiration (Lange et al, 1971). Maier-Maecker (1979) demonstrated a similar response, with stomatal aperture decreasing somewhat linearly with increasing vpd. Meinzer (1982) observed that new needles of Douglas-fir responded readily to changes in the vpd while one year-old needles did not. Wax removal increased the response to vpd, allowing peristomatal transpiration (Meinzer, 1982).

The vpd response is also modified by other factors. At low light levels, stomata of Engelmann spruce were responsive to changing vpd as long as Ψ_T was greater than -1.5 MPa (Kaufman, 1979). At low vpd and high Ψ_T, there was little response to changes in light levels (Kaufmann, 1976). Douglas-fir also responded to changes in vpd only at high Ψ_T (Johnson & Ferrell, 1983). There is some evidence of increased ABA concentrations with high vpd, but not until after 24 to 48 hours (Henson, 1984); too slow to affect the feed-forward mechanism. However high ABA concentrations appear to contribute to the continued low k_s even though bulk leaf Ψ_T increases (Henson, 1984). Under non-stress conditions, stomata respond to vpd with low k_s at high vpd and high k_s at low vpd. But this still results in high transpiration rates at high vpd (Whitehead et al, 1983). Stomatal response to water stress in conifers is reviewed by Jarvis (1980).

Indications exist of other factors, not previously cited, that may influence k_s of wounded trees. Branches of Abies amabilis with less then 50% of their xylem severed, had reduced k_s about 1 hour later; whereas severing the branch did not produce the effect (Teskey et al, 1983). The decrease in k_s of partially severed branches was independent of both Ψ_T and vpd. The speed and uniformity of the response suggested that the stimulus was linked to the xylem flow. A similar effect of wounding was observed with seedlings of Sitka spruce (Coutts, 1980). Decreases in transpiration were proportional to the severity of root damage even though Ψ_T was higher in the damaged seedlings.

Carbon Relations

Stored reserves are essential for conifer growth in the spring. Although rapid carbohydrate accumulation in needles occurs in early spring due to current photosynthates, winter grafting of ornamental conifers occurs prior to this when the needle carbohydrate storage is at a minimum. Therefore, during union development conservation of carbon reserves and scion water is balanced against photosynthesis and photosynthate accumulation. As in the case of water relations, inferences of scion carbon metabolism are based on studies of seedlings and detached branches.

Photosynthetic rates of conifers are much lower than decidious angiosperms (Kozlowski, 1966). Photosynthetic rates for Sitka spruce range from 6.9 mg CO_2/g dwt/hr in southwestern England (Fry & Phillip,

1977) to 17 mg CO_2/g dwt/hr in the Pacific Northwest (Krueger & Ruth, 1968), while dark respiration rates ranged from 0.6 mg CO_2/g dwt/hr (Fry & Phillip, 1977) to 1.78 mg CO_2/g dwt/hr (Krueger & Ruth, 1968). Rates estimated for Picea pungens, were 8.8 mg CO_2/g dwt/hr and 5 ug CO_2/g dwt/min for photosynthesis and respiration, respectfully (Verduin, 1959). Factors such as light intensity, temperature, water stress, previous growing conditions, canopy position, and season influence photosynthetic rates (Levering & Jarvis, 1979; Little & Loach, 1973). Photosynthesis occurs whenever air temperature is above -3° C if the root zone is not completely frozen (Kozlowski, 1966). Light intensity requirements of conifers are low compared with angiosperms (Krueger & Ruth, 1968), the light saturation point occurring at, or below 2800 fc (Freeland, 1944; Krueger & Ruth, 1969).

Water stress affects the photosynthetic rate by inducing stomatal closure and by lowering mesophyll conductance (k_m) to CO_2. As Ψ_T declined, k_m declined and was always lower than k_s for lodgepole pine (Dykstra, 1974) and Sitka spruce (Beadle and Jarvis, 1977).

Photosynthetic rates respond to declining Ψ_T in two phases. Net assimilation rate changes little between 0 MPa to some threshold Ψ_T value (Melzack et al, 1985); neither k_s nor k_m are affected (Beadle et al, 1981). Below the threshold value, assimilation rates decline rapidly to zero (Melzack et al, 1985). The threshold value is not fixed, but depends on environmental conditions (Melzack et al, 1985).

Representative Ψ_T values for threshold and zero assimilation levels have been reported for several conifer species. For Pinus sp.,

threshold Ψ_T range from -0.4 MPa for loblolly pine (Brix, 1962) to -0.8 MPa for Pinus halepensis (Melzack et al, 1985); with zero assimilation below -1.1 MPa (Brix, 1962) to -1.5 MPa for loblolly pine (Kaufman, 1968) and -1.5 to -1.6 MPa for lodgepole pine (Dykstra, 1974) and Pinus halepensis (Melzack et al, 1985). For four Abies sp., photosynthetic rates were constant to -0.9 to -1.1 MPa. With water stress, photosynthesis declined linearly with the decline in Ψ_T to -1.2 MPa for Abies balsamea to -3.4 MPa for Abies grandis, at which rates were 10 to 20% of watered controls. Thereafter, the decline in photosynthetic rates slowed to reach zero at -2.2 MPa for both Abies balsamea and Abies lasiocarpa to -4.3 MPa for Abies grandis (Puritch, 1973). The threshold value for Sitka spruce has been reported as -1.5 MPa with zero assimilation occurring between -2.2 and -2.6 MPa (Beadle & Jarvis, 1977; Watts & Neilson, 1978; Beadle et al, 1981). The decline in photosynthesis was not linear with the decline in k_s; a 95% decline in k_s reduced photosynthesis by 72% (Watts & Neilson, 1978).

Respiration rates generally decline parallel to photosynthetic rates to a point, then remain constant with increasing water stress. For the four Abies sp., respiration rates declined of 45 to 75 % of the watered control and became constant below -1.6 to -2.0 MPa (Puritch, 1973). Respiration rates of Pinus halepensis stabilized at 50% of the control with a decline in Ψ_T below -1.6 MPa (Melzack et al, 1985). However the respiration rate of loblolly pine, after declining between -0.8 to -1.6 MPa, increased to peak at -2.8 MPa; 40% above the rate of the control, before a sharp decline with additional stress (Brix, 1966).

Translocation of photosynthates in the scion, but mainly in the

rootstock, affects graft success. After ten days, most callus produced and differentiated in the developing union occurs by the rootstock (Copes, 1969; Kostoff, 1928). Factors that affect translocation would, therefore, effect the speed of union formation and graft success.

The current theory of photosynthate translocation is based on a source-sink relationship (reviewed by Nelson, 1963; Wardlaw, 1968; Noble, 1983). Osmoticum, loaded at the source and then unloaded at the metabolically active sink produces positive hydrostatic pressure gradients in the phloem. Sucrose is the major translocated carbohydrate (Shiroya et al, 1962a; Shiroya et al, 1962b; Kozlowski, 1966; Wardlaw, 1968; Ericsson, 1979; Mooney, 1972; Thorne & Koller, 1974) and generally considered to be the osmoticum (Nobel, 1983). However, Long (1983) suggest potassium ions are the major osmoticum. This hypothesis is based on observations of reduced sugar accumulation and translocation in leaves with mild or temporary K^+ deficiency, and better correlation of K^+ rather than sucrose concentration with translocation. Ureugdenhil (1983) contributed indirect support when he established that applied ABA inhibits translocation of sucrose. The effects of ABA on K^+ transport are well documented (Outlaw, 1983).

Factors that influence photosynthesis also affect translocation. Low light levels decrease translocation from leaves independent of photosynthate concentration (Wardlaw, 1968). Limited light results in larger accumulation and utilization of photosynthates by the shoot (Mooney, 1972), whereas low temperatures decrease shoot growth resulting in more translocation to roots and buds (Wardlaw, 1968). Sink strength affects both the rate of photosynthesis and translocation (Thorne & Koller, 1974). If water and/or minerals are limiting, more

photosynthate is translocated to the roots (Mooney, 1972).

The allocation priority of photosynthates among the various meristematic sinks in woody perennials plants was established based on growth correlations (Loomis, 1953) and ^{14}C distribution analysis (Gordon & Larson, 1968; Ursino et al, 1968; Larson & Gordon, 1969; Balakinecz et al, 1966; Shiroya et al, 1962b; Zieme, 1971). The primary sink is the developing shoot apex, followed by the main root apex, then the secondary shoot and root apexes and finally the cambium.

In all perennial plants, photosynthate is stored in the fall for spring growth (Mooney, 1972). Tree growth is better correlated with photosynthesis the previous year than that of the current growing season (Kozlowski, 1963; Larson, 1964; Kozlowski, 1966; Kozlowski & Keller, 1966, Clausen & Kozlowski, 1967; Olofiboba & Kozlowski, 1973; Kimura, 1969). Using defoliation and girdling, Olafiboba and Kozlowski (1973) estimated that stored reserves accounted for 50% of the spring growth of Pinus resinosa. Earlier, Kozlowski and Winget (1964) estimated that root, stem and trunk reserves accounted for only 12.5% of the spring growth Pinus resinosa. In Pinus palustris, stored carbon in stems accounted for 31%, root storage 15 to 24% and photosynthates from previous needles were estimated to account for 40 to 54% of the spring shoot growth (Allen, 1964).

The accumulation of storage compounds begins in the fall as accumulated starch in the needles; which later declines to near zero until early spring (Fry & Phillips, 1977; Kandler & Hopf, 1980; Krueger & Trappe, 1967; Pomeroy & Siminovitch, 1969; Sinnott, 1918; Winjum, 1963). The fall decline in needle starch occurs in conjunction with

increasing sugar content in the needles and stems (Kimura, 1969; Winjum, 1963; Fry & Phillip, 1977; Ronco, 1972; Krueger & Trappe, 1967; Holl, 1985; Pomeroy & Siminovitch, 1969), but most of the stored reserves accumulate in the roots (Glerum & Balatinecz, 1980; Olofiboba & Kozlowski, 1973; Ursino et al, 1968; Ritchie, 1982; Parker, 1959; Shiroya et al, 1966) and twigs (Olofiboba & Kozlowski, 1973; Parker, 1959) and occassionally needles (Schier, 1970) as ethanol insoluble compounds (i.e. non-sugars). Maximum sugar levels occur during the coldest weather (Krueger & Trappe, 1967; Little, 1970; Parker, 1959; Ericsson, 1979; Ritchie, 1982).

In spring, needle carbohydrates, derived from current photosynthates mainly as starch, increase rapidly prior to bud break (Little & Loach, 1973; Ritchie, 1982; Fry & Phillip, 1977; Ronco, 1977; Little, 1970d; Ericsson, 1978; Webb & Kerchesy, 1977; Little, 1970c; Kureger & Trappe, 1967; Kimura, 1969; Pomeroy & Siminovitch, 1969; Ericsson, 1979). Increased carbohydrate content also occurs in the sapwood (Holl, 1985; Little, 1970c). In all conifers, root growth, utilizing stored root reserves, precedes shoot bud break. Concurrent starch accumulation in the needles is due to slow translocation and high photosynthetic rates (Ericsson, 1979; Webb & Kerchesy, 1977; Little, 1970c). As shoot elongation initiates, root activity declines and cambial activity is induced. During shoot elongation, there is a rapid decline in the starch levels of both older needles (Little & Loach, 1973; Ritchie, 1982; Fry & Phillip, 1977; Ronco, 1977; Little, 1970d; Ericsson, 1978; Webb & Kerchesy, 1977; Little, 1970c; Kureger & Trappe, 1967; Kimura, 1969; Pomeroy & Siminovitch, 1969; Ericsson, 1979; Meyer & Spittstoesser, 1971) and roots as the carbohydrates are

translocated into the developing shoots. When shoot growth slows, more carbon compounds are diverted to the cambium and the roots (Loach & Little, 1970b; Krueger & Trappe, 1967). During shoot elongation, net carbon assimilation of the developing shoots is negative because respiration exceeds the photosynthesis of the existing needles (Loach & Little, 1970b; Nevwirth, 1959; Fry & Phillip, 1977). Maturation of the current season's needles and a net export of photosynthates from the developing needles reduces the photosynthetic rates of the previous years' needles (Loach & Little, 1970b; Gordon & Larson, 1968; Ursino et al, 1968).

Maximum spring starch levels have been reported for some species. For Abies sp., branch starch levels were 5 to 10% of dry weight (Kimura, 1969) while needle levels reported, range from 6 to 13% (Kimura, 1969; Little & Loach, 1973; Little, 1970d). Starch content of Douglas-fir and noble fir needles reach 15 to 20% of the needle dry weight prior to budbreak (Winjun, 1963). Similar contents of 14% to 17% of the needle dry weight were found for red pine (Pomeroy & Siminovitch, 1969) and 25% for Scots pine (Ericsson, 1979). Though the importance of spring starch accumulation appears evident, Little (1974) reported that current shoot growth was not related to pre-bud break starch levels for balsam fir or Norway spruce.

The major carbohydrates in spruce scions at grafting are sugars. Four major sugars, glucose, fructose, sucrose and raffinose, account for 60% of the needle sugars in the summer and 85% in the winter in balsam fir (Little, 1970d). In addition, pungenin is also a major winter sugar in blue spruce (Neish, 1958). Of the four major sugars, only raffinose concentration changes are consistent among species.

Raffinose occurs only during the winter months and rapidly disappears at bud break (Neish, 1958; Holl, 1985; Parker, 1959; Rast et al, 1969; Kandler & Hopf, 1980; Krueger & Trappe, 1967; Pomeroy & Siminovitch, 1969; Aronsson et al, 1976; Little, 1970d). Two compatable hypotheses for its occurrence have been put forth. Neish (1958) hypothesized that both raffinose and pungenin functioned mainly as readily utilized storage carbohydrates. Each constituted 3% of the needle dry weight while the other major sugars combined comprised 3.8% of the needle dry weight (Neish, 1958). In Picea mariana raffinose constituted 2% of the needle fresh weight (Rast et al, 1963). Raffinose is also associated with cold hardiness of conifers. Raffinose and pungenin contribute equally with sucrose and the hexoses to the Ψ_{π} of blue spruce (Neish, 1958). The induction of raffinose by short days and cool temperatures was associated with increased cold hardiness (Kandler & Hopf, 1980), while the rate of dehardening of Norway spruce and Scots pine has been correlated with the decline of raffinose (Aronsson et al, 1976). Differences in raffinose and stachyose concentrations were correlated with differences in cold hardiness between longleaf and white pines (Parker, 1959).

Seasonal change of sucrose content depends on the species. Sucrose concentrations are relatively constant in blue spruce (Neish, 1958). But sucrose levels of Norway spruce (Holl, 1985; Aronsson et al, 1976), Scots pine (Ericsson, 1979, Aronsson et al, 1976), red pine (Pomeroy & Siminovitch, 1969) and Douglas-fir (Winjum, 1963; Krueger & Trappe, 1967; Ritchie, 1982) peak in mid-winter, then decline during shoot elongation to a yearly low shortly thereafter to increase again in the fall. Similar seasonal patterns were observed for glucose and

fructose in Scot pine (Ericsson, 1979) and red pine (Pomeroy & Siminovitch, 1969), but levels in blue spruce (Neish, 1959) were relatively constant. In Douglas-fir the concentrations of glucose and fructose were relative constant (Krueger & Trappe, 1967) or too variable to discern seasonal fluctuations (Winjum, 1963).

Carbohydrates are assumed to be the major winter storage compounds. However, lipids are the major storage compounds in trunk and branch tissues in some species. Trees can be divided into two general groups based on the form of the carbon storage compound; starch trees, trees that store starch, and fat trees, trees that store neutral lipids (Sinnott, 1918; Ziegler, 1964). Generally, angiosperms are starch trees while gymnosperms are fat trees. Based on histochemical analysis, Sinnott (1918) concluded that the thickness of ray parenchyma cells walls governed the type of storage compound. Thick wall cell store starch while thin wall cells store lipids. However, in the roots of all trees species, the storage compound is still starch (Ziegler, 1964).

Lipid compounds in fat trees increase in autumn, peak during the winter months and then decline in late spring (Zieger, 1964; Sinnott, 1918; Ronco, 1972; Kimura, 1969; Holl, 1985). Lipid production in Scots pine increases as temperature declines (Ziegler, 1964). The seasonal change is most pronounced in the twigs and young branches, with the lipid compounds most abundant in the phloem (Sinnott, 1918). In pines, lipid content in the winter was 2.58% in the sapwood and 10.9% in the bark (Ziegler, 1964).

Few quantative reports of seasonal changes in neutral lipid content of trees exist. Ronco (1972) reported that ether-soluble

lipids declined from 4% dry weight to 2.8% dry weight, based on whole seedling extraction between December and April. Lipid content in branches of young Abies veitchii trees peak at 17% dry weight prior to bud break then declined to 7% dry weight in the summer (Kimura, 1969). When red pine seedlings were labelled in October, 71% of the labelled assimulate fixed in the non-cell wall fraction was found in an ether extract and water soluble non-sugar fraction. In the ether fraction, 65 to 95% were metabolically active compounds (Schier, 1970).

Plant Growth Substances

The differentiation of callus to form the graft union appears to be controlled by the interaction of the various endogenous plant growth substances (PGS) and sucrose. Studies of vascular differentiation of both the cambia and callus cultures have revolved around the application of PGS to replace developing buds. The results of these studies are reviewed as they relate to vascular differentiation and wound healing.

Cambial growth in the spring is initiated by the basipetal translocation of indoleacetic acid (IAA), produced by developing buds (Wareing, 1951). In tissue culture systems, naphthaleneacetic acid (NAA) was equally effective as IAA in initiating cambial activity in excised radish roots (Torrey & Loomis, 1967). Neither gibberellins (GA) or cytokinins applied to stem segments of Pinus sylvestris (Zajaczkowski, 1973) or willow (Robards et al, 1969) stimulated cambial activity. In decapitated plants, applications of cytokinins did not

stimulate xylem formation in Pinus sylvestris (Alleznowicz & Tomaszewski, 1969) or oak (Zakrzewski, 1983). Neither did similar applications of gibberellins to Pinus sylvestris (Alleznowicz & Tomaszewski), Populus robusta (Digby & Wareing, 1966a) or dwarf pea (Kisarishi & Muir, 1964). However applications of GA to the dormant cambium of white pine in vitro did produce differentiation and maturation of sieve tube cells (Demaggio, 1961). Gibberellins also stimulated phloem production in decapitated Populus robusta (Digby & Wareing, 1966a) and xylem formation in bean (Harrison & Klein, 1979).

Sucrose appears to be instrumental in the regeneration of cambial tissue in wound callus. Sucrose, in conjunction with auxin, enhances the differentiation of vascular tissue (Overbeek, 1966; Robards et al, 1969; Terry et al, 1971). Wentmore and Rier (1963) influenced differentiation of angiosperm parenchyma callus by varying sucrose concentration. Sucrose concentrations of 2.5% to 3.5% favored xylem, cambial, and phloem differentiation, lower concentrations produced xylem while higher concentrations produced phloem. Identical responses occurred with Phaselous vulgaris and camellia callus cultures (Jeffs and Northcote, 1966), and by grafting a developing bud onto the callus (Robards et al, 1969). Optimal cambial activity in Pinus sylvestris stem segments occurred with the addition of two percent sucrose in the medium (Zajaczkowski, 1973). Only disaccharides in conjunction with IAA stimulate the complement of vascular tissue in bean callus. Non-reducing sugars other than glucose have no effect on tissue differentiation, while two percent cellobiose, lactose and raffinose with IAA result in some xylem formation (Jeffs & Northcote, 1967).

The gradient induction hypothesis attempts to explain the

auxin/sucrose interaction on the induction of cambia (Wilson, 1978). It proposes that in severed vascular bundles, auxin and sucrose are transported at high rates and diffuse into the ground tissue, resulting in the formation of wound callus. With the diffusion of these two compounds, a lateral concentration gradient develops. At some point along this gradient cambium is induced. For bean callus, the ratio was approximatly 25 ug/liter IAA to 0.75% sucrose (Jeffs & Northcote, 1967).

Interactions of ABA with auxin appear to regulate cambial activity in vivo. Like IAA, ABA is synthesized in the needles and translocated into the trunk (Little & Wareing, 1981; Wodzicki & Wodzicki, 1980). In Picea sitchensis, IAA and ABA were present in three forms in the cambium throughout the year: an acidic form, a diffusible form and a conjugated form (Little & Wareing, 1981). IAA concentration remained relatively stable throughout the year, but free ABA concentrations declined during late winter, reaching a minimum concentration during initation of spring growth (Little and Wareing, 1981). In contrast, in woody angiosperms Digby and Wareing (1966b) observed higher concentrations of IAA in stems at bud-break than while dormant. These high auxin concentrations were associated with cambial differentiation and maximum activity. IAA concentrations also varied considerably during the year in the stems Pinus sylvestris (Zajackowski, 1973). The induction of cambial dormancy in Picea sitchensis did not result from declining IAA concentrations; rather, it was concluded that changes in the response of the cambium IAA governed cambial activity (Little & Wareing, 1981). Decreased cambial activity due to high ABA was also shown for balsam fir (Little & Eidt, 1968; Little & Edit, 1970) and

white spruce (Little and Edit, 1970). Seasonal changes in ABA concentrations were also observed and directly correlated with late wood production in Pinus sylvestris (Wodzicki & Wodzicki, 1980). ABA is thought to inhibit the effect of IAA resulting in delayed maturation of late tracheids.

Water stress inhibits cambial growth by decreasing turgor, inhibiting photosynthesis and translocation, and by stimulating the synthesis and translocation of ABA (Hsiao, 1975). Elevated levels of ABA were related to internal water stress in Abies balsamea (Little, 1975) and Picea sitchensis (Little & Wareing, 1981).

The application of PGS to decapitated shoots is used to study the interaction of these compounds on cambial differentiation in vivo. In Picea abies, continued cambial activity appears to depend on a constant supply of IAA from the buds (Denne & Wilson, 1977; Torrey, 1971). The application of 5.7×10^{-3} M IAA to the debudded shoots of Picea abies initially substituted for the buds but later, tracheid diameter and wall thickness declined (Denne & Wilson, 1977). NAA at 10^{-5} M has also replaced stem apices but with no increase in xylogensis over the control plants (Harrison & Klein, 1979).

Mixtures of auxins and cytokinins have proved effective at replacing apical buds. Kinetin and IAA (0.1% in lanolin) applied to debudded shoots inhibited lateral bud break three times longer than IAA alone (Davies et al, 1966). One mg each (in lanolin) of IAA and a cytokinin applied to debudded Pinus sylvestris trees effectively replaced the buds' effect on wood formation (Allejnowicz & Tomaszewski, 1969). In Pinus sitchensis, $3 \times 10_{-2}$ M IAA and benzylaminopurine (BAP) (in lanolin) also replaced decapitated buds. But the addition of BAP,

while contributing the most to the increase in stem diameter, was only effective in increasing the cortical parenchyma (Phillipson & Coutts, 1980). The mixture of kinetin (0.92 uM) and IAA (28.5 uM) increased cambial activity of oak trees in December but inhibited activity in June (Zakrzewski, 1983).

Mixtures of gibberellins and auxins (in lanolin) varied considerably in effect. In Populus robusta, the effect on vascular differentiation was dependent on both the concentration and ratio of gibberellin to IAA (Digby & Wareing, 1966a), but with Picea abies (Denne & Wilson, 1967), and dwarf peas (Kuraishi & Muir, 1964), there was no additional xylem formation over IAA application alone. A mixture of 10^{-5} M each of NAA and GA applied to decapitated beans resulted in phloem, but not xylem formation; when the concentration of each was increased to 10^{-3} M, the reverse was found (Harrison & Klein, 1970). Maximum cambial activity occurred in Quercus robur cuttings with IAA (28.5 uM) applied to the decapitated apex and 2.9 uM GA_3 in the basal solution (Zakrzewski, 1983).

Mixtures of auxins, cytokinins and gibberellins produced twice the number of tracheids compared to normal bud production one mg of each (in lanolin) applied to debudded Pinus sylvestris shoots (Allejnowicz & Tomaszewski, 1969). Similar findings were observed in decapitated bean plants (Harrison & Klein, 1970). It was hypothesized that under natural conditions auxin is the principal factor in induction of xylem formation. Gibberellins (Allejnowica & Tomaszewski, 1969) and cytokinins (Davis et al, 1960; Allejnowica & Tomaszewski, 1969) improve the efficiency of auxin and affect the distribution of it within the stem.

Wound Healing

Wound healing, much like grafting, requires PGS's to stimulate callus formation before vascular differentiation can be initiated. IAA appears to be the limiting factor for the formation of wound callus (Doley & Leyton, 1970), and the subsequent differentiation into xylem (Terry et al, 1971) and phloem (Jacobs, 1952). Fourteen times more IAA is required for tracheid cell regeneration than for normal differentiation from cambial initials (Jacobs, 1970). Sieve tube cell regeneration is faster than that of xylem cells because lower IAA is required.

The stimulation and development of callus tissue in wound healing is influenced by several factors other than auxins. Traumatic acid is thought to be formed in injured cells and stimulate localized cell division (Lipetz, 1970). Once it was called the wound hormone; but lack of strong evidence has led to doubts about its importance (Lipetz, 1970). Cytokinins also influence callus formation and differentiation. A ten-fold increase in cytokinin concentration in root cortical tissue, resulted in a 5-fold increase in callus formation and a 50-fold increase in tracheary element formation (Terry et al, 1971). It was suggested that a change in the auxin/cytokinin ratio stimulated cells around a wound to divide (Lipetz, 1970). In Fraxinus species, a decrease in Ψ_T, independent of IAA concentration, resulted in a marked decline in wound callus formation (Doley & Leyton, 1970).

PGS and related compounds have been applied to tree wounds and grafted plants to accelerate the healing process and/or increase grafting success. The application of either NAA or indole-3-butyric

acid (IBA) at 100 ppm applied 3 times in nine days to the apex of cacti scions significantly improved graft success. The addition of auxin promoted vascular connections rather than stimulated callus production (Shimomura and Fuzihara, 1977). Glutathione, (a tripeptide of glutamic acid, glycine, and cysteine) applied at rates of 50 and 100 mg per gram talc to retanglar wounds in sugar maple also stimulated wound healing significantly (5%) (Davis, 1949). Davis suggested that the sulfur content was the main reason for the stimulation.

Most treatments of applied compounds, however, have proven inclusive or detrimental to wound healing. The application of: IBA, o-chlorophenoxyacetic acid, p-chlorophenoxyacetic acid, 2,4-dichlorophenoxyacetic acid, and traumatic acid at 0.1 to 200 mg/g talc to sugar maple wounds were detrimental to wound healing (Davis, 1949). Applications of IAA, IBA, NAA, naphthoxyacetic acid and naphthalene acetamide in concentrations ranging from 0.1% to 4% in both lanolin and orange shellac to wounds were also detrimental at concentrations above 1% (McQuilkin, 1950). Concentrations of 1% or less, or applications of traumatic acid were inconclusive (McQuilkin, 1950). Bark patches of shortleaf pine painted with a 200 ppm IBA solution also produced inconclusive results (Jackson & Zak, 1949). Grafted loblolly and slash pine scions on slash pine rootstocks were treated with either 25 ppm IBA, 100 ppm ascorbic acid, 100 ppm nitrogen, 50 ppm 6-mercaptopurine or 50 ppm dexamethasome 21-phosphate. These compounds were applied using scion stump infiltration in field grafts and as a foliar spray on pot grafts. All the treatments failed to significantly improve grafting success (Smith et al, 1972).

Photoperiod and Temperature Effects

Most studies of photoperiod responses of conifers have been limited to seedlings, yet the few studies with conifer grafts indicate an influence of photoperiod and temperature on success and post-union scion growth.

Increasing photoperiod usually increases plant growth. Photoperiods of 12 hours or less stop active growth in conifers and days longer than 16 hours generally re-activate growth (Cram & Lindquish, 1963). Photoperiods are increased by either, light breaks during the dark period, or increasing the natural daylength. High intensity light or low intensity light of 25 f.c. or more have been used (Nitsch, 1957b). The response to light breaks depends on the species and provenance (Arnott, 1974; Heide, 1974). Neither Norway spruce or rhododendron respond to light breaks (Nitsch, 1957b), but the height of seedling loblolly pine and yellow popular was twice that achieved under natural daylength (Zahner, 1955). Blue spruce seedlings became dormant under 8 hour days with a 2 hour light break (Cram & Lindquist, 1963), but grew continuously under 12 hour days with a 2 hour light break, if the energy level was 200 - 250 u watts/minute/cm^2 or greater (Young & Hanover, 1971; Tinus, 1971). Long days of 18 to 20 hours have resulted in continuous growth or several growth flushes without a chilling requirement in Pinus sp. (Wareing, 1951a; Wheeler, 1979), Norway spruce (Nienstaedt, 1959; Nitsch, 1957b), blue spruce (Hanover, 1975; Hanover & Reicosky, 1972), and larch (Wodzicki, 1964). Blue spruce has also been grown continuously with a 24 hour day (Young

& Hanover, 1977). However an 18 hour day was found to be optimum for shoot and cambium growth in Norway spruce (Nitsch, 1957a; Heide, 1974).

The effect of long days on graft success has been demonstrated for some species. A continuous photoperiod of high intensity light increased graft success 35% and scion growth 58% for Lodgepole pine (Wheeler, 1979). September-grafted Norway spruce in a greenhouse, with a 20 hour photoperiod from mid-October till June, had higher success and more growth than similiar trees receiving 2 to 5 months of chilling after grafting before being returned to the greenhouse under normal or long day conditions (Nienstaedt, 1959). Long days also accelerated growth and delayed dormancy for blue spruce scions (Hanover, 1975).

The response to lengthened photoperiods is age dependent. Young and Hanover (1976) found that Picea seedlings less than 2 years-old responded to continuous light with continuous growth. Once the seedlings were 3 year-old, a bud chilling requirement developed even under constant light. An established scion of a 50 year-old 'Hoopsi' blue spruce also went dormant under a continuous photoperiod and required chilling to initiate growth (Young & Hanover, 1976).

Concentrations of plant growth substances change with photoperiod. Larch grown under 16 hours of high intensity light (800 - 1000 fc), had significantly more growth promoting substances in their apices then those grown with continuous low intensity light or 12 hours of high intensity light (Wodzicki, 1964). As light intensity and day length decreased, growth inhibitor concentrations in the cortex increased (Wodzicki, 1964). The inhibitory substance was later determined to be ABA (Wodzicki & Wodzicki, 1980). Similar findings with Betula pubescens have been reported (Kawase & Nitsch, 1959). Long days do not

interfere with shoot or root development, or reversion to natural growth habits (Hanover & Reicosky, 1972).

Greenhouse temperatures influence photoperiod response. Optimum greenhouse temperatures for photoperiod responses are 15° to 25° C, the lower range for short days and the upper range for long days (Nitsch, 1957b). With long days, more assimilate was used for height growth in Scots pine at temperatures less than 65° F, higher temperatures resulted in increased needle length and less shoot height (Jensun & Gatherum, 1965). For Norway spruce seedlings' maximum growth occurred at 21° C with 18 hour days (Heide, 1974). Day/night temperatures have a significant effect on scion growth after union development. With 16 hour days, the best overall scion development occurred with a 20° C day temperature irregardless of night temperature (11° - 17° C). Maximum growth occurred at 30°/21° C day/night temperatures (Booth & Saunders, 1979).

Chapter 3

MATERIALS AND METHODS - WATER

The materials and methods are divided into three sections. The first section provides a description of the plant material. The second section list and describes the experiments and the measurements recorded for each, while the third describes in detail the methods and equipment employed.

Plant Materials

Dormant scions of Picea pungens 'Hoopsi' (Colorado blue spruce) and dwarf Pinus strobus (white pine) were collected near Salem, OR. Abies procera (noble fir) and Taxus media 'Hicksii' were collected in Corvallis, OR. Scions, consisting of the branch tips of the current season's growth (11 - 17 cm), were stored in sealed polyethylene bags placed in ice, and side-grafted (Diagram 1) within 48 hours after collection. Rootstocks were 2-1 or 2-2 Picea abies (Norway spruce) in 7.6 x 7.6 x 15.3 cm plastic pots, Pinus strobus in 8.9 x 8.9 x 7.6 cm plastic pots, Abies balsamea (balsam fir) in 8.9 x 8.9 x 12.7 cm plastic pots and Taxus media (yew) in 4 l plastic containers. Greenhouse grafting was done from early January until mid-February on actively growing rootstocks that had been placed in the greenhouse ($18.3^{o}/12.8^{o}$ C, day/night) five weeks prior to grafting. Dormant rootstocks remained in the unheated polyethylene-lined lath house.

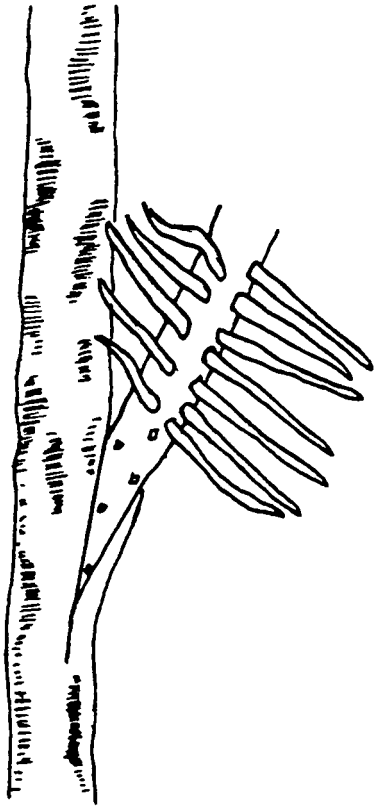

Diagram 1. Sketch of a conifer side graft.

After initiating shoot growth, all rootstocks were fertilized weekly at 472 ppm nitrogen with Peter's 20-20-20 liquid fertilizer (W. R. Grace & Co., Fogelsville, Pa).

Experiments

Experiments were conducted from 1981 to 1986 during the winter and spring months. Unless otherwised noted, all plant material consisted of dormant Picea pungens 'Hoopsi' scions grafted onto actively growing Norway spruce rootstocks and grown in the greenhouse.

Greenhouse Water Relations - 1983. (GWR-83). Two treatments of 32 grafts each were grafted in early January 1983. In one treatment, all rootstocks were debudded initially and every 2 to 3 weeks thereafter. Rootstocks in the other treatment were not debudded. Weekly measurements of scion total water potential (Ψ_T) were made on alternate weeks for 12 weeks on needles from one-half of the scions in each treatment. Osmotic potential (Ψ_π) and starch content were determined in each sample. Scion growth parameters were recorded.

Greenhouse Water Relations - 1984. (GWR-84). Sixty-four trees were grafted in mid-January 1984. Total water potential, Ψ_π and needle starch were determined for 10 weeks as in GWR-83. Scion growth parameters were recorded.

Lath House Water Relations - 1984. (LWR-84). Sixty-four dormant rootstocks were grafted in mid-January 1984 and were overwintered in a polyethylene-covered lath house. Samples for Ψ_T, Ψ_π and needle starch

were collected as in GWR-83 each 1.5 weeks for the first 7.5 weeks. Additional measurements were made weekly until 16.5 weeks after grafting. Scion growth parameters were recorded.

Established Graft Water Relations. (EGWR). Dormant three year-old Picea pungens 'Hoopsi' grafts in 12 1 pots were brought into the greenhouse in early January 1984. Total water potential, Ψ_π, transpiration rates and needle starch were measured for 7 weeks.

Greenhouse Twig Transpiration. (GTT). Midday measurements of Ψ_T and transpiration were made on eight twigs upright to ring stands in the greenhouse. The experiment was conducted in March 1983.

Mis-aligned Graft Water Relations. (MGWR). In late January 1985, 13 scions were grafted so that the cambia of stock and scion were not properly aligned. Measurements of Ψ_T, Ψ_π and accumulated starch were made weekly for eight weeks.

Temperature and Transpiration. (T&T). Forty-eight scions were grafted in late January 1985 and divided into two treatments of 24 trees each. Twenty-four grafts each were placed in two growth chambers set for 10 hr photoperiods (84 uE/m-sec, PAR), 15.6° C nights, and with either 21.1° or 26.7° C photoperiods. Each scion was sampled weekly for Ψ_T and Ψ_π. Transpiration was measured concurrently one hr, five hr and nine hr into the photoperiod. After bud break (6 weeks after grafting), measurements were stopped and the grafts moved to the greenhouse to complete growth. Scion growth parameters were recorded.

Scion Water Loss. (SWL). Scions were collected in early January 1986. Treatments consisted of aligned and mis-aligned grafts and detached ungrafted twigs. Each treatment consisted of 80 twigs

weighed at grafting and at the time of harvest. The cut end of the ungrafted twigs was sealed with petroleum jelly before weighing. For 7 weeks, Ψ_T (by pressure chamber, PMS Instruments, Corvallis, OR) and Ψ_π of 10 twigs per treatment were measured weekly. Twigs were oven dried (70°, 24 hours) and reweighed.

Dye - 1983. (Dye-83). Scions were grafted in early February 1983. Beginning two weeks after grafting and continuing until eight weeks after grafting, eight trees per week were cut at the crown and placed in a 50 ml beaker containing the dye solution. Total water potential was determined and the dye in the scion was extracted and measured. Bud break date was recorded.

Dye -1984. (Dye-84). Grafts of noble fir on balsam fir, dwarf white pine on white pine and 'Hicksii' yew on yew were made in late January 1984. Five grafts of each species were placed in the dye solution weekly. The dye was extracted, quantitated, and bud break recorded.

Dye - 1985. (Dye-85). Scions were grafted onto dormant rootstocks in early January 1985 and overwintered in the lath house. Eight trees were placed in the dye solution in the greenhouse every 1.5 weeks during the first six weeks and weekly thereafter until 18 weeks after grafting. At the same time, Ψ_T was measured.

Rootstock Translocation - (RSTrans). Rootstocks were grafted in mid-January 1983. On alternate weeks, all foliage of ten grafted rootstocks were isolated in polyethylene bags and 10 uCi of $^{14}CO_2$ ($Na_2{}^{14}CO_3$ form; Amersham, Arlington, IL) was generated in each bag. The bags were removed after 3.5 hours and the trees left under the lights (75 uE/m^2-sec, PAR) for 20 hours. The portion of each tree

containing the graft union and scion was cut from each rootstock, held at -70° C for 10 minutes, split in half, and autoradiographed for 2 weeks.

Scion Starch Accumulation Requirement. (SSAR). Early February 1984, 200 grafts were divided into 8 treatments. Beginning at grafting and weekly thereafter, groups of 25 scions were sampled for needle starch analysis, then covered with aluminium foil. Six weeks after grafting, all foil was removed and the scions resampled for starch. Scion growth parameters were recorded.

Scion Translocation. (ScTrans). Two hundred scions were grafted in late January 1985. Ten scions were treated each with 1.22 uCi of $^{14}CO_2$ released from $Na_2{}^{14}CO_3$ each half week, for 7.5 weeks. Scions were isolated in polyethylene bags, treated, and placed under high-pressure sodium vapor lamps (125 uE/m^2-sec at scion level). After 5.5 hr, the bags were removed and the trees returned to the greenhouse around midnight. Five scions were harvested 24 and the remaining scions 48 hr after treatment. At harvest, each scion was subdivided into the graft union, lower buds, middle buds and terminal buds, dried (70° C) for 24 hr, weighed and oxidized (Packard Tri-Carb Sample Oxidizer, Packard Instrument Co., Downers Grove, IL). Needles and bark were removed, weighed, and a weighed subsample coarsely chopped and then boiled five minutes in 1 ml of 2-propanol in a capped 50 ml tube.

Bark and needle subsamples were first extracted and separated into lipids and sugars. The dried chloroform fraction was redissolved in 1.0 ml hexane and a subsample counted as the lipid fraction in a toluene-based fluor (6 g/l PPO + 150 mg/l POPOP). The dried aqueous

phase was dissolved in 1.0 ml distilled water, subsampled and counted in an aqueous fluor (Quantafluor, Mallinckrodt Inc., Paris, KY) as part of the sugar fraction.

The solid residues were scraped from the filters into 50 ml centrifuge tubes and extracted with hot 80% ethanol for starch/sugar separation. The supernatant was dried, dissolved with 1.0 ml distilled water, subsampled, and counted as the second portion of the sugar fraction in aqueous fluor. The bark pellet was oven dried (70° C), weighed, oxidized and counted as the bark solid fraction. The needle residue was digested with amyloglucosidase (Sigma Chemical Co., St Louis, MO) as for starch analysis. After digestion, the tubes were centrifuged for 5 min at 10,000 g and the supernatant decanted. The residue was washed with 80% ethanol, heated in a boiling water bath and cooled in ice four minutes, centrifuged, decanted, combined and dried. The dried supernatant was dissolved in 1.0 ml distilled water, subsampled and counted as the starch fraction in the aqueous fluor. The residue was oven dried (70° C), weighed, oxidized and counted as the needle solid fraction. Samples were counted with a Beckman LS 7000 liquid scintillation counter (Beckman Instruments Inc., Fullerton, CA).

Total Carbon Reserves - Covered. (TCRC). In mid-January 1985, 200 scions were grafted and covered with aluminium foil. Each week bark samples (approximately 1 cm^2) and needle samples (10 to 15 needles) were removed from 25 scions. Eight weeks after grafting, the foil was removed. Needle samples were assayed for starch and sugar content. Bark samples were extracted for lipid and sugar analysis. The lipid fraction was separated into polar and non-polar lipids, with

the non-polar fraction quantitated. Scion growth parameters were recorded.

Total Carbohydrate Reserves. (TCR). Procedures were the same as for TCRC except that scions were not covered with aluminum foil and the non-polar lipid fraction was not quantitated.

Scion Respiration. (SR). In mid-January 1985, 100 scions were grafted with respiration rates for ten scions measured weekly for eight weeks using a Gilson Differential Respirometer (Gilson Medical Electronic, Middleton, MI). Respiration was measured on a subsample, consisting of 1 to 1.5 cm long section of stem with needles and a bud, for three hours after equilibration. Total water potential was determined for eight of the scions.

Scion Photoperiod Response. (Photo). Ninety scions were grafted in early January 1984 and divided into three treatments. One group was placed immediately under high-pressure sodium vapor lamps in the greenhouse with a 20 hour photoperiod (125 uE/m^2-sec, PAR, from the lamps at scion level). The other two treatments were grown under normal greenhouse light conditions until approximately half of the scions had broken bud, then one of two treatments was moved under the lamps. Scion growth parameters were recorded.

Extended Scion Dormancy. (Dorm). Four treatments of 30 grafts each were grafted in late January 1984. Treatments consisted of a control, 1 x 10^{-4} M ABA and 1 x 10^{-5} M ABA applications and scions stored for 6 weeks in sealed polyethylene bags packed in ice. A fifth group was grafted late February with scions that had been stored for 10 weeks near 0^{o} C. ABA was applied in solution to the basal end of the scions for 16 hours under the sodium vapor lamps. Scion growth

parameters were recorded.

Scion Antitranspirant Application. (Antitrans). In early February 1984, 30 scions were dipped in 1% Vapor Guard (Poly-1-p-menthene-8-9-diyl; Miller Chemical & Fertilizer Co., Hanover, PA) and allowed to dry 30 min before grafting. Scion growth parameters were recorded.

Polyethylene-enclosed Grafts. (Tent). Thirty scions were grafted in early February 1984. The trees were enclosed until bud break within a sheet of polyethylene (0.4 mm) until bud break. Scion growth parameters were recorded.

Rootstock Root Warming. (Root-warm). In mid-January 1985, 100 grafts were made on dormant rootstocks, 50 by an inexperienced grafter. Twenty-five grafts from each grafter were returned to the lath house while the others were heeled-in with sawdust in open, bottom-heated, rooting boxes ($21.1^{\circ} \pm 2^{\circ}$ C) in the lath house. Scion growth parameters were recorded.

Unestablished Rootstocks. (Lifted-84). Sixty grafts were made in early January 1985 on rootstocks lifted and potted in September, 1984. Scions were grafted on twenty actively growing rootstocks in the greenhouse. The remaining 40 were kept dormant. Twenty remained in the lath house, the other twenty were placed in a bottom heat rooting box the same as Root-warm grafts. Scion growth parameters were recorded.

Environment During Union Development. (Environ). In early January 1984, 120 trees were grafted; forty were grafted on actively growing rootstocks and remained in the greenhouse, the other eighty were on dormant rootstocks. Of the eighty dormant rootstocks, forty

were returned to the lath house, the other 40 placed outdoors. Scion growth parameters were recorded.

Plant Growth Substance Applications - 1982. (PGS-82). In mid-December 1981, solutions of indolebutyric acid (IBA), benzyladenine (BA) and gibberellic acid (GA_3) were applied to the cut surfaces of the scions prior to grafting as a 3 sec quick-dip or a 3 min dilute soak. Soak concentration were 0.01 mM, 0.1 mM and 1.0 mM. Quick-dip concentrations for IBA and GA_3 were 0.5 mM, 5.0 mM and 50.0 mM, while the BA concentrations were 0.1 mM, 1.0 mM and 10.0 mM. The quick-dip solutions were in 40% ethanol, the soak solutions in 20% ethanol. Controls consisted of a 40% ethanol quick-dip and untreated scions. Each treatment contained of 10 scions and was evaluated for graft success.

Plant Growth Substance Applications - 1983. (PGS-83). Three hundred trees were grafted in mid-January 1983. Scions were given three min soaks of BA at 0.01 mM, 0.1 mM and 1.0 mM; and IBA and naphthaleneacetic acid (NAA) at 0.1 mM, 1.0 mM and 10.0 mM in 20% ethanol prior to grafting. Treatments consisted of 30 grafts each plus an untreated control. Scion growth parameters were recorded.

Plant Growth Substance Applications - 1984. (PGS-84). Thirty scions per treatment were grafted in mid-January, 1984. Treatments consisted of BA (0.1 mM), IBA (1.0 mM) and control (no PGS) solutions soaked for 3, 9 or 27 min. Solution were in 33.3% ethanol. Scion growth parameters were recorded.

Rootstock Shoot Reduction - 1983. (RSRed-83). Scions were grafted in mid-January 1983 on dormant rootstocks and returned to the lath house. Eight treatments, of 24 or 30 scions each, consisted of

applications of 1000 ppm maleic hydrazide (Sigma Chemical Co., St Louis, MO) and 1000 and 2000 ppm Atrinal (HLR Sciences Inc., Vero Beach, FL) to the rootstocks, an untreated control and rootstock bud removal at grafting, twice, three times and continued removal. The buds on the rootstocks were removed after grafting when they were one to two cm long. Applications of the growth retardants were made prior to grafting by immersing inverted rootstocks into the solution to the lowest branches, and laying them on their side until the foliage dried. Growth retardant treatments were repeated on actively growing rootstocks in the greenhouse. Scion growth parameters were recorded.

Rootstock Shoot Reduction - 1984. (RSRed-84). Thirty scions for each of the four treatments were grafted in early January, 1984. Treatments consisted of continued removal of the rootstock buds, an untreated control and Atrinal applied as before at 1000 ppm to both actively growing (greenhouse) and dormant (lath house) rootstocks. Scion growth parameters were recorded.

Methods

Scions were checked on alternate days for bud break. Bud break was recorded when needle color could be seen through the bud scales. In late summer, the number of scion branches, total branch length and terminal length were measured and graft success recorded.

Total water potential, except were noted, was measured using a Richard's model, wet-ring thermocouple psychrometer (25^{o} + 0.0001^{o} C) on five to seven randomly selected needles per scion per sample.

After the Ψ_T measurement, needle samples were sealed in Parafilm (American Can Co., Greenwich, CT) envelopes and stored at -70° C. Osomtic potential of the needles was measured on expressed sap using a vapor pressure osmometer (5100C, Wescor, Inc., Logan, UT). Concentrations were corrected for errors due to previous psychrometic measurements (0.064 MPa) and apoplastic dilution (Appendix 1). Turgor pressure (P) was then calculated using the standard water relations equation: $\Psi_T = \Psi_\pi + P$. Transpiration was measured using a steady-state porometer (Li-Cor 1600, Li-Cor Inc., Logan, UT). Values were standardized by measuring the area of the wide side of each needle (Li-Cor Leaf Area Meter, Li-Cor Inc., Logan, UT) and multiplying the area by the mean ratio of the needle perimeter to the width of the wide side of 75 needles.

To detect xylem connections across the graft, rootstocks were severed at the crown, then recut under water and placed in a 50 ml beaker containing approximately 20 ml of the dye solution (0.5% (w/v) acid fuchsin (Sigma Chemical Co., St. Louis, MO) in distilled water). Trees were left in the dye in the greenhouse for four hr before the graft union and scion were harvested. The dye was extracted from scion segments (approximately 1 cm) removed just above the graft union. The segments were ground with washed sand in a mortar and pestle and extracted in 15 ml of distilled water with a microsoxhlet apparatus for 1.5 hr. The extract was centrifuged and absorbance of the supernatant read at 545 nm using a dual-beam spectrophotometer (Beckman Model 34, Beckman Instrument Co., Fullerton, CA). Dye concentration was normalized to cm^3 of tissue.

Starch and sugar separation procedures were adapted from the

methods of Potter and Breen (1980). All weights were determined with a Mettler electronic balance (AC100, Mettler Instrument Co., Hightstown, NJ). Weighed needle samples (50 to 120 mg Dwt) were soaked in 80% ethanol for one hour and then homogenized with a microblender (Tissumizer, Tekmar Co., Cincinnati, OH). The homogenate was boiled for 4 min and cooled in ice 4 min twice, then centrifuged at 10,000g for 5 min. The supernatant was decanted and the extraction repeated twice on the precipitate with 3 min intervals of boiling and cooling. If sugar analysis was required, supernatant fractions were combined and dried in a water bath (70° C) with forced hot air. The pellet was oven dried over night at 70° C.

For starch analysis, the pellet was dispersed in 10 ml of 0.1 M acetate buffer (pH 4.5) and digested with a suspension of amyloglucosidase (2 mg/2 ml) (Grade II, Sigma Chemical Co., St Louis, MO) at 55° C for one hr. The digest was centrifuged at 10,000 g for five min and a 1.0 ml aliquot of supernatant placed in a 15 ml test tube. Two ml of glucose oxidase reagent (Statzyme, Worthington Diagnostic Systems Inc., Freehold, NJ) were added to the test tube and incubated in a water bath (36° C) for 15 min. Absorbance at 500 nm was measured with the spectrophotometer. Starch substrate (Sigma Chemical Co., St Louis, MO) was used for the standard.

Sugar was purified using the method of Boersig and Negm (1985). The dried supernatant was rehydrated with 1.0 ml distilled water, applied to a micro-column consisting of 1 ml each: Dowex 50W (acid form), Amberlite IRA-45 (acetate form), polyvinylpolypyrrolidone, (top to bottom) and eluted with 5.5 ml of distilled water. The elutant was dried as before. The dried sugars were dissolved in 40 ml distilled

water. Sugar analysis was adapted from the method of Yemm and Willis (1954) as modified from Dreywood (1946). Five ml of cold anthrone solution (2.5 mg/5 ml 72% H_2SO_4) were pipetted into 15 mm glass test tubes in an ice bath and the tubes capped with holed-septa. One ml of the dilute sugar solution was transfered to the 15 mm test tubes, vortexed and returned to the ice bath. Thirty tubes at a time were placed in a rapidly boiling water bath for 12 minutes, then cooled in water at room temperature for five min. Absorbance was read using the spectrophotometer and quantitated as glucose equivalents.

Lipids and sugars were extracted and separated using a modification of the method of Bligh and Dyer (1959). Bark samples were chopped and then soaked in a 50 ml stoppered test tube containing 15 ml chloroform:methanol (2:1) at 60° C for 1.5 hours. The tissue was homogenized with a microblender and filtered through a coffee filter under vacuum into a screw-top 50 ml culture tube. The orginal tube and microblender were washed with an additional 15 ml of the chloroform:methanol solution, then used to wash the filter/solid residue. Distilled water was added, the tubes capped, shaken and centrifuged at 2000 g for 5 min and the sugar containing aqueous (upper) phase removed. Methanol:water (10:8; 9.5 ml) was then added to the chloroform fraction, the mixture partitioned, centrifuged and separated. The two aqueous phases were combined and dried for sugar analysis. The chloroform phase was dried at 55° C with a stream of N_2 gas and the lipids redissolved in 0.5 ml hexane.

Non-polar lipids were separated from the total lipid fraction with 2 ml silicic acid (100 mesh) (VWR Scientific) in hexane packed in columns which were eluted with 7.5 ml of 18% diethyl-ether in hexane.

The elution was dried at 55° C with a N_2 gas stream and redissolved in 0.5 ml of hexane. The non-polar lipids were separated with a Perkin-Elmer Liquid Chromatograph (Perkin-Elmer, Norwalk, CN) with a concave gradient at 500 curvature to 50% (18.5 min.) 2-propanol:water (99:1) in hexane with a 30 cm silica column (SI-10A, Brownlee Labs, Santa Clara, CA). Compounds were detected using a Perkin-Elmer spectrophotometer (LC-55) at 212 nm. Sample peaks were quantitated by a Hewlett-Packard 3390A Integrator (Hewlett-Packard Co., Avondale, PA) connected in parallel to a chart recorder.

Chapter 4

RESULTS

Water Relations

Statistical analyses of the parameters from the greenhouse grafts for 1983 (GWR-83, Fig. 1) between the budded and debudded treatments were generally non-significant. Therefore the two treatments were combined. A frequency distribution of the bud break dates suggested partitioning of the data into four groups, Group 1 (G-1), scions that required 46 to 54 days for bud break, Group 2 (G-2), scions that required 56 to 64 days for bud break, Group 3 (G-3), scions that required greater than 65 days for bud break and Group 4 (G-4), the unsuccessful grafts.

At grafting, Ψ_T were high, but rapidly declined the first week to reach a minimum at week 2 before increasing. In G-1, Ψ_T increased slowly until bud break at week 7, when Ψ_T increased rapidly and remained at -1.0 MPa during shoot elongation. Groups 2 and 3 followed a similar pattern, except that Ψ_T was slightly lower than in G-1 from week 4 until near bud break. Total water potentials of the unsuccessful scions began a general decline after 5 weeks, becoming significantly different from successful grafts after six weeks (Appendix 2.1). In successful grafts, Ψ_T remained above -1.9 MPa.

Osmotic potentials of all the grafts declined rapidly the first week, after which the Ψ_π of the successful grafts was relatively constant until three weeks prior to scion bud break. Thereafter, a

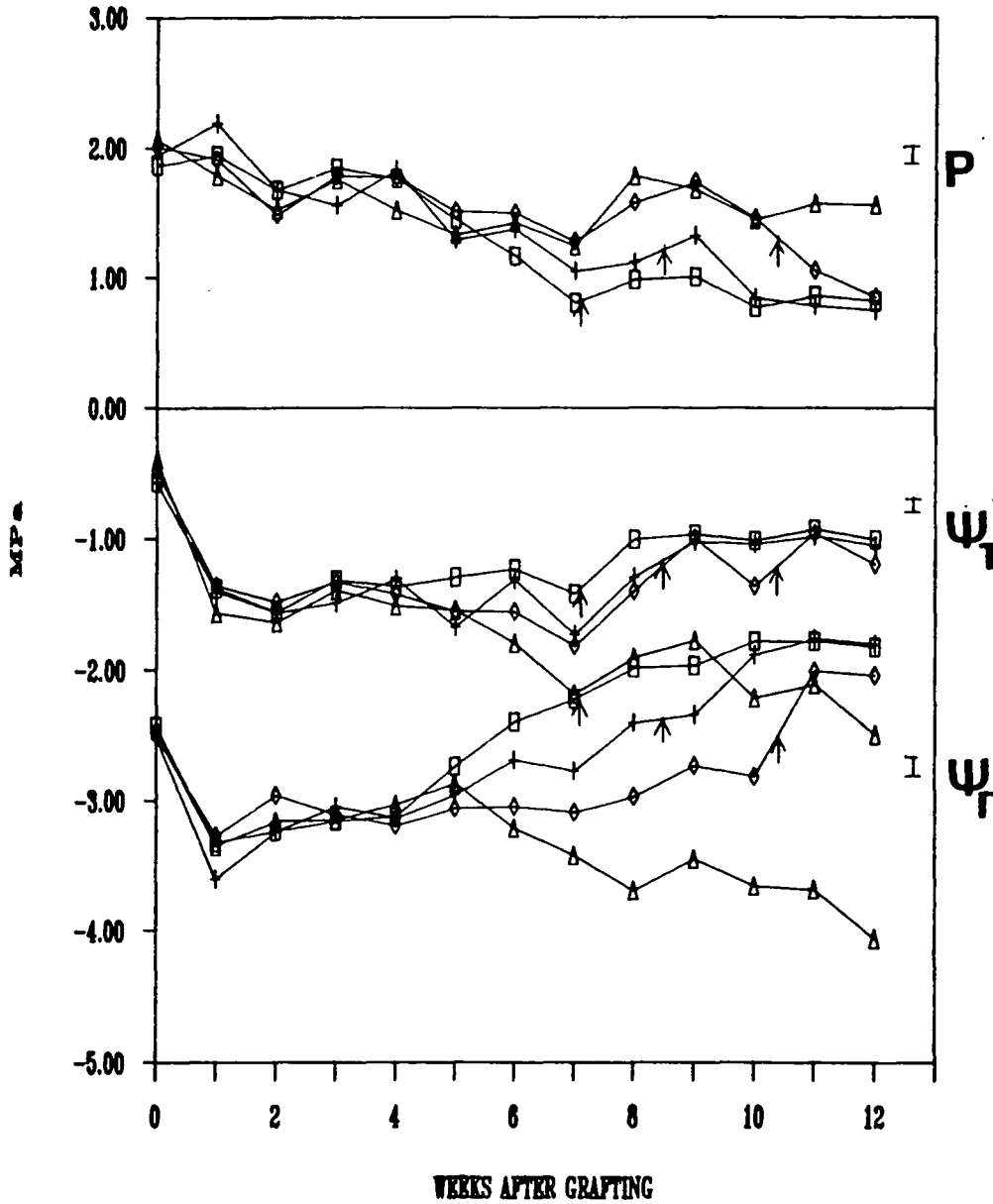

Fig. 1. Mean total water potentials, osmotic potentials and turgor pressures for 12 weeks after grafting for the experiment Greenhouse Water Relations - 1983. Group 1 (□) grafts required 46-54 days for bud break, Group 2 (+) required 56-64 days, Group 3 (◇) required greater than 65 days and Group 4 (△) grafts were unsuccessful. Arrows indicate mean bud break. Vertical bars indicate standard errors.

marked increase in Ψ_π occurred that stabilized two weeks after bud break. However, the Ψ_π of G-4 declined after week 5, becoming significantly different from the successful grafts after week 6 (Appendix 2.2). The decline in the Ψ_π of G-4 generally parallelled that of the Ψ_T.

Turgor pressures declined slowly after the first week and stabilized at about 0.8 MPa in G-1 scions during shoot elongation. However, P increased in G-4 after week 7,scions due to more rapid declines in Ψ_π than Ψ_T, and remained significantly higher than G-1 scions (Appendix 2.3). Turgor pressures of G-4 scions remained near 1.5 MPa.

The water relations of successful greenhouse grafts in 1984 (GWR-84, Fig. 2) were similar to 1983. The data were again grouped according to bud break dates. Group 1 (G-1) scion broke bud in less than 44 days (mean = 41 days), Group 2 (G-2) scions required longer than 44 days for bud break (mean = 48 days), and Group 3 (G-3) consisted of unsuccessful grafts.

Total water potentials declined rapidly the first two weeks, but remained above -1.7 MPa for all groups. By week 3, Ψ_T had increased sharply (0.2 to 0.3 MPa) for all groups. Thereafter the Ψ_T of G-1 increased gradually until mean bud break, when the Ψ_T increased sharply to -1.0 MPa. Group 2 scions maintained Ψ_T of -1.4 to -1.55 MPa until near bud break. After bud break, Ψ_T also increased to -1.0 MPa. There were no statistically significant differences in Ψ_T among the groups until the seventh week (Appendix 2.4), when the Ψ_T of G-3 began to decline. Total water potentials of the successful grafts remained at -1.0 MPa during shoot elongation.

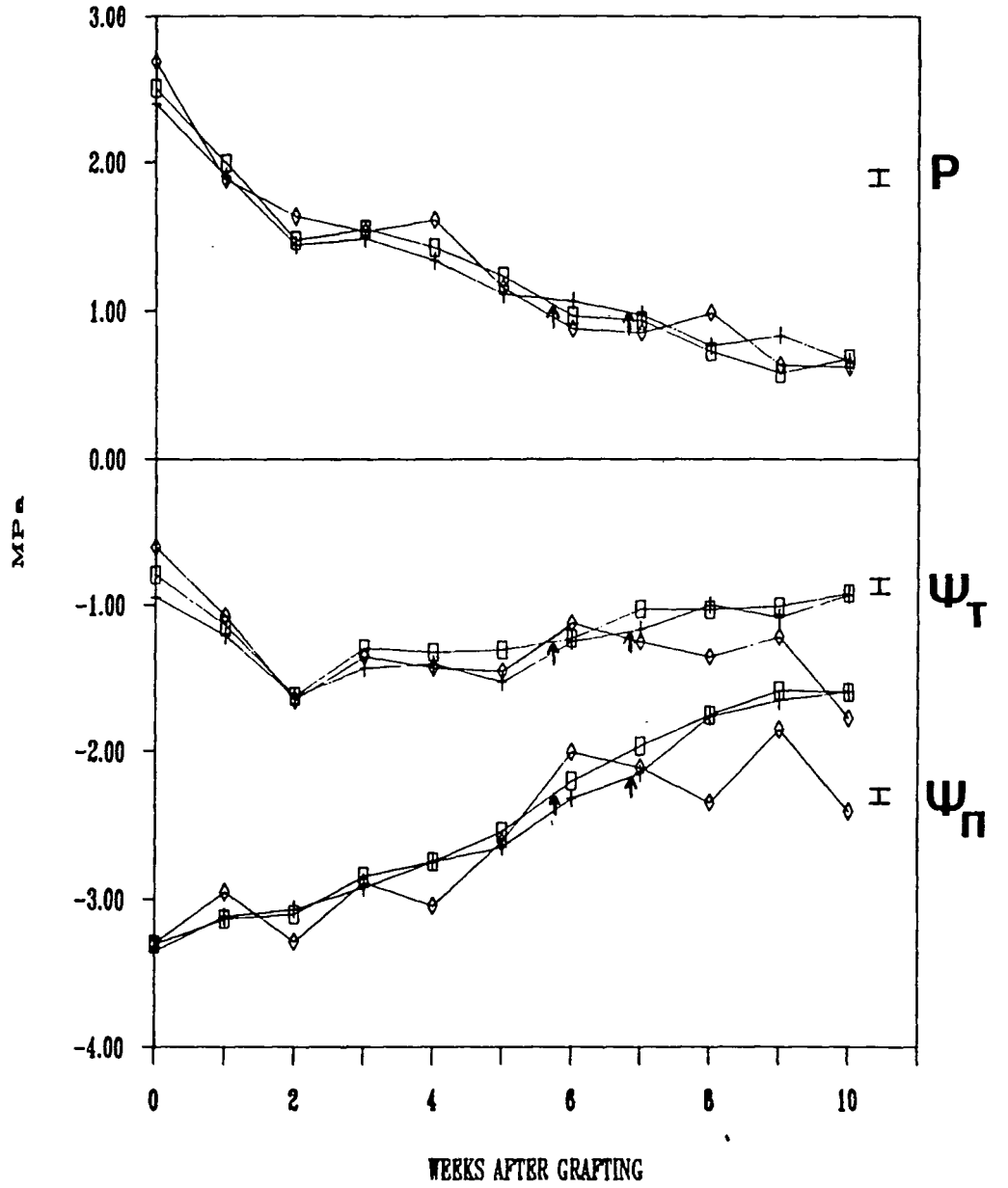

Fig. 2. Mean total water potentials, osmotic potentials and turgor pressures for 10 weeks after grafting for the experiment Greenhouse Water Relations - 1984. Group 1 (□) grafts required less than 44 days for bud break, Group 2 (+) required greater than 44 days, while Group 3 (◇) grafts were unsuccessful. Arrows indicate mean bud break. Vertical bars indicate standard errors.

Osmotic potentials were lowest at grafting, increasing gradually thereafter until two weeks prior to bud break, when rapid increases in Ψ_π occurred. Osmotic potentials of the successful grafts stablized after eight weeks, and were generally not significantly different between groups until after week 7, when the Ψ_π of G-3 began to decline (Appendix 2.5).

Turgor pressures were high at grafting and declined rapidly the first two weeks, after which P declined gradually to stabilize between 0.55 to 0.75 MPa the last 2 weeks. Statistically, P were significantly different among groups and between weeks, but not the interaction (Appendix 2.6).

Water stress in the lath house grafts (LWR-84, Fig. 3) was not as severe as in GWR-84 grafts. The data for the LWR-84 grafts were grouped according to scion bud break. Group 1 (G-1) scions broke bud in less than 103 days (mean = 98.5 days), Group 2 (G-2) scions required greater than 103 days (mean = 108 days) and Group 3 (G-3) consisted of the unsuccessful grafts.

Total water potentials of G-1 and G-2 declined gradually after grafting, generally reaching a minimum of -1.4 MPa after six weeks, then slowly increased to reach -1.0 MPa approximately 3 weeks prior to scion bud break. For G-1, this occurred at approximately 50% bud break on the rootstocks, while for G-2, rootstock buds were beginning to elongate when Ψ_T of -1.0 MPa were reached. After bud break, Ψ_T generally were maintained at -1.0 to -1.15 MPa. Unsuccessful scions had lower Ψ_T, but parallelled changes in the Ψ_T of G-1 and G-2 between weeks 3 to 12.5, after which Ψ_T generally declined until measurements were stopped. Total water potentials were significantly different

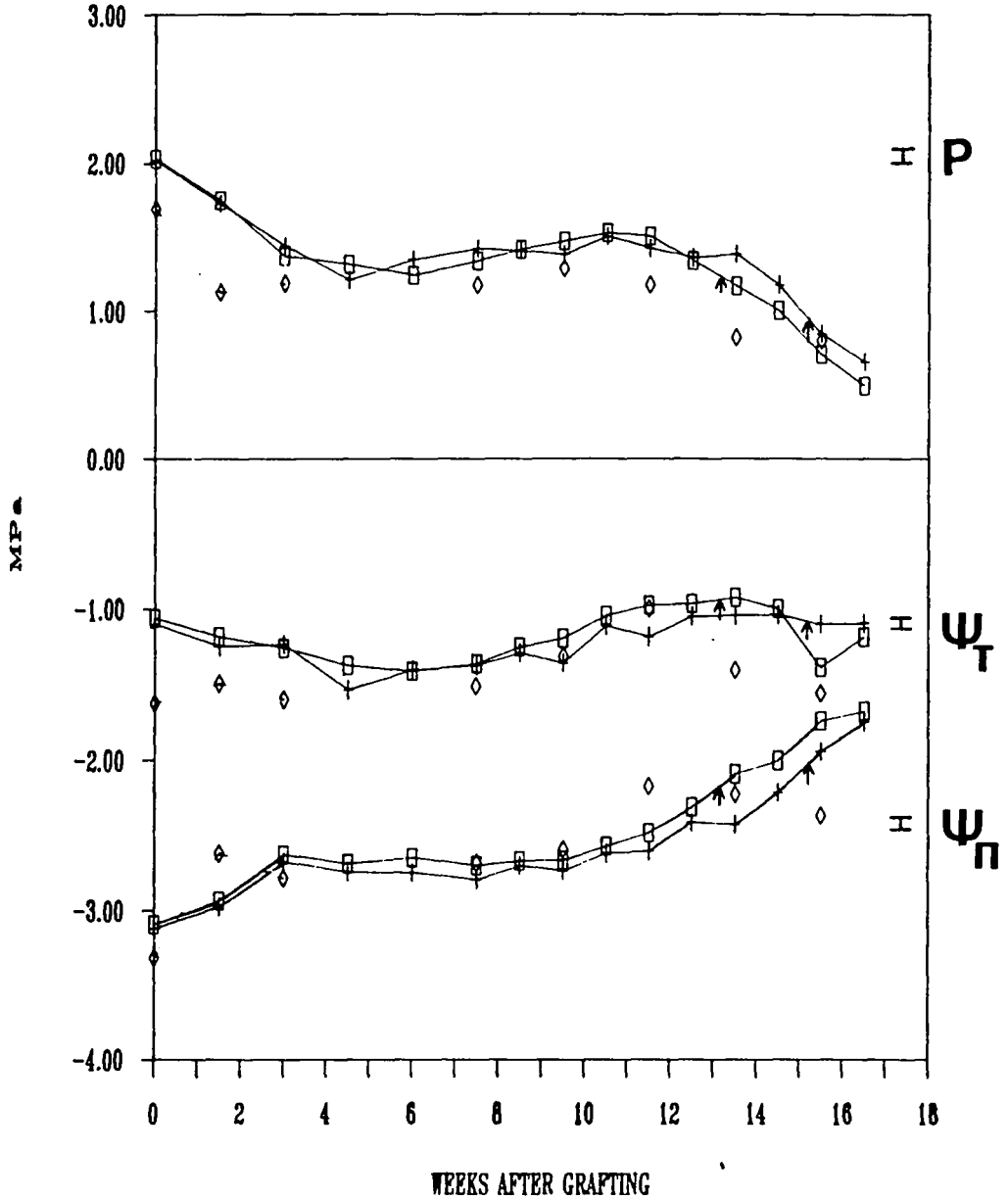

Fig. 3. Mean total water potentials, osmotic potentials and turgor pressures for 16.5 weeks after grafting for the experiment Lath house Water Relations - 1984. Group 1 (□) grafts required less than 103 days for bud break, Group 2 (+) required greater than 103 days, and Group 3 grafts (◇) were unsuccessful. Arrows indicate mean bud break. Vertical bars indicate standard errors.

between groups and between weeks; however the interaction was not significant (Appendix 2.7).

At grafting, Ψ_π of all groups were low, then increased over the first three weeks before stabilizing. Osmotic potentials remained stable until week 11.5 (Appendix 2.8), when the Ψ_π of G-1 increased. The Ψ_π of G-2 showed a marked increase after week 13.5, 2 weeks prior to bud break.

Turgor pressures declined the first three weeks with the increase in Ψ_π, then remained between 1.2 to 1.5 MPa. Two weeks prior to bud break, P of both G-1 and G-2 declined. Group 3 P parallelled the changes in G-1 and G-2 until week 14.5, when P of G-3 stopped declining. Turgor pressures were significantly different between groups and weeks, but the interaction was not significant (Appendix 2.9).

Total water potentials of dormant, 3 year-old established grafts brought into the greenhouse (EGWR, Fig. 4) increased during the first two weeks to remain near -0.7 MPa until two weeks after bud break. Osmotic potentials remained constant until bud break, after which Ψ_π increased rapidly during shoot elongation. Turgor pressures increased 0.3 MPa the first week and remained between 2.0 to 2.4 MPa until bud break, declining rapidly thereafter. Transpiration achieved maximum rates after 1 week in the greenhouse (16° to 21° C; Fig. 5), with maximum rates of 0.8 ug/cm^2-sec recorded.

Twigs brought into the greenhouse in March, 1983 (GTT) dried very rapidly (Fig. 6), dropping in Ψ_T from -1.4 MPa to -5.4 MPa in 23 days. The rate of decline was very rapid the first two days (-0.7 MPa/day), then slowed to an average of -0.126 MPa/day. Transpiration

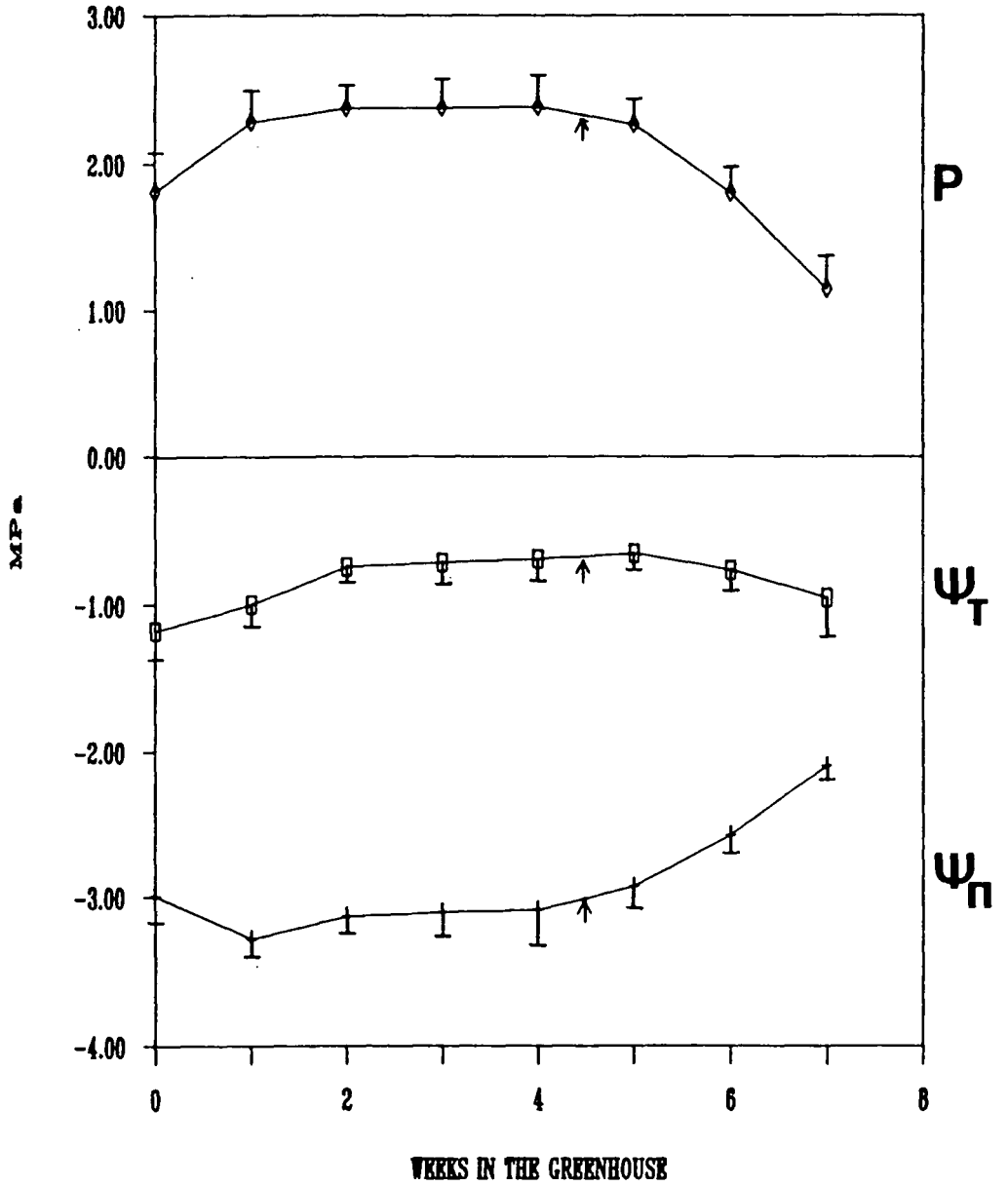

Fig. 4. Mean total water potentials, osmotic potentials and turgor pressures for 7 weeks for the experiment Established Graft Water Relations. Arrows indicate mean bud break. Vertical bars indicate standard errors.

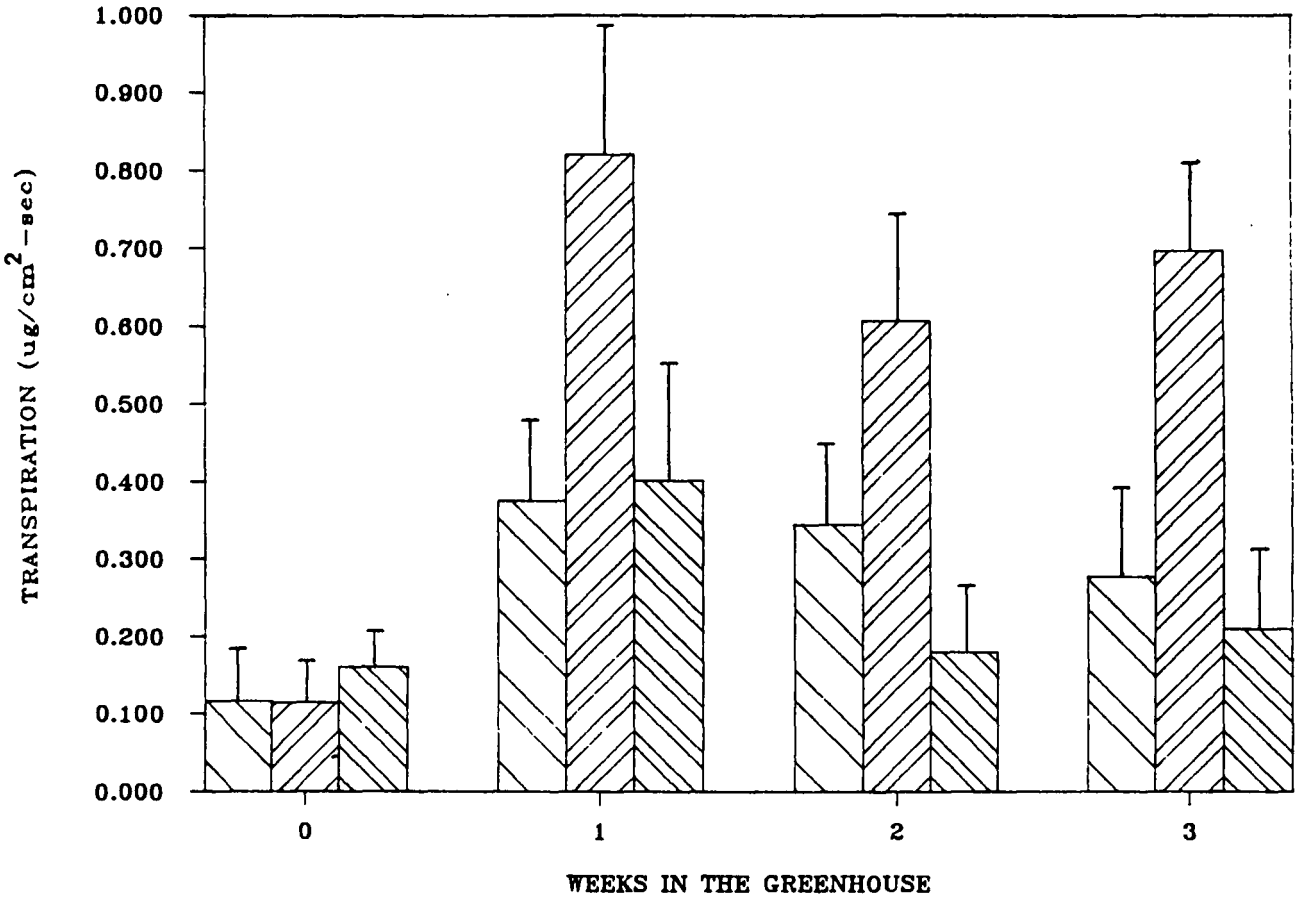

Fig. 5. Mean transpiration rates of the trees of in the experiment Established Graft Water Relations, prior to bud break. Measurements were made at 0930 ( ), 1300 ( ) and 1700 ( ). Vertical bars indicate standard errors.

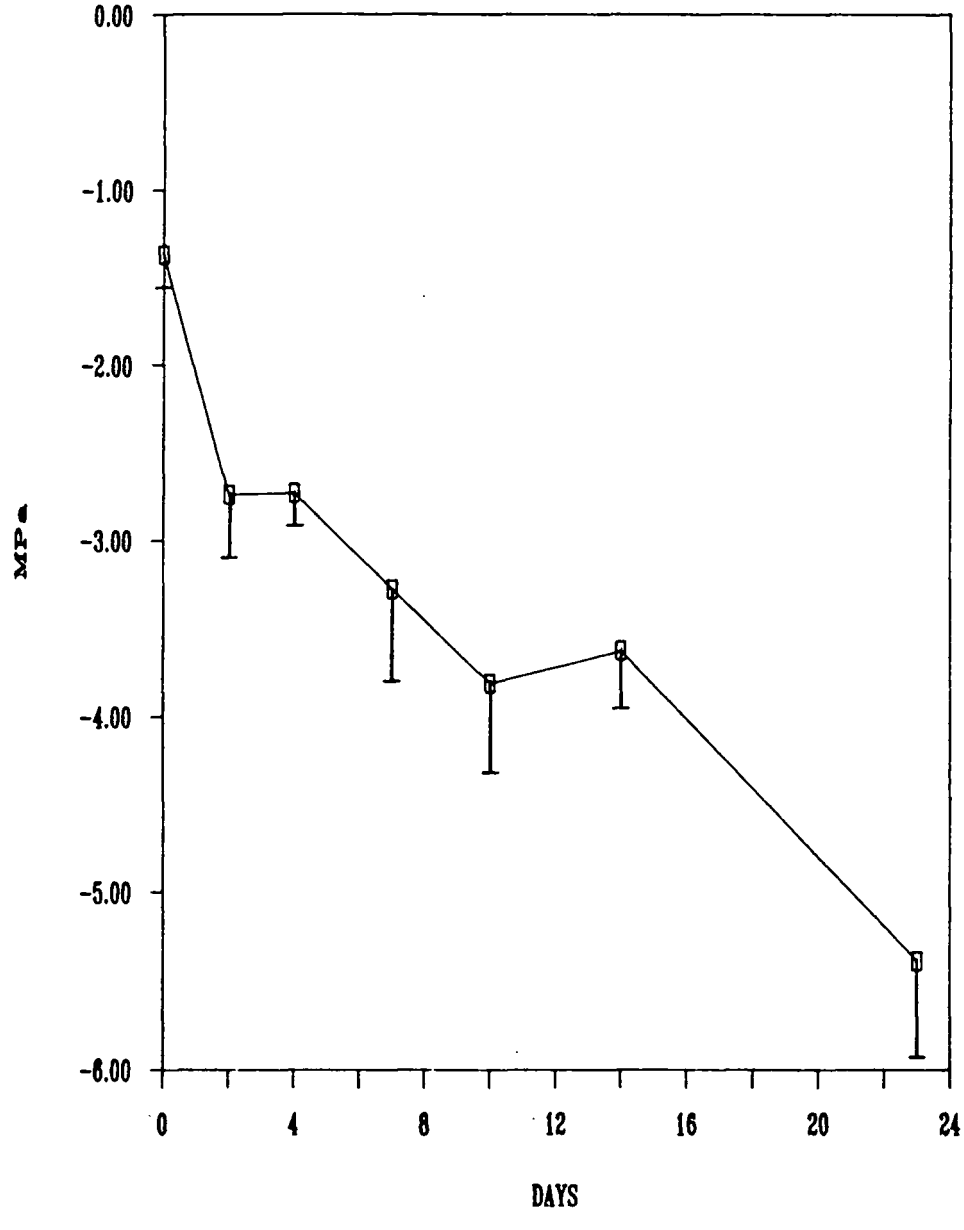

Fig. 6. Mean total water potentials for the twigs in the experiment Greenhouse Twig Transpiration. Vertical bars indicate standard errors.

rates appear to decline during the first four days (Fig. 7), but rose to a measured maximum on day 7, concurrent with Ψ_T measured at -3.28 MPa. By day 10, transpiration had declined to a constant level while Ψ_T continued to decline.

When grafts were intentionally misaligned (MGWR, Fig. 8), declined to -2.3 MPa during the first two weeks. Over the next four weeks, Ψ_T remained near -2.5 MPa, before again declining. Osmotic potentials declined linearly during the eight weeks. After two weeks, P were maintained at 1.0 to 1.3 MPa during the measurement period due to the linear decline in Ψ_π.

The data for the 21.1° and 26.7° treatments in the growth chambers (T&T) were divided into successful and unsuccessful grafts. At 21.1° C, total water potentials declined the first two weeks to a minimum of -1.79 MPa (Fig. 9). Subsequently the Ψ_T of the successful grafts increased during the remainder of the measurement period, while that of the unsuccessful grafts continued to decline, but at a slower rate. Osmotic potentials remained relatively constant for the first two weeks, then increased in the successful grafts (3 weeks prior to bud break), but declined in the unsuccessful grafts. Turgor pressures were constant after an initial decline, until week 5, when the P of successful grafts declined to 0.7 MPa while unsuccessful P increased to 1.47 MPa. Differences in Ψ_T and Ψ_π between successful and unsuccessful were significant after week 2, but differences in P were not significant until the fifth week (Appendix 2.10).

Transpiration rates at 21.1° C declined during the first 2 weeks, then increased thereafter (Fig. 10). Only the transpiration rates of the successful grafts at 0815 hr changed significantly between weeks 4

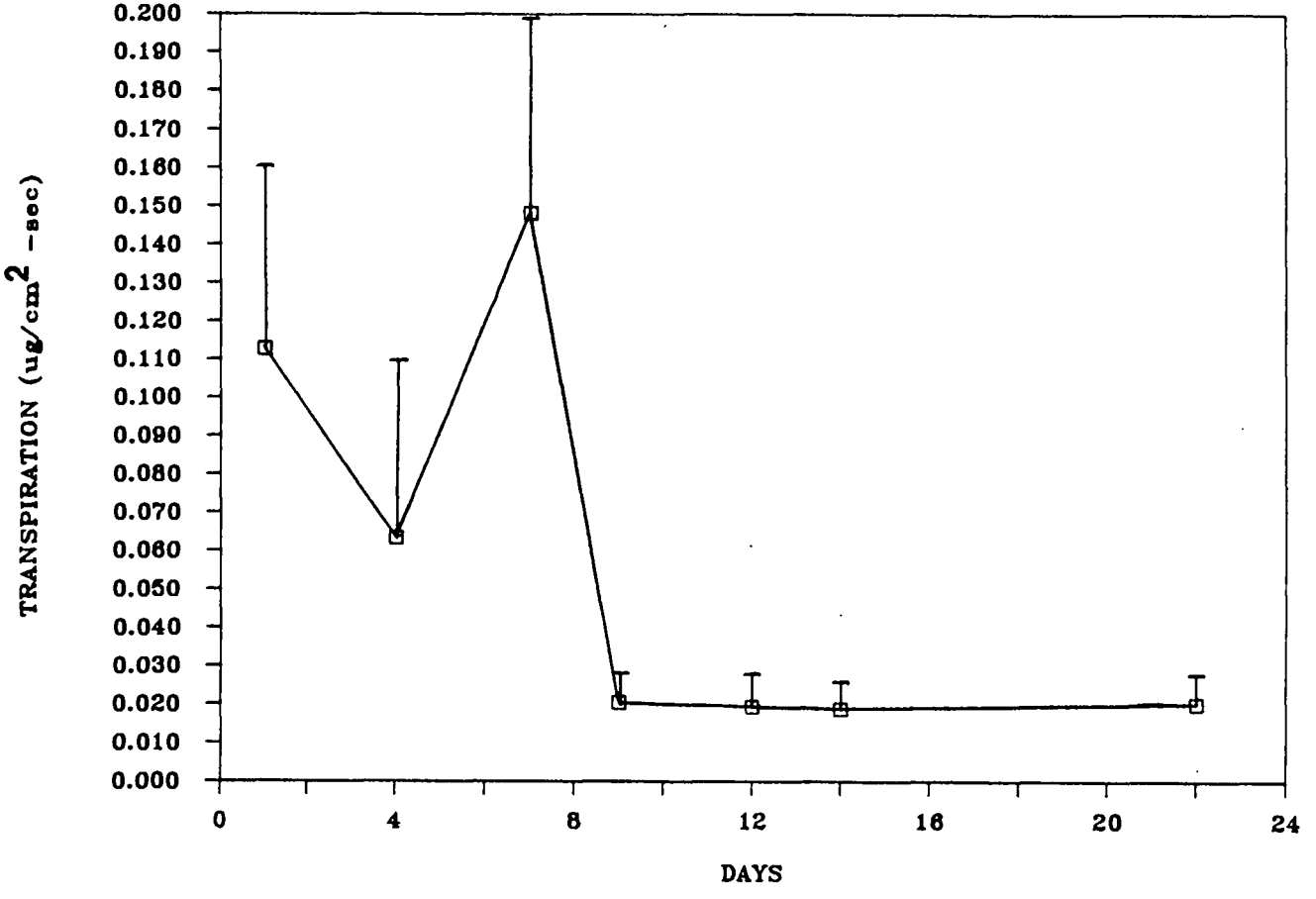

Fig. 7. Mean transpiration rates for the twigs in the experiment Greenhouse Twig Transpiration. Vertical bars indicate standard errors.

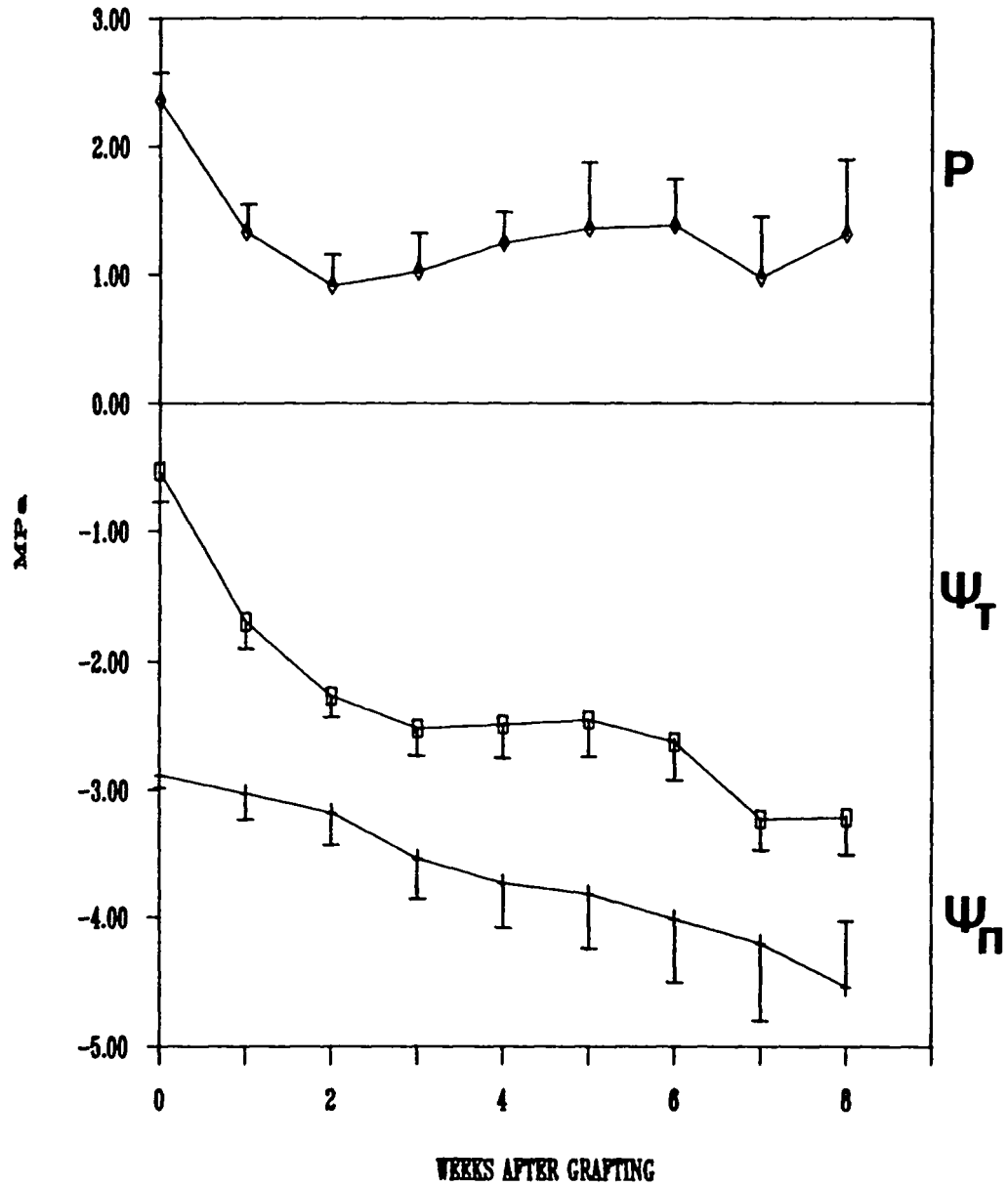

Fig. 8. Mean total water potentials, osmotic potentials and turgor pressures for eight weeks after grafting for the experiment Mis-aligned Graft Water Relations. Vertical bars indicate standard errors.

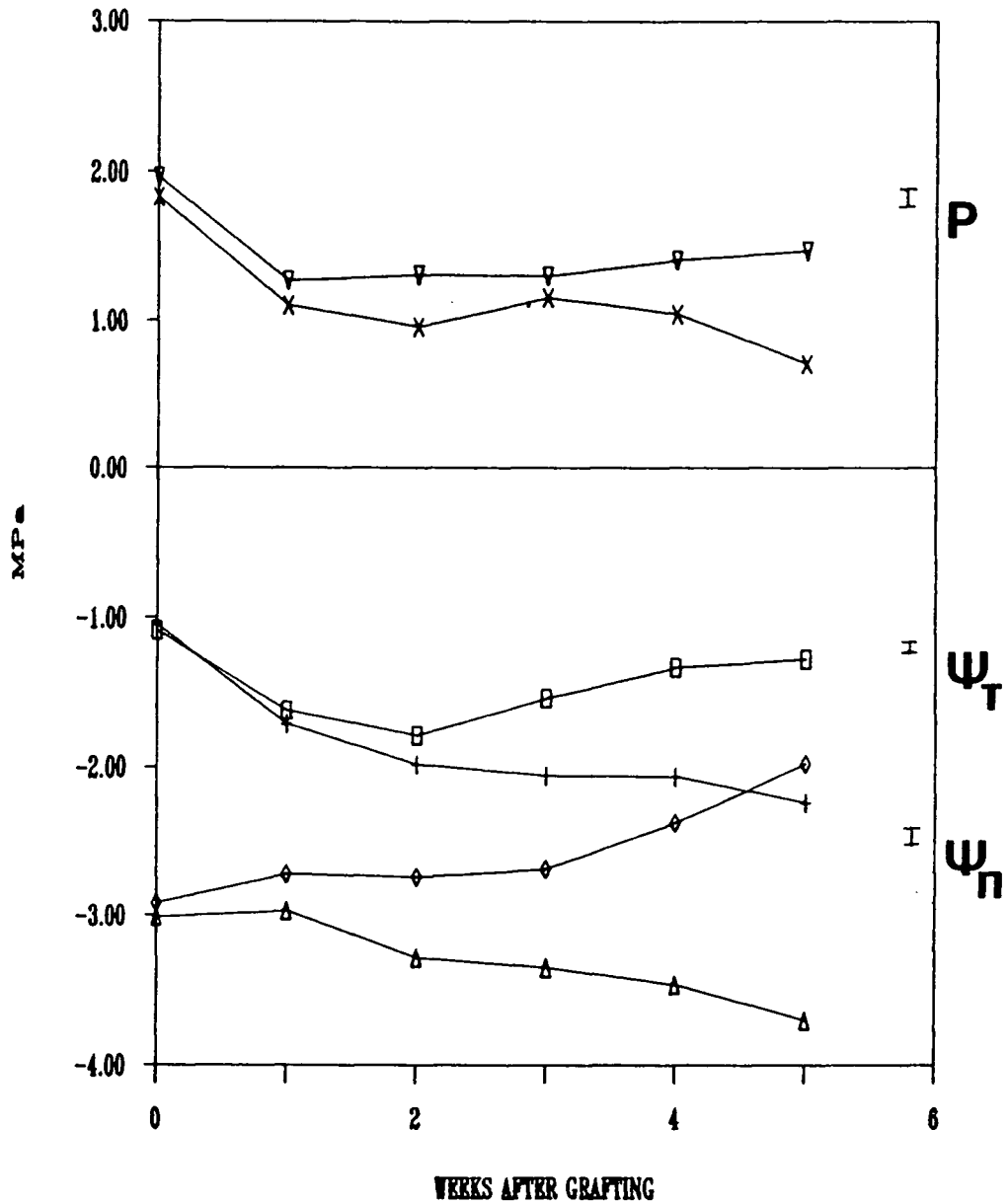

Fig. 9. Mean total water potentials, osmotic potentials and turgor pressures prior to bud break for the 21.1° C treatment of Temperature and Transpiration. Scions were partitioned into successful (□, ◇, X) and unsuccessful (+, △, ▽) grafts. Vertical bars indicate standard errors.

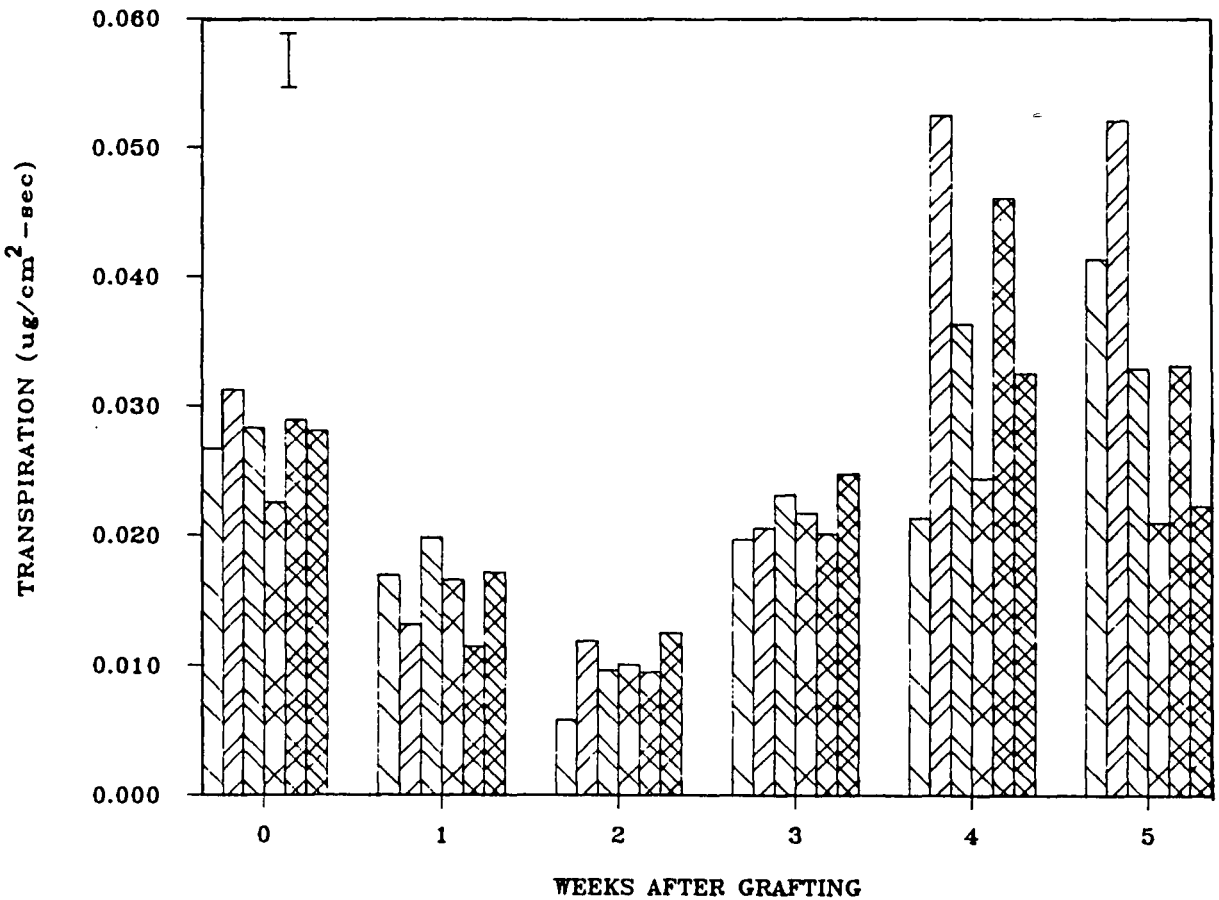

Fig. 10. Mean transpiration rates prior to bud break for the 21.1° C treatment of Temperature and Transpiration. Transpiration rates were partitioned into successful (0815, ▨, 1200, ▨, 1600, ▨) and unsuccessful (0815, ▨, 1200, ▨, 1600, ▨) grafts. The vertical bar indicates the standard error.

and 5 (Appendix 2.11). Transpiration rates of successful and unsuccessful grafts were significantly different only at week 5 at 0815 hr and 1600 hr, but at both weeks 4 and 5 at 1200 hr. Transpiration rates of the successful grafts were correlated with Ψ_T. Decreases in Ψ_T the first week, resulted in decreases in the transpiration rates. After week 2, increases in Ψ_T brought increases in the transpiration rate, with marked increases occurring above -1.4 MPa (Fig. 11). Transpiration rates of the unsuccessful grafts were also correlated with Ψ_T the first two weeks, but thereafter transpiration rates continued to increase as Ψ_T declined further (Fig. 12). Transpiration rates during the course of the day were not significantly different during the first three weeks, but thereafter the highest rates were recorded at 1200 hr (Appendix 2.11).

Unlike at 21.1° C, Ψ_T of successful grafts for the 26.7° treatment (Fig. 13) reached a minimum the first week. After the second week, Ψ_T increased in the successful grafts, but at a slower rate than at 21.1°; while the Ψ_T of the unsuccessful grafts declined. The Ψ_π of the successful grafts began increasing after 2 weeks (three weeks prior to bud break). The Ψ_π of unsuccessful grafts declined continuously after the first week. Both Ψ_T and Ψ_π were significantly different between successful and unsuccessful grafts after the first week; P were not significant (Appendix 2.12). Turgor pressures remained steady between 1.0 to 1.37 MPa after an initial decline.

Transpiration rates at 26.7° C were ten-fold higher than at 21.1°. Minimum transpiration rates occurred during weeks 1 and 2 when the Ψ_T of the successful grafts were lowest (Fig. 14). Transpiration

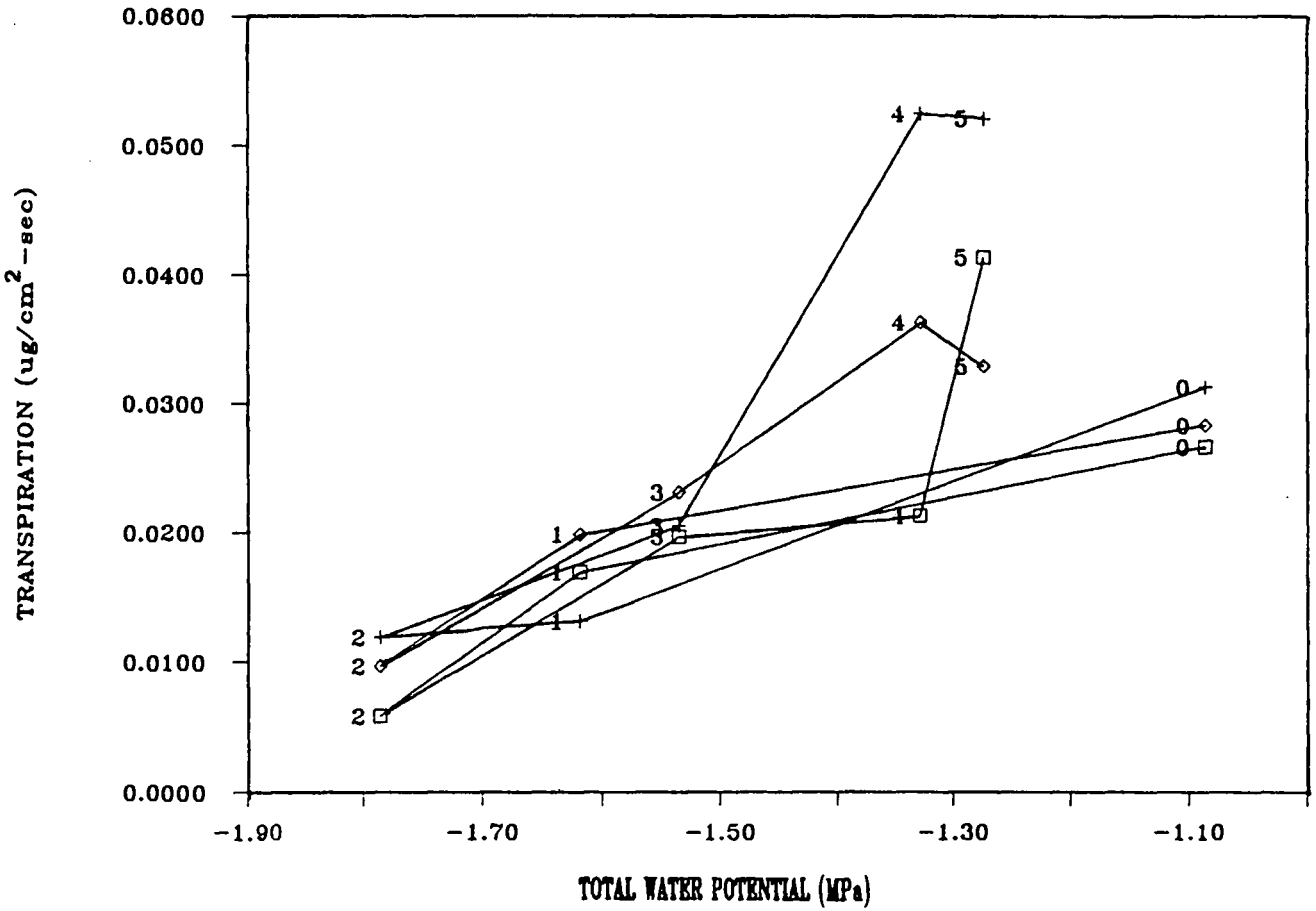

Fig. 11. Mean transpiration rates of the successful grafts from the 21.1° treatment of Temperature and Transpiration plotted against their total water potentials. Mean rates at 0815 (□), 1200 (+) and 1600 (◇) are shown. The number beside each point indicates the week of measurement.

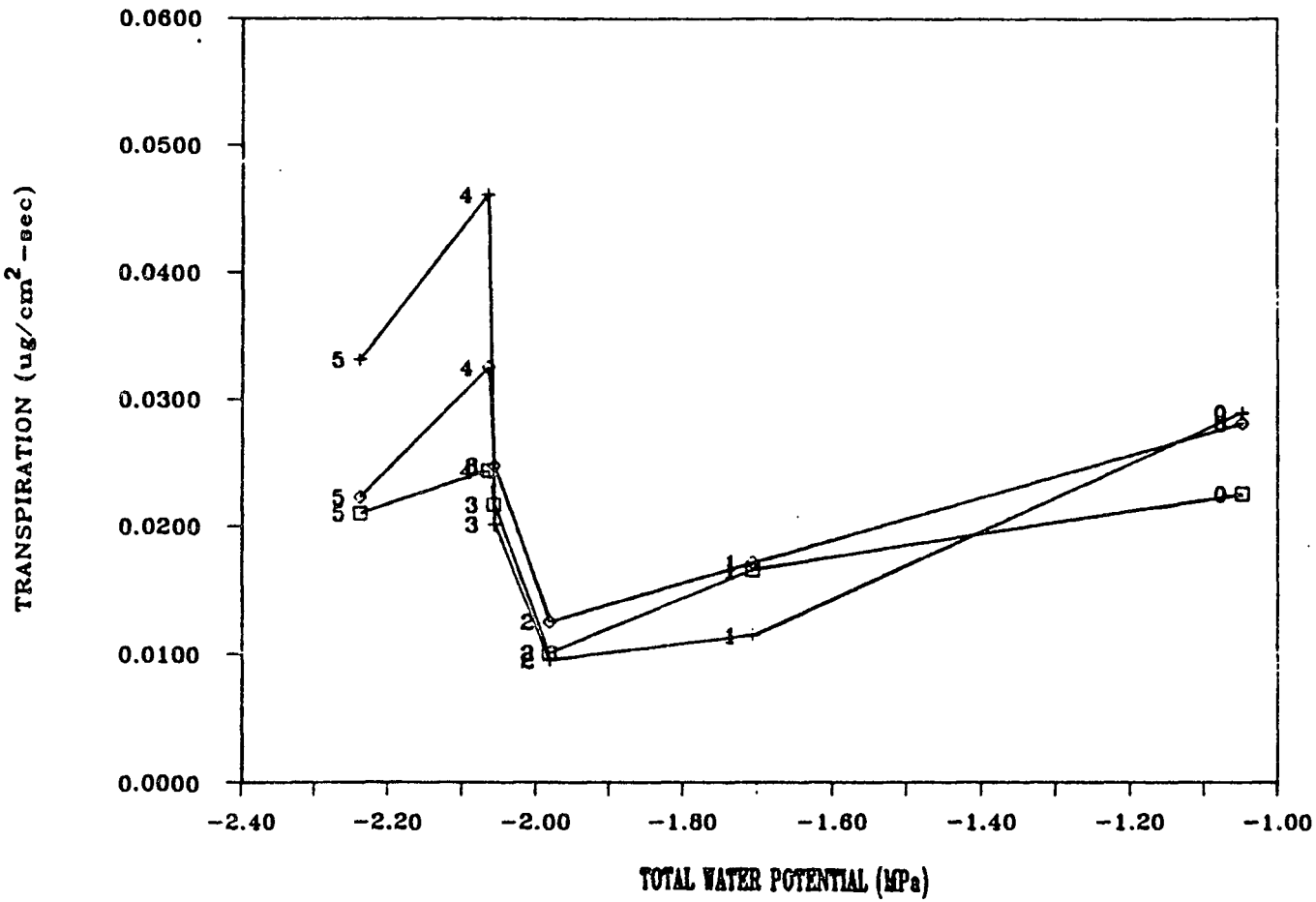

Fig. 12. Mean transpiration rates of the unsuccessful grafts from the 21.1° treatment of Temperature and Transpiration plotted against their total water potentials. Mean rates at 0815 (□), 1200 (+) and 1600 (◇) are shown. The number beside each point indicates the week of measurement.

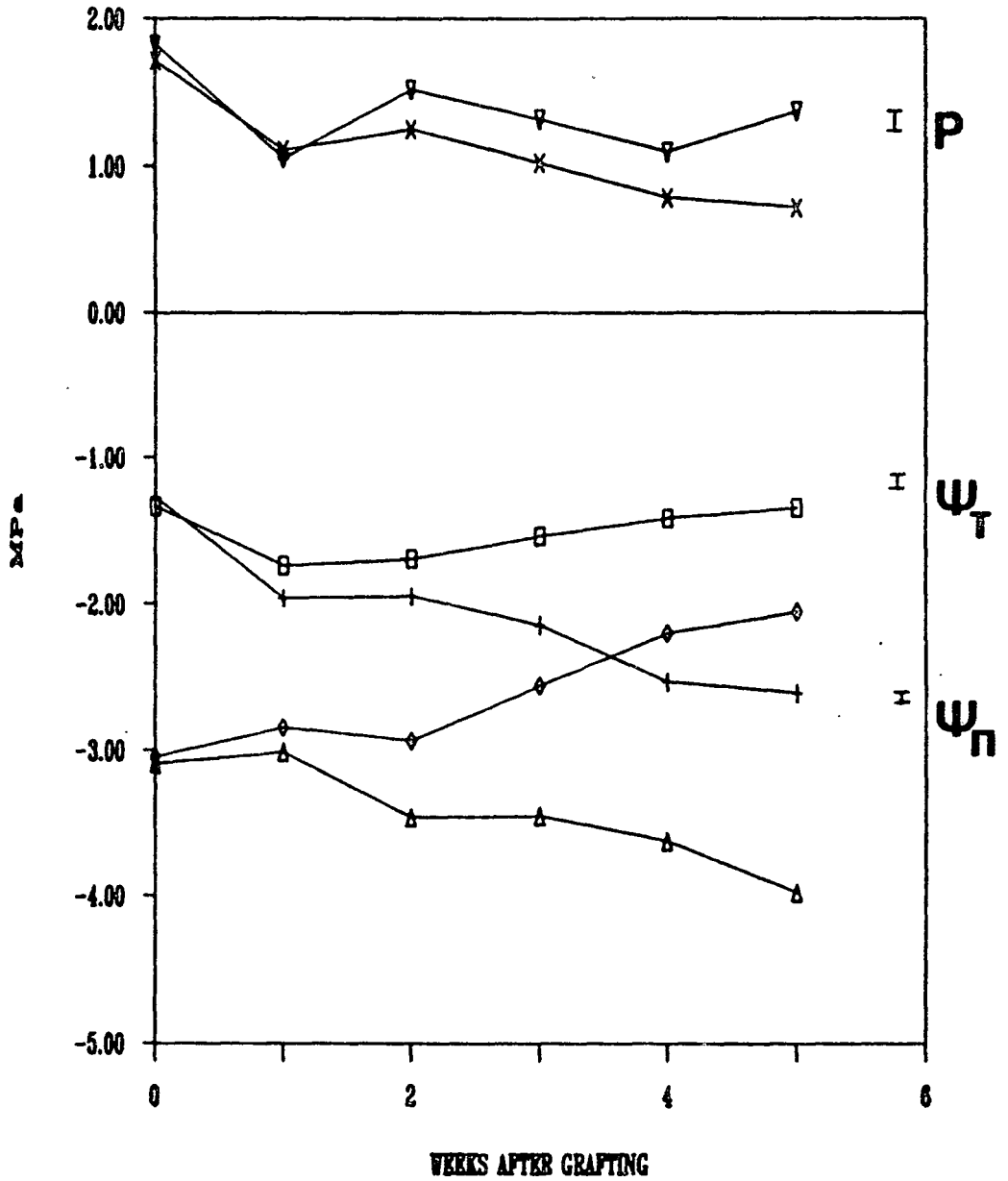

Fig. 13. Mean total water potentials, osmotic potentials and turgor pressures prior to bud break for the 26.7° C treatment of Temperature and Transpiration. Grafts were partitioned into successful (□, ◇, ⊗ and unsuccessful (+, △, ▽) grafts. Vertical bars indicate standard errors.

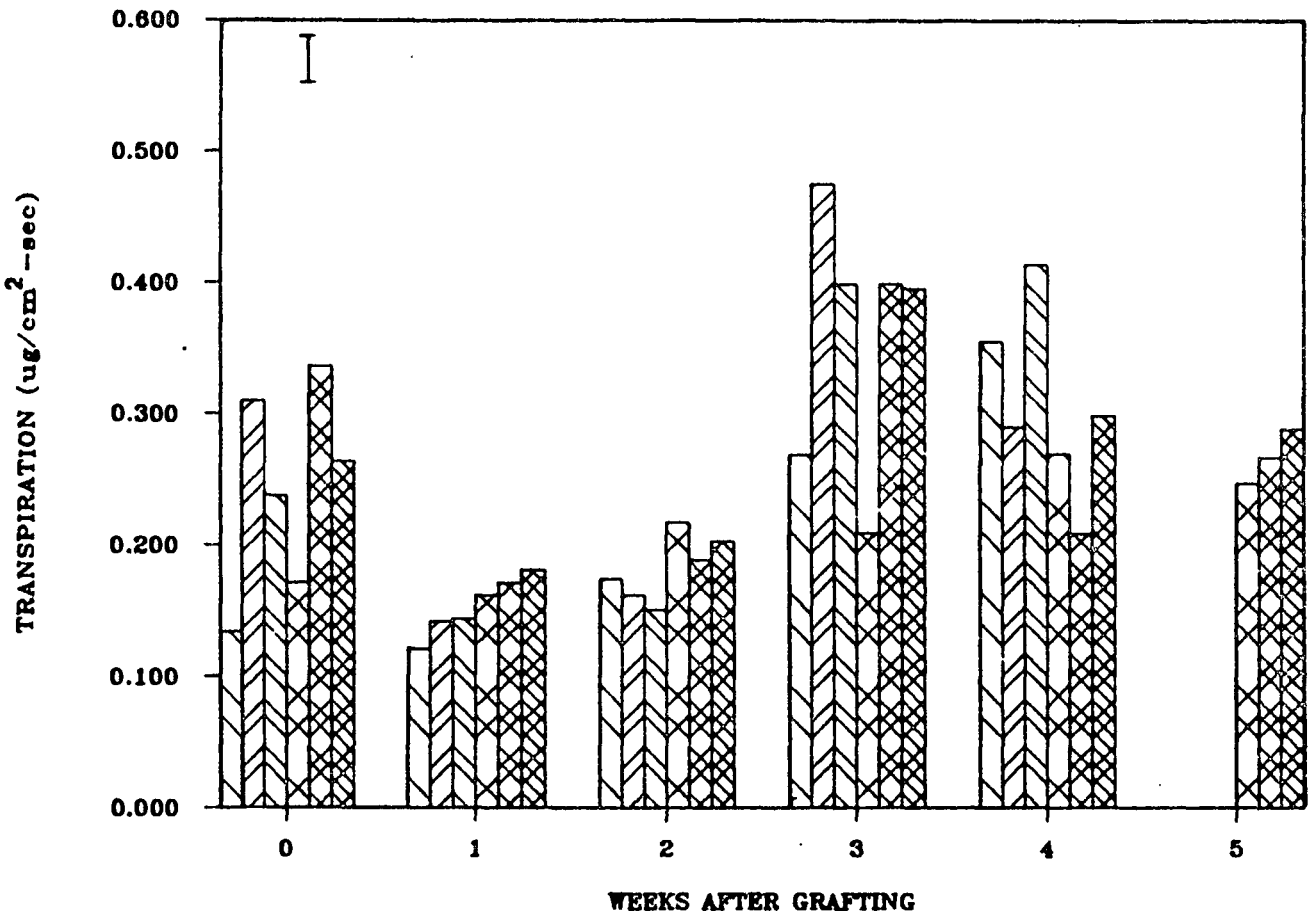

Fig. 14. Mean transpiration rates prior to bud break for the 26.7° C treatment of Temperature and Transpiration. Grafts were partitioned into successful (0815, ▨, 1200, ▨, 1600, ▨) and unsuccessful (0815, ▨, 1200, ▨, 1600, ▨) grafts. The vertical bar indicates standard error.

rates were not significantly different between weeks 1 and 2 (Appendix 2.13). After week 2, transpiration rates increased for all grafts. Transpiration rates between successful and unsuccessful grafts were not significantly different except during the fourth week at 0815 hr and 1600 hr. Transpiration measurements were not made on the successful grafts the fifth week because of bud break. Transpiration rates of successful grafts increased with increasing Ψ_T (Fig. 15), and increased over time with decreasing Ψ_T of unsuccessful grafts (Fig. 16) analogous to the observations at 21.1°.

The analyses of the growth parameters of T&T are in Table 1. Success rates of 33 and 46% were low compared to the average of 80% achieved by untreated grafts in the greenhouse. Only the time required for bud break were significantly different between the two treatments, both of which were lower than that of greenhouse grafts.

In the Scion Water Loss experiment (SWL), water loss from successful scions during union formation reduced the relative water content (RWC) to 83% before recovering (Fig. 17). The RWC declined 13% the first week, gradually reaching a minimum at week 4. The RWC of the unsuccessful grafts parallelled those of the successful grafts during the first 5 weeks, but continued to decline thereafter. In ungrafted twigs, RWC declined continuously during the seven weeks. However, the RWC were not significantly different until after the second week (Appendix 2.14).

The changes in the Ψ_T of the successful grafts (SWL) were similar to those described previously for greenhouse grafts (Fig. 18). The Ψ_T of unsuccessful grafts were generally lower, but not significantly different from those of the successful grafts until after week 5

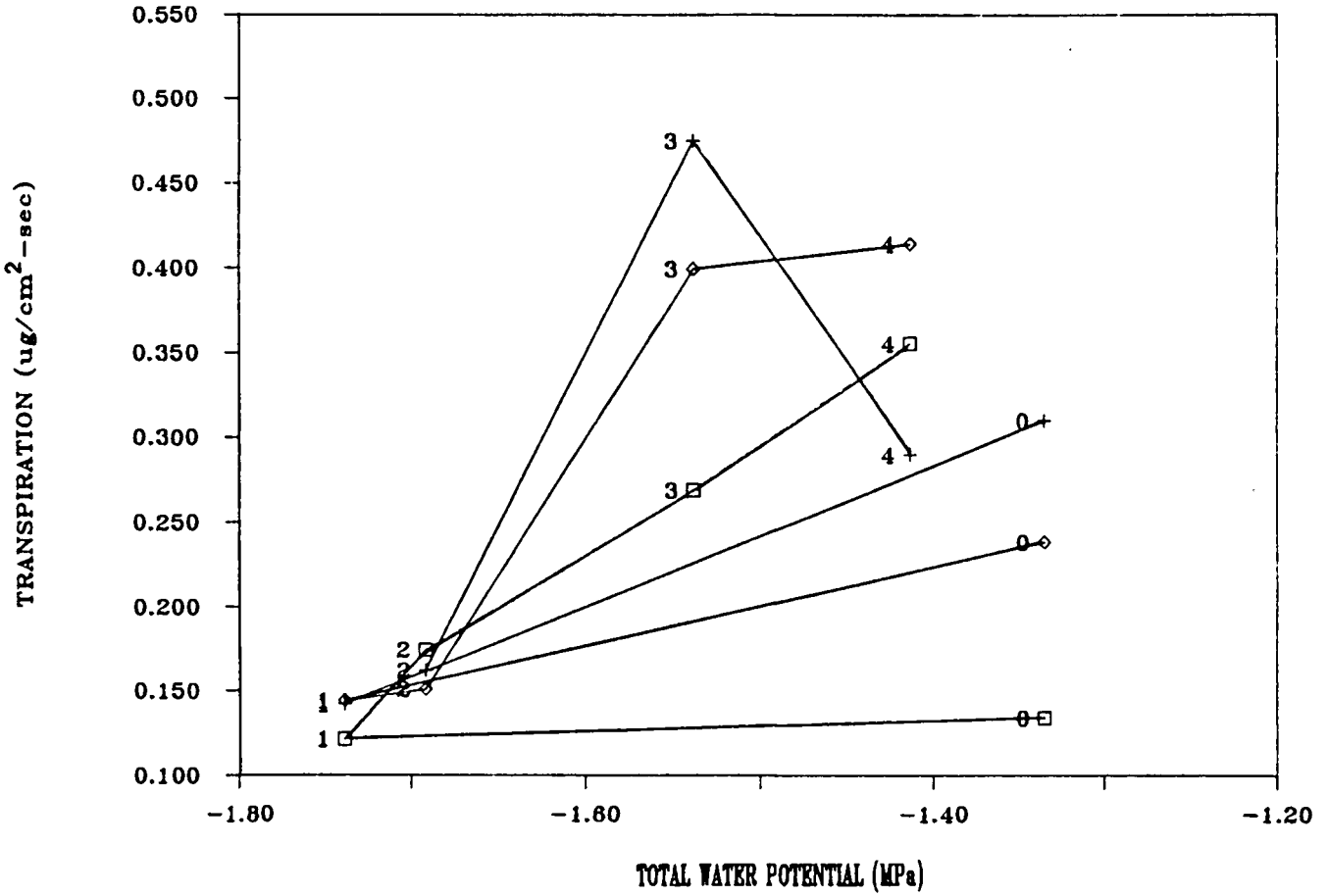

Fig. 15. Mean transpiration rates of the successful grafts from the 26.7° treatment of Temperature and Transpiration plotted against their total water potentials. Mean rates at 0815 (□), 1200 (+) and 1600 (◇) are shown. The number beside each point indicates the week of measurement.

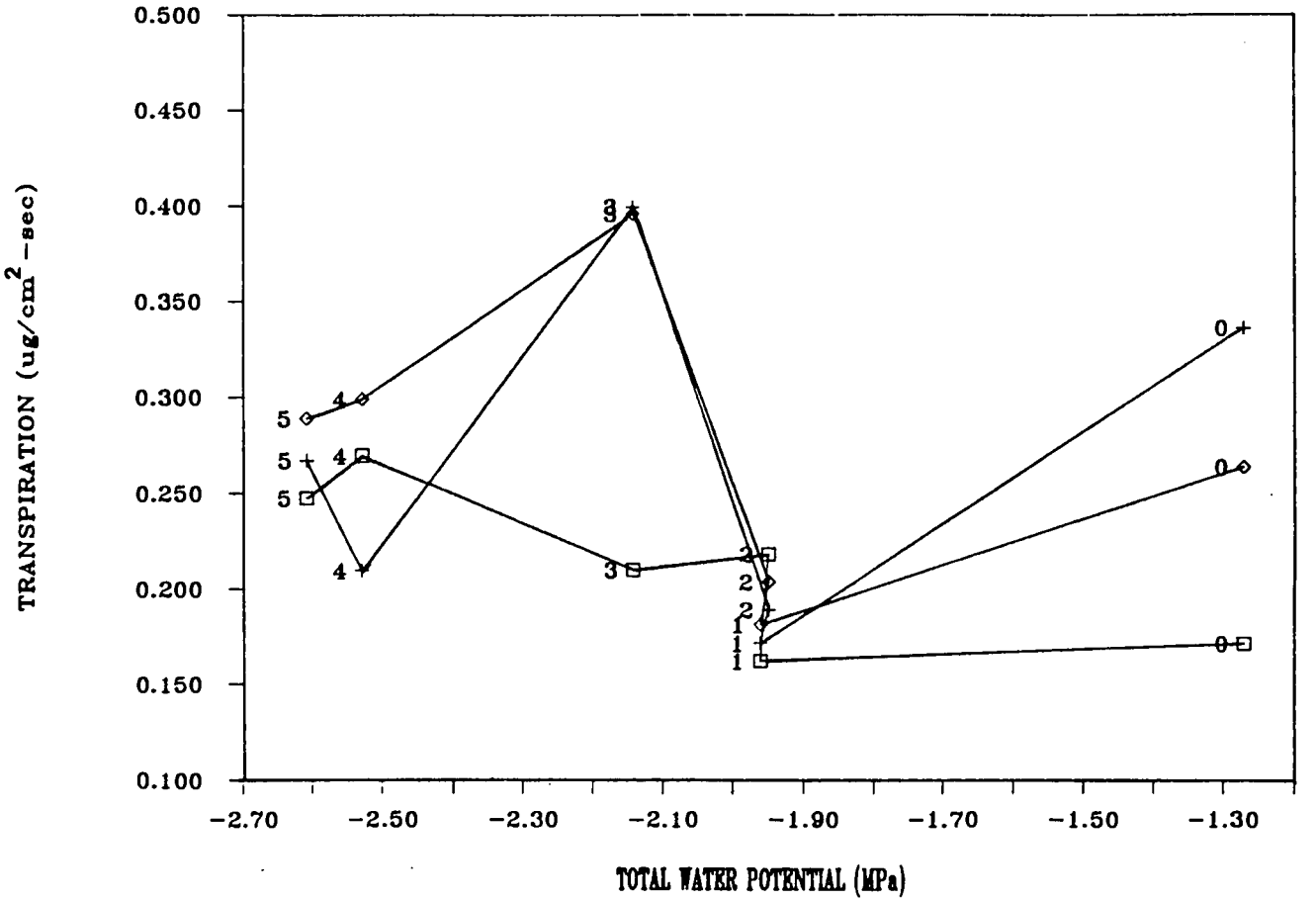

Fig. 16. Mean transpiration rates of the unsuccessful grafts from the 26.7^{o} treatment of Temperature and Transpiration plotted against their total water potentials. Mean rates at 0815 (□), 1200 (+) and 1600 (◇) are shown. The number beside each point indicates the week of measurement.

Table 1. Means of growth parameters of Temperature and Transpiration. Means with the same letter are not significantly different at the 5% level as separated by Protected LSDs. Growth parameters are given in cm, bud break as days after grafting.

Treatment	% Success	Bud Break	Average Branch	Growth/ cm Scion	Branch Number	Terminal Length	Total Growth
21.1	33.3	43.7±4.6 a	6.0±1.5	2.1±1.0	5.8±2.5	4.0±2.8	36.4±16.4
26.7	45.8	37.8±4.7 b	5.8±0.8	2.4±0.7	7.3±1.7	5.5±2.8	42.8±12.9

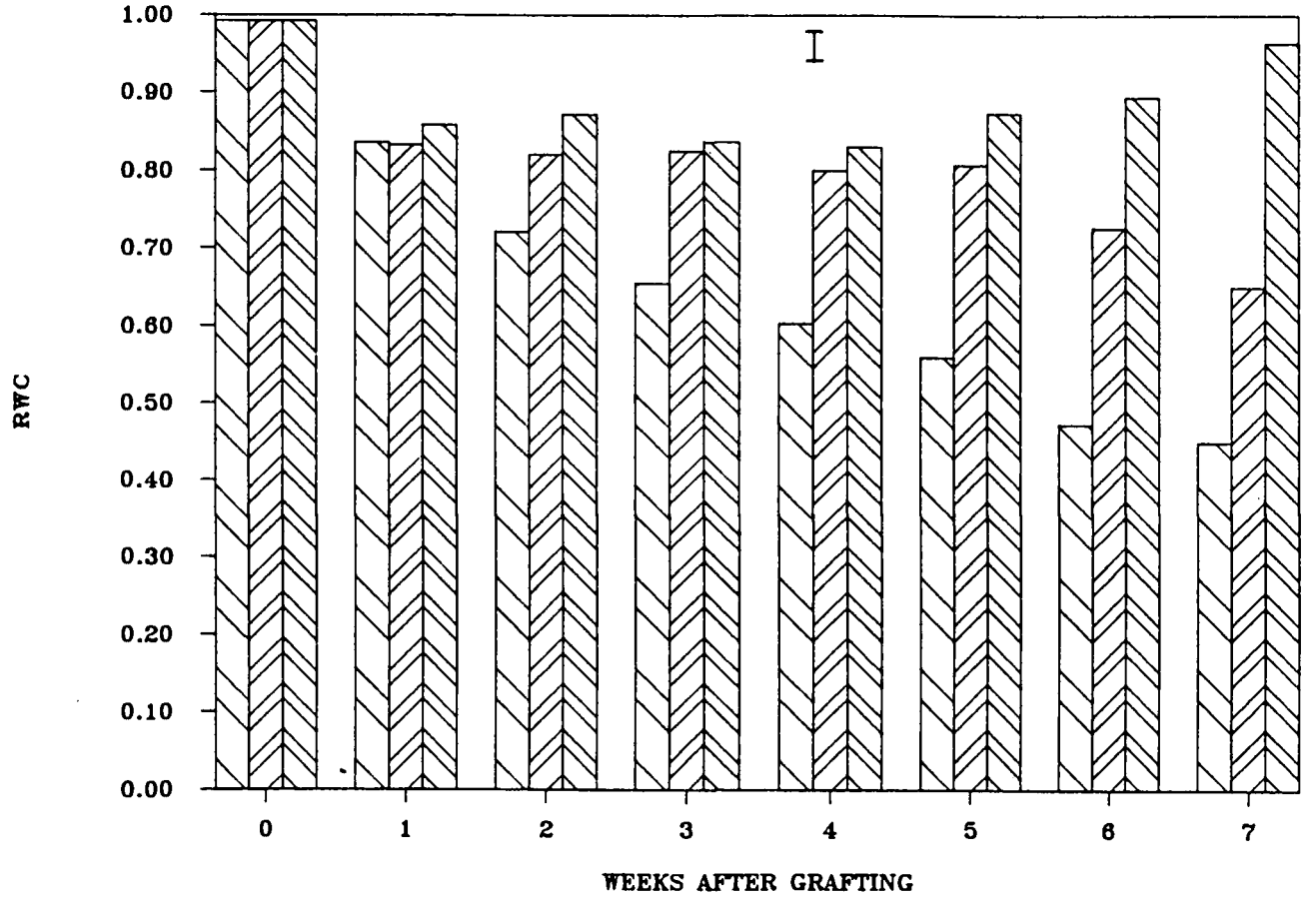

Fig. 17. Mean relative water contents from grafting until bud break for the treatments of Scion Water Loss. Treatments are represented as (▨) successful, (▨) unsuccessful and (▨) ungrafted twigs. Vertical bars indicate standard errors.

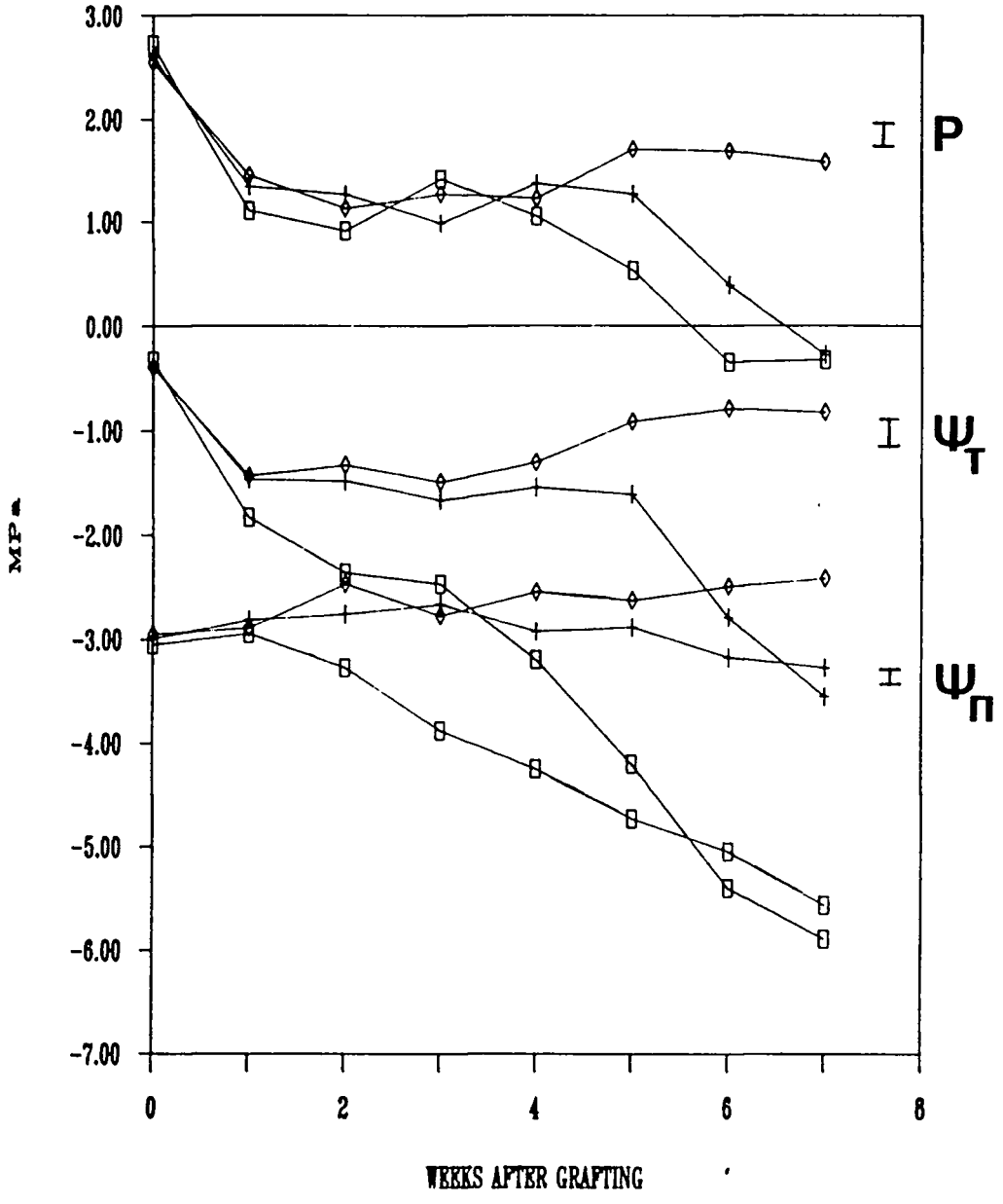

Fig. 18. Mean total water potential, osmotic potential and turgor pressures until bud break for the treatments of Scion Water Loss. Treatments are respresented as (◇) successful, (+) unsuccessful and (□) ungrafted twigs. Vertical bars indicate standard errors.

(Appendix 2.14), when Ψ_T of the unsuccessful grafts declined markedly. The Ψ_T of the detached twigs declined continuously, becoming significantly different from those of the scions after the first week. The Ψ_π of the successful grafts gradually increased over the seven weeks while those of the unsuccessful grafts declined after week 3. Twig Ψ_π declined linearly after the first week, becoming significantly different from the scions thereafter (Appendix 2.14). Turgor pressures for the three treatments declined rapidly the first week, then stabilized and were not significantly different between treatments until after week 4. Osmotic adjustment maintained P above 0.5 MPa through week 5, thereafter, more rapid declines in Ψ_T than Ψ_π resulted in negative P the last two weeks for the twigs and the last week for the unsuccessful grafts. Turgor pressures of the successful grafts remained high (1.59 MPa) at the onset of bud break, which began at week 6.

Dye was first recovered in small amounts from the 'Hoopsi' scions (Dye-83) by week 4 (Fig. 19). The dye content for weeks 5 through 8 was not significantly different, even though this time spanned 1 week prior to bud break to two weeks of shoot elongation. The Ψ_T for weeks 4 and 5 were near -1.4 MPa (Fig. 20). At week 6, bud break was recorded and the Ψ_T were greater than -1.0 MPa.

The correlation of dye content with scion bud break in the scions of other species (Dye-84) was not as well defined. As shown in figure 21, trace amounts of dye were found in all species after three weeks, with the exception of Pinus at week 6. Apparent mean bud break occurred around week 7 for all species. The exact date is questionable for Pinus because a distinguishing bud break

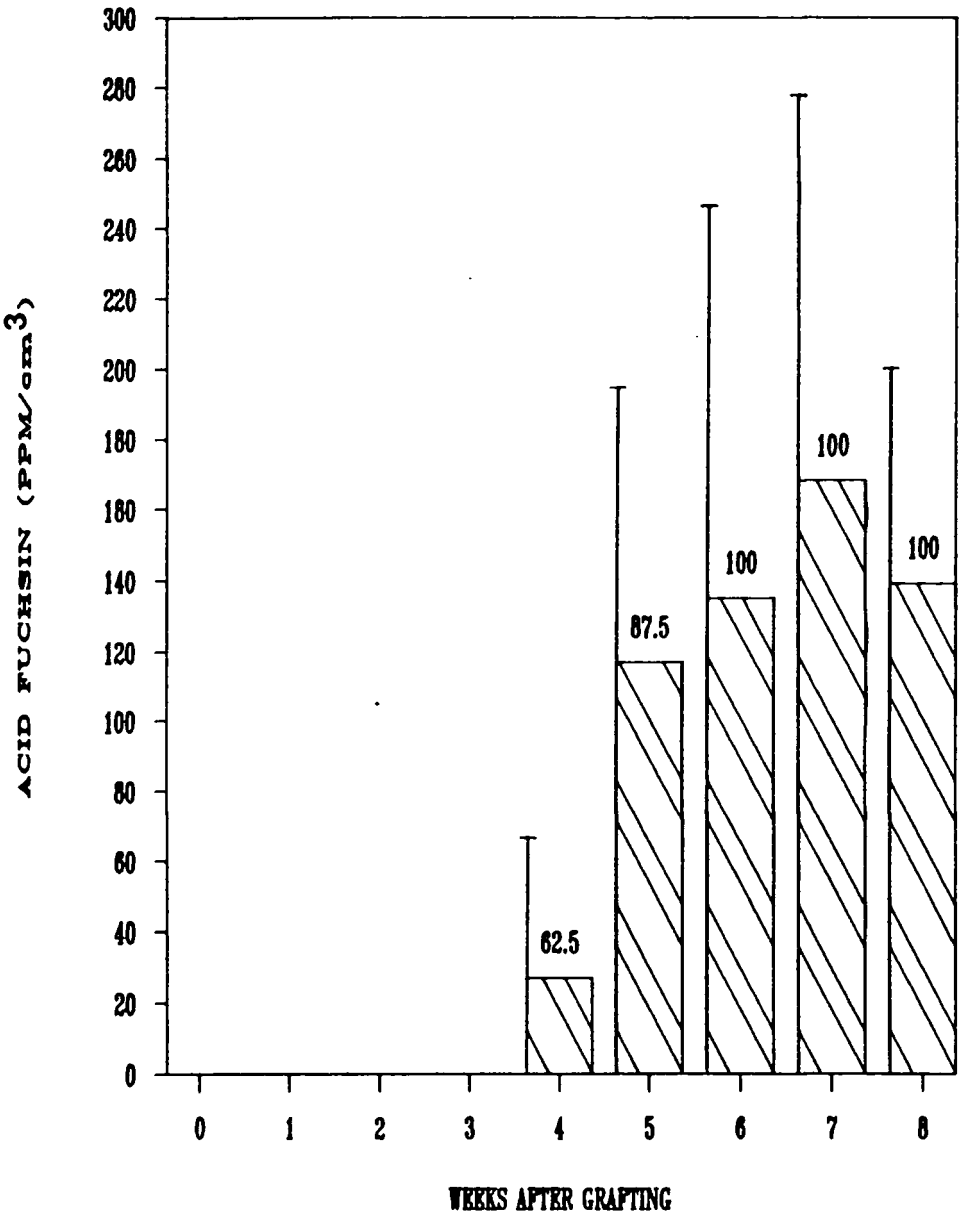

Fig. 19. Dye content for 8 weeks for the spruce scions of experiment Dye 1983. Vertical bars indicate standard errors. The numbers indicate days after bud break.

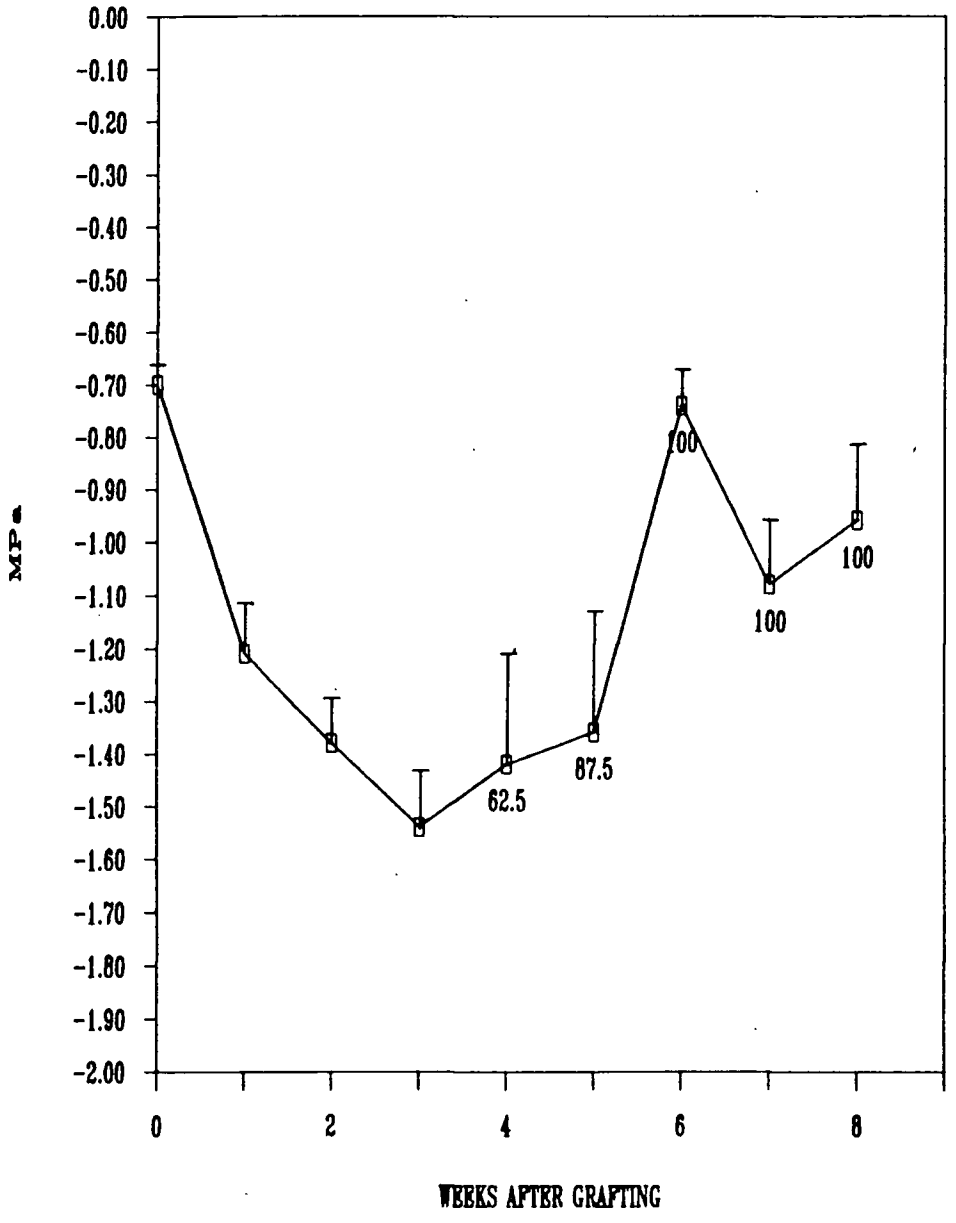

Fig. 20. Mean total water potential for 8 weeks for the spruce grafts of Dye 1983. Vertical bars indicate standard errors. The numbers indicate the percentage of grafts with dye.

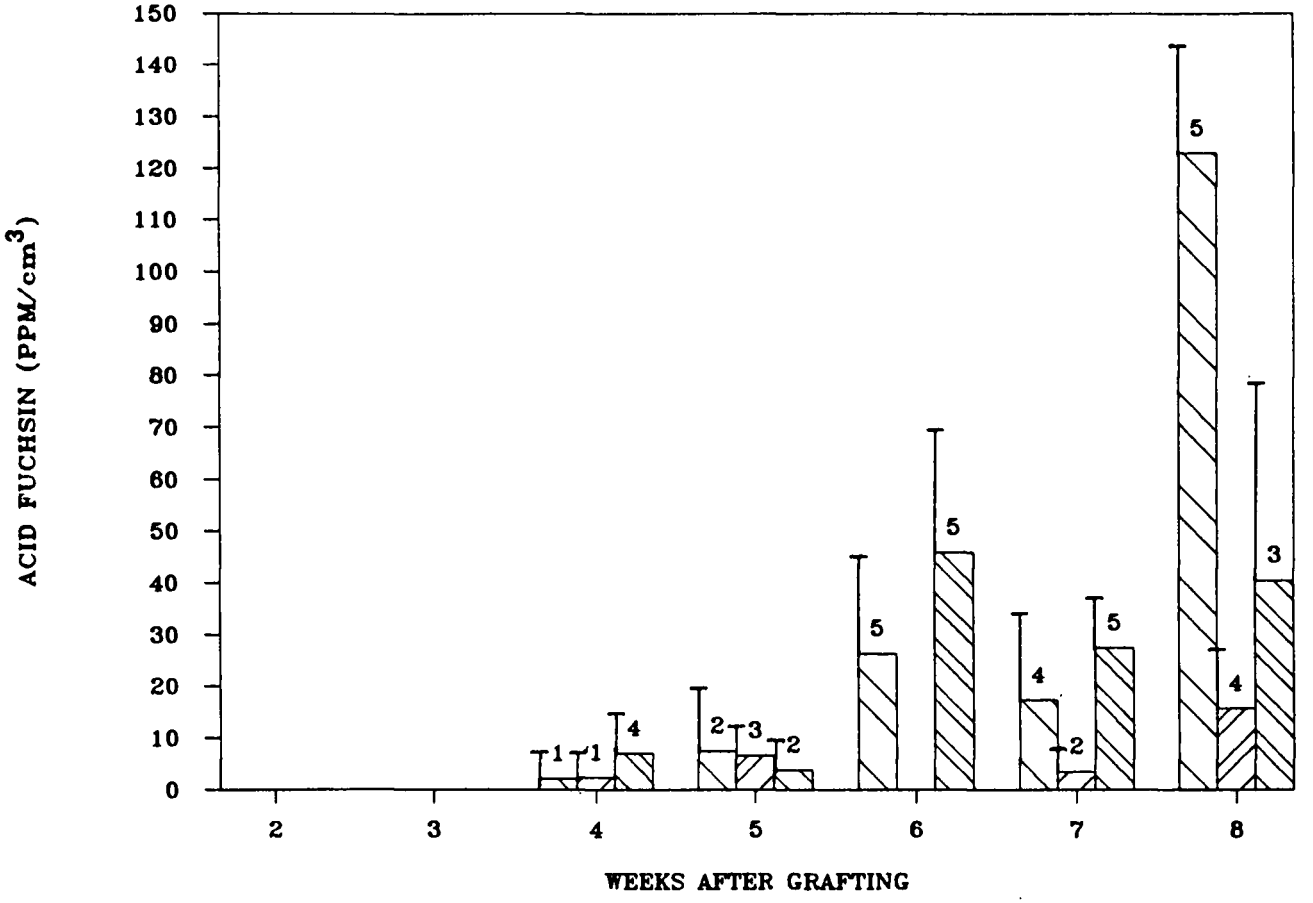

Fig. 21. Dye content of Abies, Pinus, and Taxus scions for 8 weeks after grafting for experiment Dye 1984. Means are represented as ( ) Abies, ( ) Pinus and ( ) Taxus. The numbers indicate the number of scions with dye content (of 5). Vertical bars indicate standard errors.

characteristic was not well defined. For the Abies grafts, dye content in the scions at bud break was not significantly different from that found two weeks prior to bud break. Similar results were observed for Taxus but one week earlier for the Pinus grafts.

Water stress in lath house grafts was higher in 1985 (Dye-85, Fig. 22) than in 1984. Total water potential dropped rapidly the first three weeks and remained between -1.6 to -1.8 MPa the following seven weeks, after week 10 increased, reaching -1.2 MPa by week 15. Scion bud break occurred 17.5 weeks after grafting. Dye content was observed in the scions beginning at week 13 (Fig. 23), approximately two weeks after 50% of the rootstock buds had broken. Dye content was correlated with Ψ_T of greater than -1.2 MPa, and was not significantly different between weeks.

Carbon Metabolism

In conjunction with the measurements of water relations, total starch content of the needles was measured in several experiments. The analysis of the starch content for GWR-83 is shown in figure 24. Starch levels increased rapidly the first week, then the rate of accumulation slowed to peak at week 6, approximately 14 mg/g Fwt for G-1 and G-2. Group 1 starch content declined rapidly 1 week prior to bud break and continued until starch levels were approximately 2.0 mg/g Fwt. Similar rapid declines occurred in Groups 2 and 3 around bud break. Starch content in G-4 also dropped rapidly between weeks 6 and 7 but without concurrent bud break. The G-4 grafts generally had

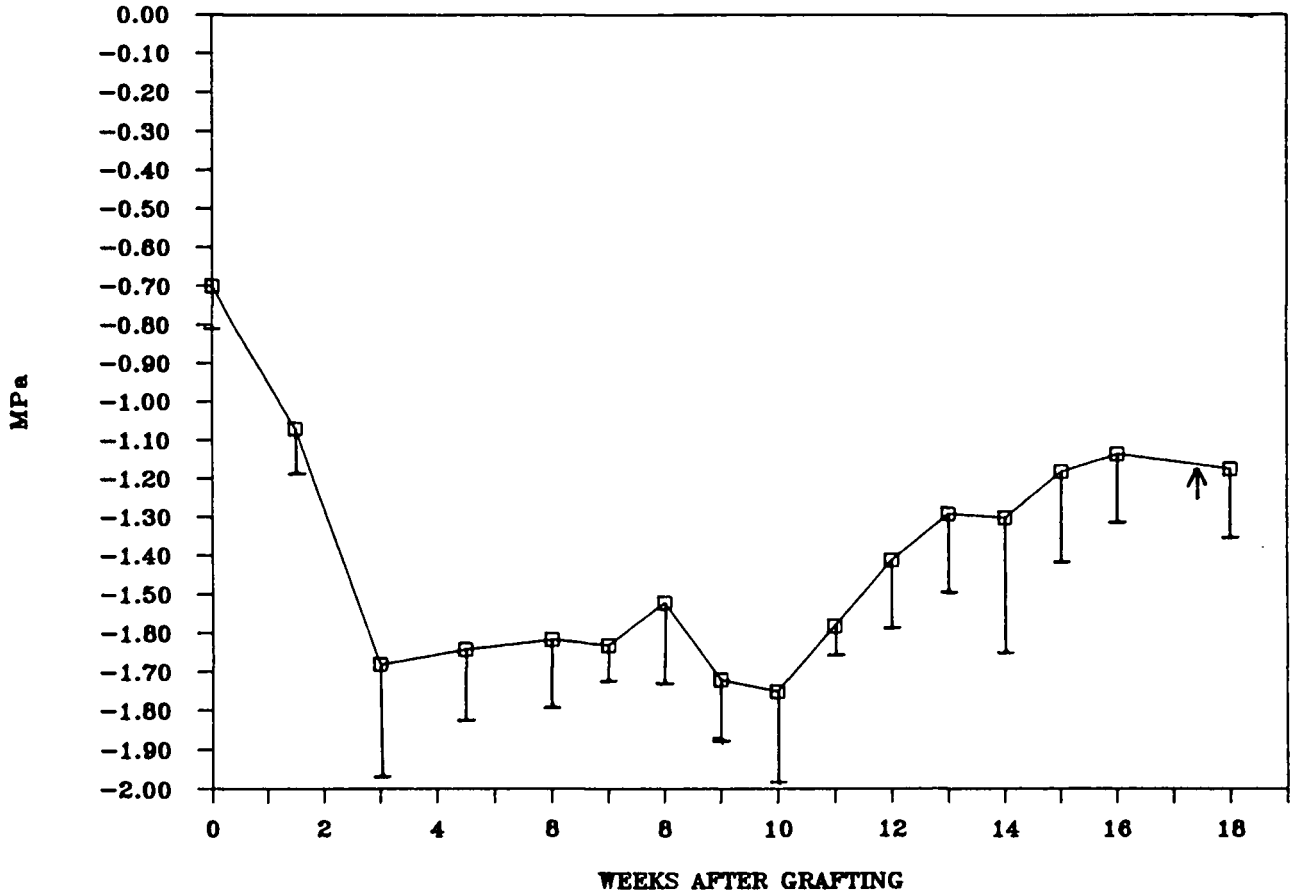

Fig. 22. Mean total water potentials for Lath house spruce grafts from Dye 1985. The arrow indicates bud break. The vertical bars indicate standard errors.

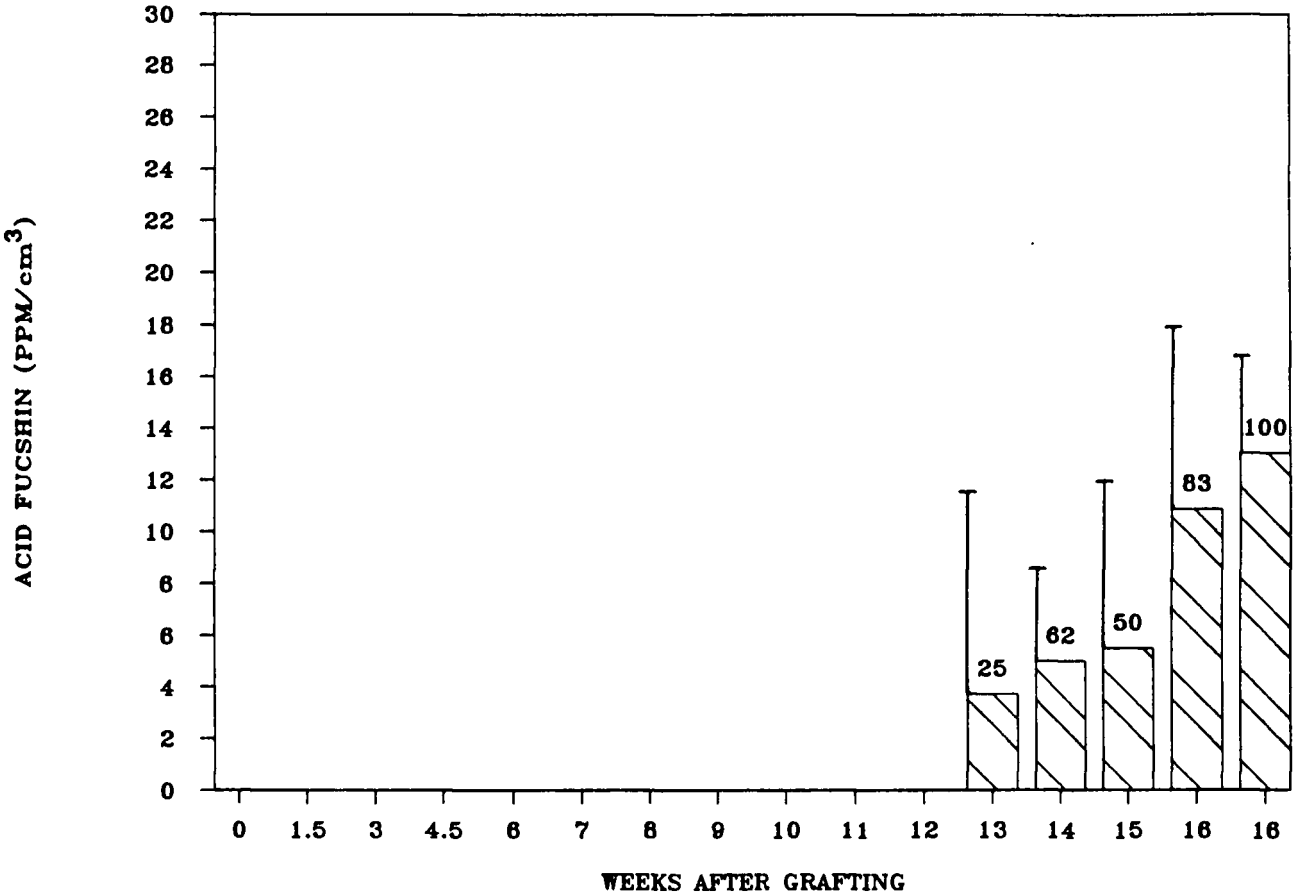

Fig. 23. Dye content for the spruce scion segments for Dye 1985. The numbers indicate the percentage of scions with dye. Vertical bars indicate standard errors.

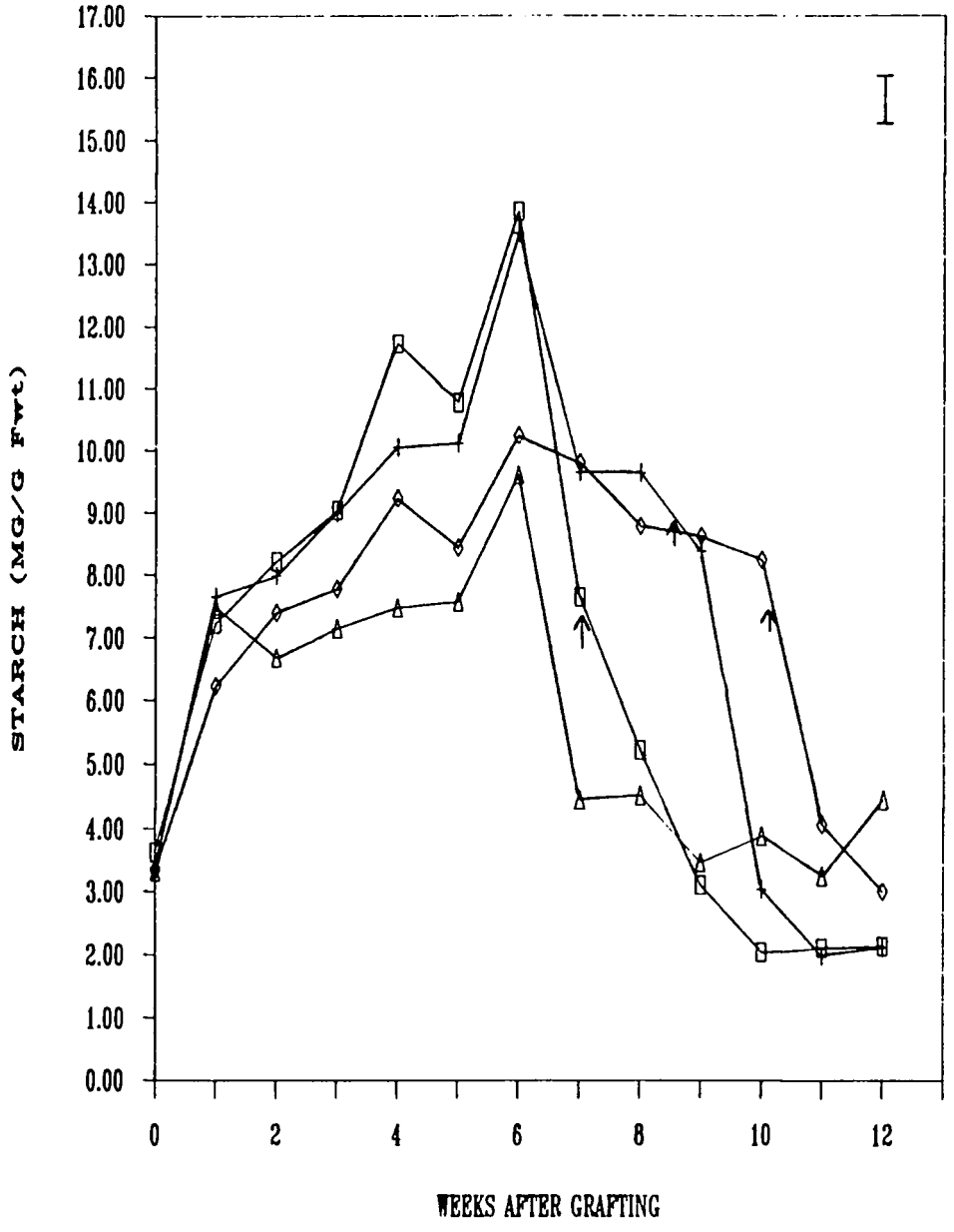

Fig. 24. Needle starch content for 12 weeks for the experiment Greenhouse Water Relations 1983. Group 1 (□) scions required 46-54 days for bud break, Group 2 (+) scions required 56-64 days, Group 3 (◇) required greater than 65 days and Group 4 (△) scions were unsuccessful. Arrows indicate mean bud break. The vertical bar indicates standard error.

slower and lower starch accumulation compared to the successful grafts through the first six weeks. Statistically, there was no significant difference among groups per week, but starch content for Group 4 was significantly lower than for successful grafts and differences between weeks were significant (Appendix 2.15). Average branch length and time required for bud break were significantly different between the Groups for GWR-83 (Table 2), but total growth parameters were not.

Starch accumulations in the greenhouse grafts in 1984 (GWR-84, Fig. 25) were lower than those observed in 1983. The rate of starch accumulation slowed after the second week, to peak two weeks prior to bud break in the successful grafts, then declined to stabilize at approximately 2 mg/g Dwt. Group 2 peaked with a higher starch content one week later than Group 1. Starch levels of the unsuccessful grafts (G-3) peaked lower and declined rapidly. However starch levels were not significantly different between Groups but were for weeks (Appendix 2.16). Growth parameters for the successful grafts were only significantly different for bud break date (Table 3).

Starch accumulations were much higher for lath house grafts (LWR-84, Fig. 26) than for greenhouse grafts, with accumulations beginning in the needles after week 6. The rate of accumulation was constant and peaked at week 12.5 for Groups 1 and 2. With the limited replication, unsuccessful grafts appeared to peak at 10.5 weeks. In all groups, starch content declined rapidly after reaching maximum content. For the successful grafts, this decline began 1.5 to 2.5 weeks prior to mean bud break. The decline in starch content for unsuccessful grafts occurred without bud break. Differences in starch content were significantly different for Groups and weeks, but not the

Table 2. Means of growth parameters of Greenhouse Water Relations 1983. Means with the same letter are not significantly different at the 5% level as separated by Protected LSDs. Growth parameters are given in cm, bud break as days after grafting.

Parameter	Group 1	Group 2	Group 3
Bud Break	49.8±3.1 a	60.2±3.6 b	72.0±7.2 c
Branch Number	5.6±2.0	5.9±1.8	5.3±1.4
Terminal Length	6.9±1.6	7.3±1.0	5.6±1.5
Total Growth	31.5±12.8	34.8±11.5	25.1±9.4
Average Branch	5.6±1.0 b	5.9±0.6 a	4.8±1.5 c
Branch : Bud	73.9±22.7	83.8±18.2	68.8±17.8

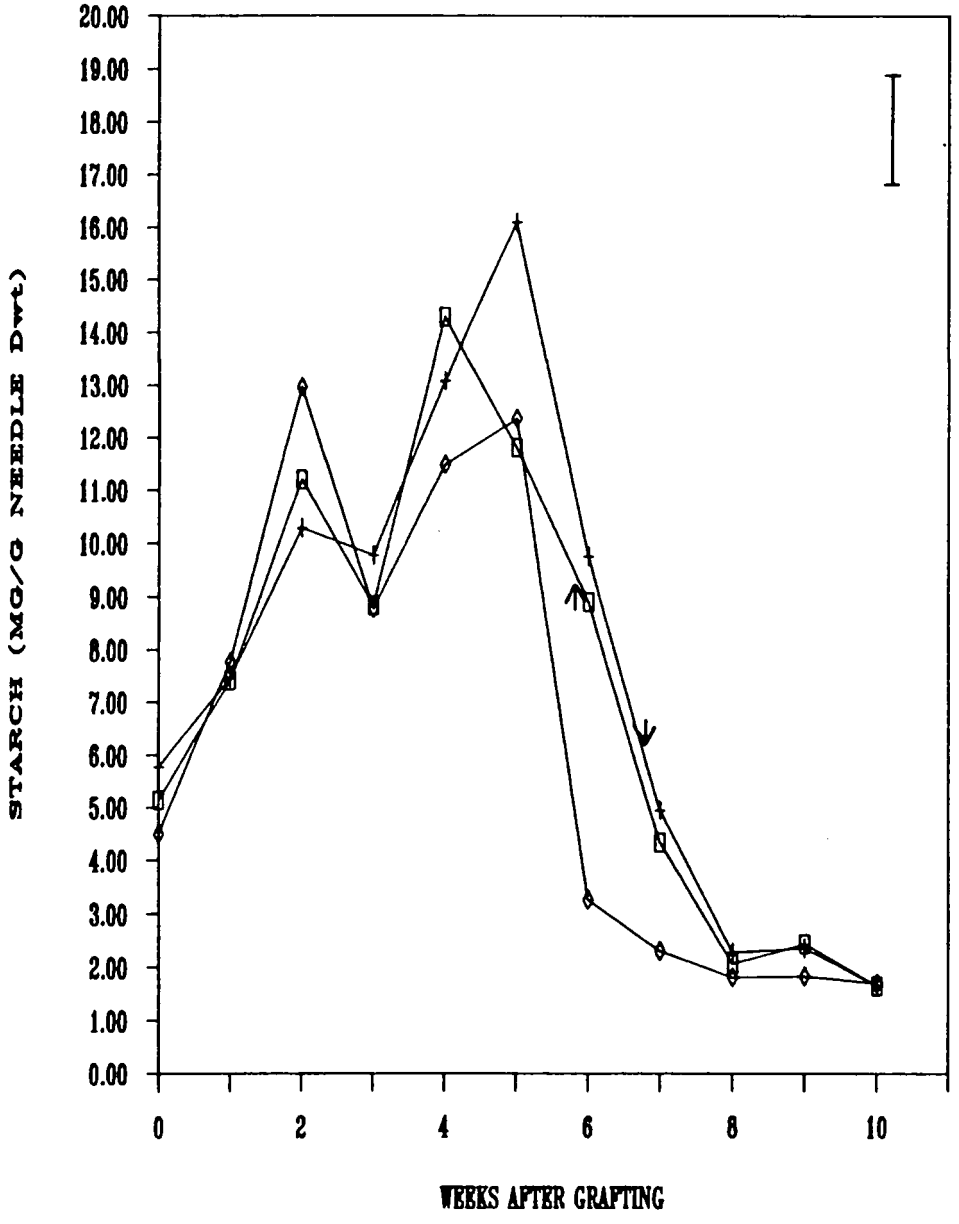

Fig. 25. Needle starch content for 10 weeks for the experiment Greenhouse Water Relations 1984. Group 1 (□) scions required less than 44 days for bud break, Group 2 (+) scions required greater than 44 days, Group 3 (◇) scions were unsuccessful. Arrows indicate mean bud break. The vertical bar indicates standard error.

Table 3. Means of growth parameters of Greenhouse Water Relations 1984 and Lath Water Relations 1984. Means with the same letter are not significant at the 5% level as separated by Protected LSDs. Growth parameters are given in cm, bud break as days after grafting.

	GWR-84		LWR-84	
Parameter	Group 1	Group 2	Group 1	Group 2
Bud Break	41.1±7.2 a	47.8±3.0 b	98.5±3.6 a	108±3.1 b
Branch Number	6.2±3.0	5.5±1.9	5.4±1.9	4.9±1.8
Terminal Length	6.2±3.5	6.2±3.2	5.7±2.5	4.2±2.0
Total Growth	36.5±14.3	34.7±12.1	30.8±11.1	25.2±7.5
Average Branch	6.3±1.5	6.5±1.5	5.8±0.9	5.3±1.1
Growth/cm_3Scion	2.1±0.8	2.0±0.7	1.8±0.6	1.4±0.4
Growth/cm^3 Scion	31.1±9.4	30.2±10.7	31.3±11.1	27.1±9.0

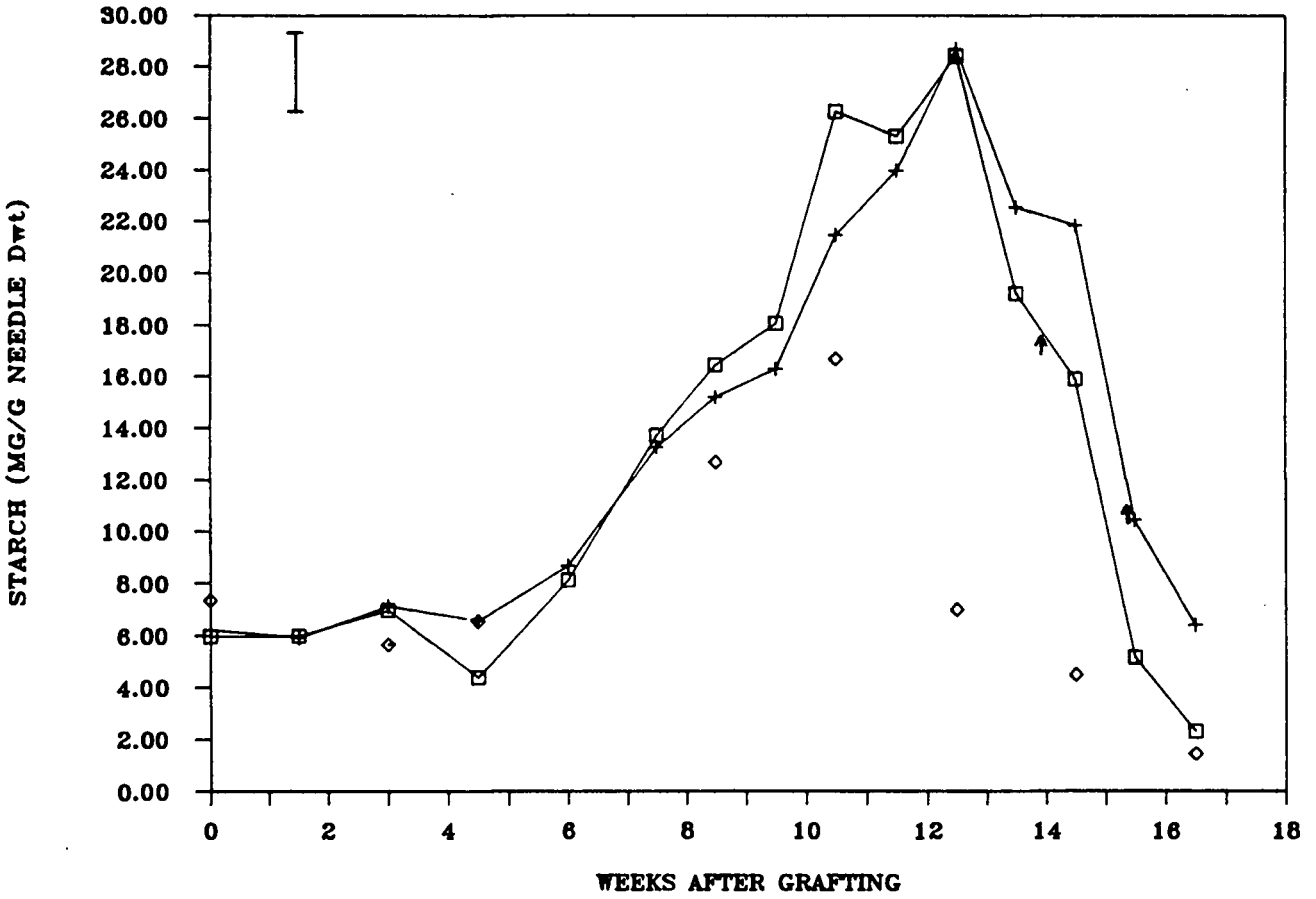

Fig. 26. Needle starch content for 16.5 weeks for the experiment Lath house Water Relations 1984. Group 1 (□) scions required less than 103 days for bud break, Group 2 (+) scions required greater than 103 days, Group 3 (◇) scions were unsuccessful. Arrows indicate mean bud break. The vertical bar indicates standard error.

interaction (Appendix 2.17). Only bud break dates differed significantly among the growth parameters measured (Table 3).

Comparisons of the growth parameters between GWR-84 and LWR-84 are listed in Table 4. Greenhouse grafts generally had significantly more post-union growth than lath house grafts.

The accumulation of starch in the needles of established grafted trees (EGWR) is similar to that of greenhouse grafts. Needle starch content increased rapidly in the greenhouse, followed by rapid declines around bud break (Fig. 27). The peak starch levels were 2- to 3-fold higher than those found in developing greenhouse grafts. Unlike the grafts, starch levels did not decline until after mean bud break.

In contrast to the starch relations described above, the rate of starch accumulation in the misaligned grafts (MGWR, Fig. 28) was high the first week, but subsequently declined to 1.5 mg/g Dwt by week 6.

The pattern of starch accumulation of the SSAR grafts (Fig. 29) though lower, is similar to that previously described for greenhouse grafts. By covering the scions with aluminum foil, photosynthesis was prevented, resulting in rapid depletion of needle starch, as exemplified by week 5. The growth parameters are compiled in Table 5. Covering the scion had no effect on graft success. There was a tendency for scions covered the longest to have the highest graft success. Other than terminal branch length, covering the scions had no effect on post-union growth or bud break.

The amount of total radioactivity recovered from the scions (ScTrans, Fig. 30) declined from 60% to 10%, for both harvest times during the first week. The radioactivity in each fraction is shown as

Table 4. Means of growth parameters of Greenhouse Water Relations 1984 versus Lath Water Relations 1984. Means with the same letters are not significantly different at the 5% level as separated by Protected LSDs. Growth parameters are given in cm, bud break as days after grafting.

Parameter	GWR-84	LWR-84
Bud Break	44.2±4.2 a	100.8±5.4 b
Branch Number	5.9±2.5	5.3±1.9
Terminal Length	6.2±3.3 a	5.3±2.5 b
Total Growth	35.7±13.2 a	29.4±10.5 b
Average Branch	6.4±1.5 a	5.7±1.0 b
Growth/cm_3Scion	2.1±0.7 a	1.7±0.6 b
Growth/cm^3 Scion	30.7±10.0	30.3±10.7

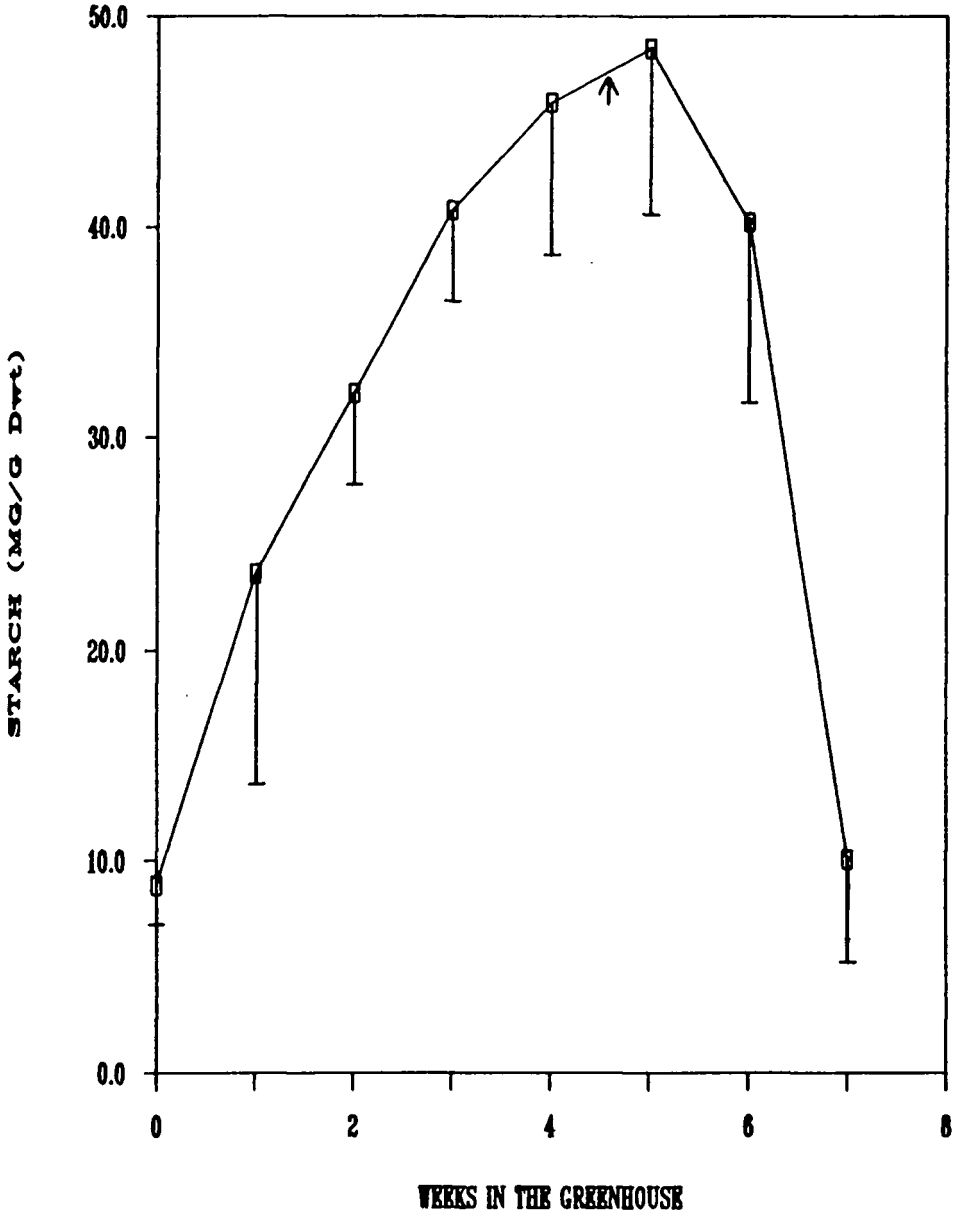

Fig. 27. Needle starch content for 7 weeks for the experiment Established Graft Water Relations. The arrow indicates mean bud break. The vertical bars indicate the standard errors.

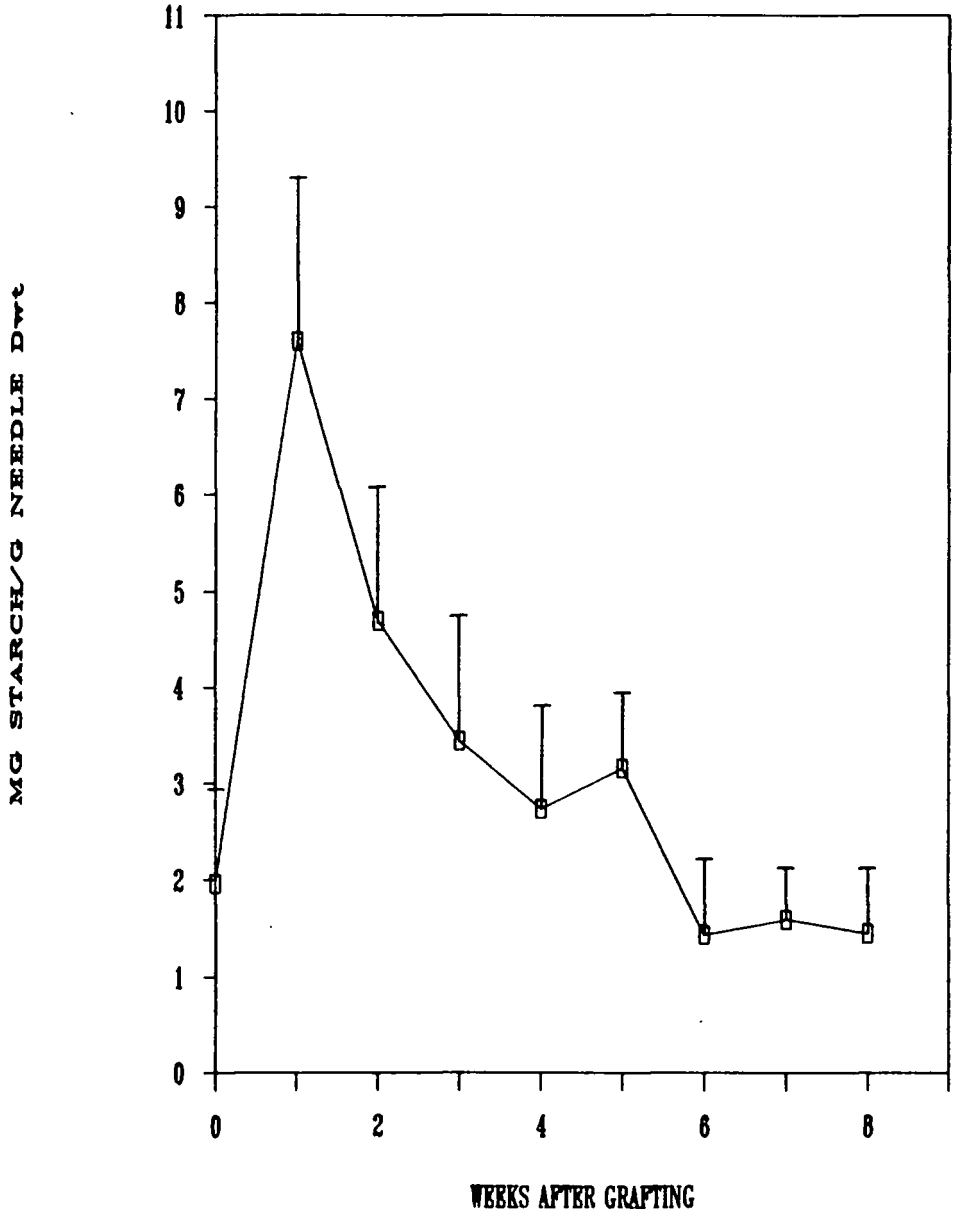

Fig. 28. Needle starch content for the grafts of the Mis-aligned Graft Water Relations experiment. The vertical bars indicate the standard errors.

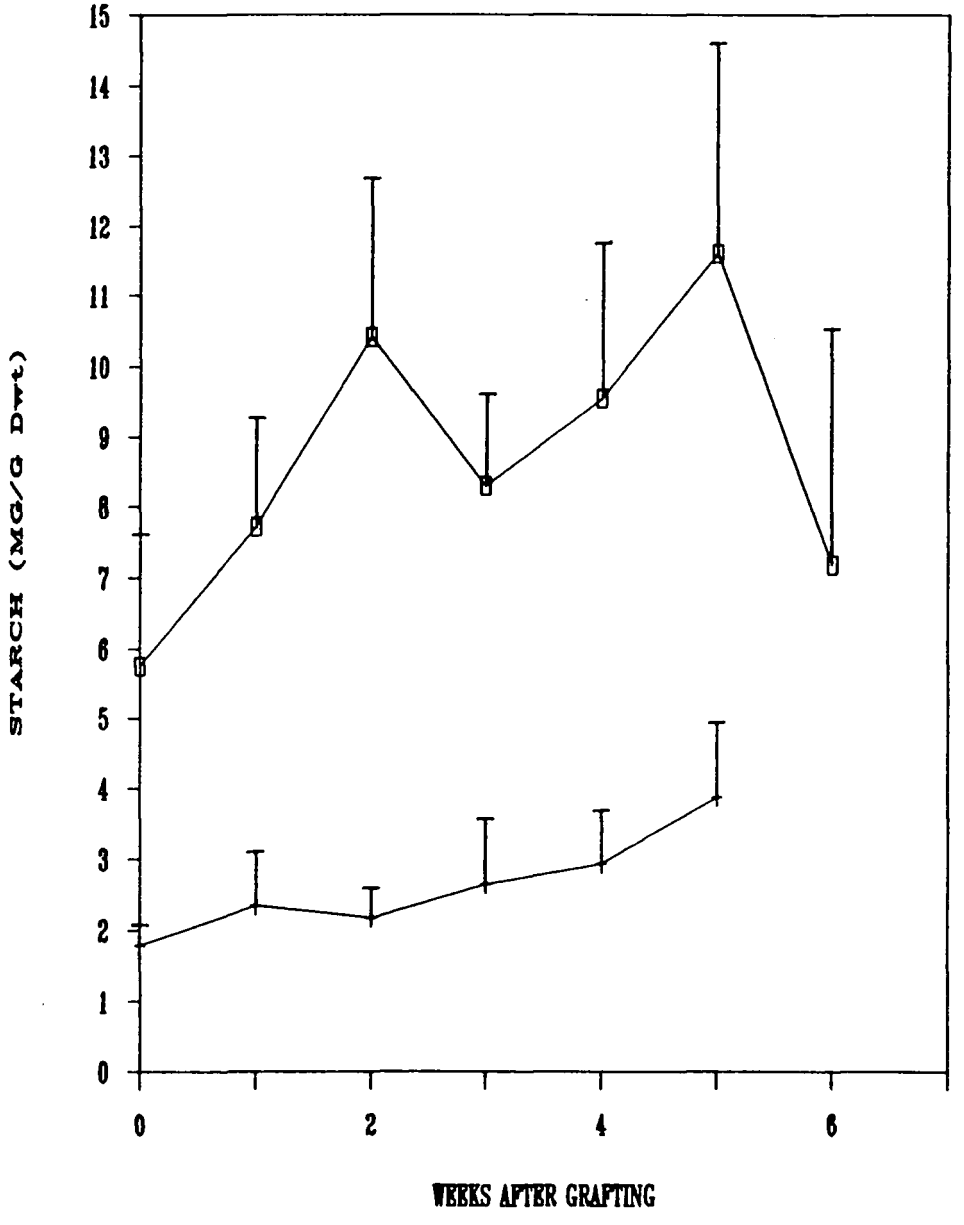

Fig. 29. Needle starch content of the experiment Scion Starch Accumulation Requirement. The upper line represents needle starch content prior to scion covering. The lower line is the needle starch content 6 weeks after grafting. The vertical bars indicate standard errors.

Table 5. Means of growth parameters of Scion Starch Accumulation Requirement. Means with the same letter are not significantly different at the 5% level as separated by Protected LSDs. Growth parameters are given in cm, bud break as days after grafting.

Treatment	% Success	Bud Break	Branch Number	Terminal Length	Total Growth	Growth/ cm Scion	Growth/ cm^3 Scion
Control	64 c	42.1±5.7	3.6±2.0	3.1±2.9 d	22.5±11.3	1.7±0.9	34.7±20.1
Week 0	96 a	44.3±3.5	4.6±1.9	6.8±2.7 a	34.8±12.1	2.2±1.7	34.6±10.2
Week 1	96 a	45.7±5.5	3.7±1.9	5.3±4.3 abc	27.0±14.5	1.8±0.9	30.6±13.1
Week 2	100 a	44.7±2.8	4.7±3.2	4.0±3.3 cd	30.8±19.8	1.9±1.0	26.5±10.1
Week 3	79.2 b	45.3±10.7	3.4±1.5	4.7±3.5 cd	23.3±12.8	1.7±1.0	32.3±16.8
Week 4	79.2 b	45.9±6.6	4.0±1.6	4.7±2.8 cd	26.6±11.8	1.8±0.7	30.1±9.5
Week 5	88 ab	43.6±4.5	4.3±2.1	5.0±3.1 bcd	28.3±14.1	2.0±0.8	39.3±16.5
Week 6	92 a	46.2±6.5	5.0±3.1	5.8±3.3 ab	30.5±17.9	2.0±1.1	31.1±14.0

	Average Branch
Control	6.8±1.7
Week 0	7.7±1.2
Week 1	7.4±1.3
Week 2	6.5±1.0
Week 3	6.7±1.7
Week 4	6.6±1.3
Week 5	6.6±1.6
Week 6	6.5±2.5

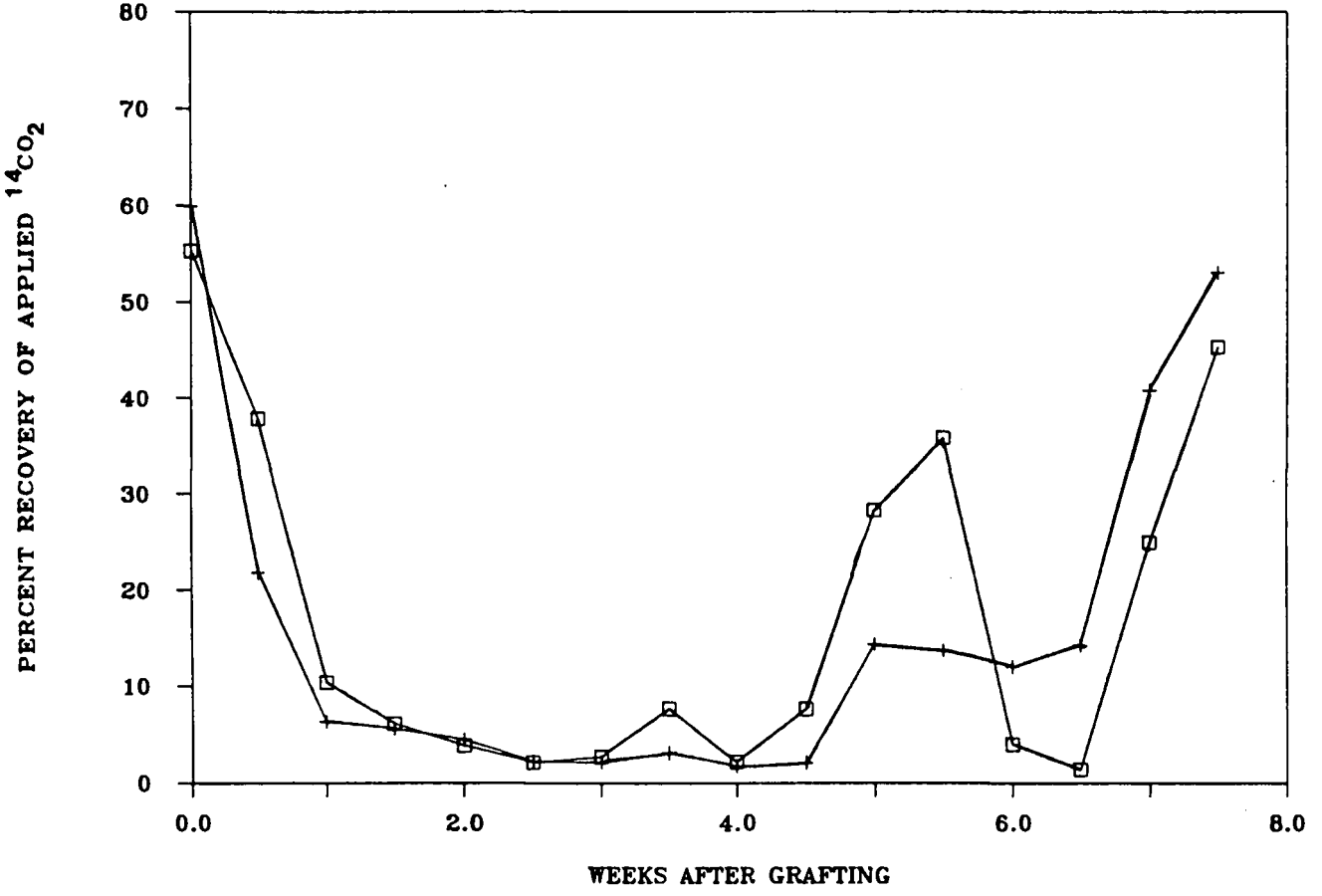

Fig. 30. Percent total recovery of the ^{14}C applied for the first 7.5 weeks after grafting. Scions were harvested 24 (□) and 48 (+) hr after exposure.

the percentage of the total radioactivity recovered. From weeks 1 to 4.5, the total recovery remained less than 10% of that applied. Thereafter, the total recovery was generally greater than 10%, increasing rapidly the last week as shoot elongation began. The variability between weeks 4.5 to 6.5 was due to the variability in the development of functional xylem connections and the inability to visually distingish between successful and unsuccessful grafts.

Total recovery of radioactivity in the graft union gradually increased during the first 5.5 weeks but was generally less than 1.5% during that period (Fig. 31). More label was normally recovered in the union after 48, than 24 hrs. After week 5.5, the percentage of recovery in the graft union increased rapidly, but was still less than 4% after 7.5 weeks.

Initially, needle starch accounted for 45 to 55% of the total recovery (Fig. 32), but thereafter, accounted for only 5-25%. The recovery in this fraction was variable, but remained in this range until 5.5 weeks, after which it accounted for less than 10% of the total recovery, declining to near zero by 7.5 weeks.

The majority of the label after week 0 was generally found in the needle sugar fraction (Fig. 33). Needle sugars at the 24 hr harvest accounted for greater than 50% of the total recovery except for week 4. Generally, more label was recovered in the needle sugar fraction at the 24 hr than the 48 hr harvest, whereas the opposite occurred in the bark sugar fraction (Fig. 34), with recovery generally ranging from 5 to 30% for the 24 hr harvest and 10 to 35% for the 48 hr harvest. After grafting, the total sugar fraction at the 48 hr harvest (needle + bark) generally accounted for 70% or more of the

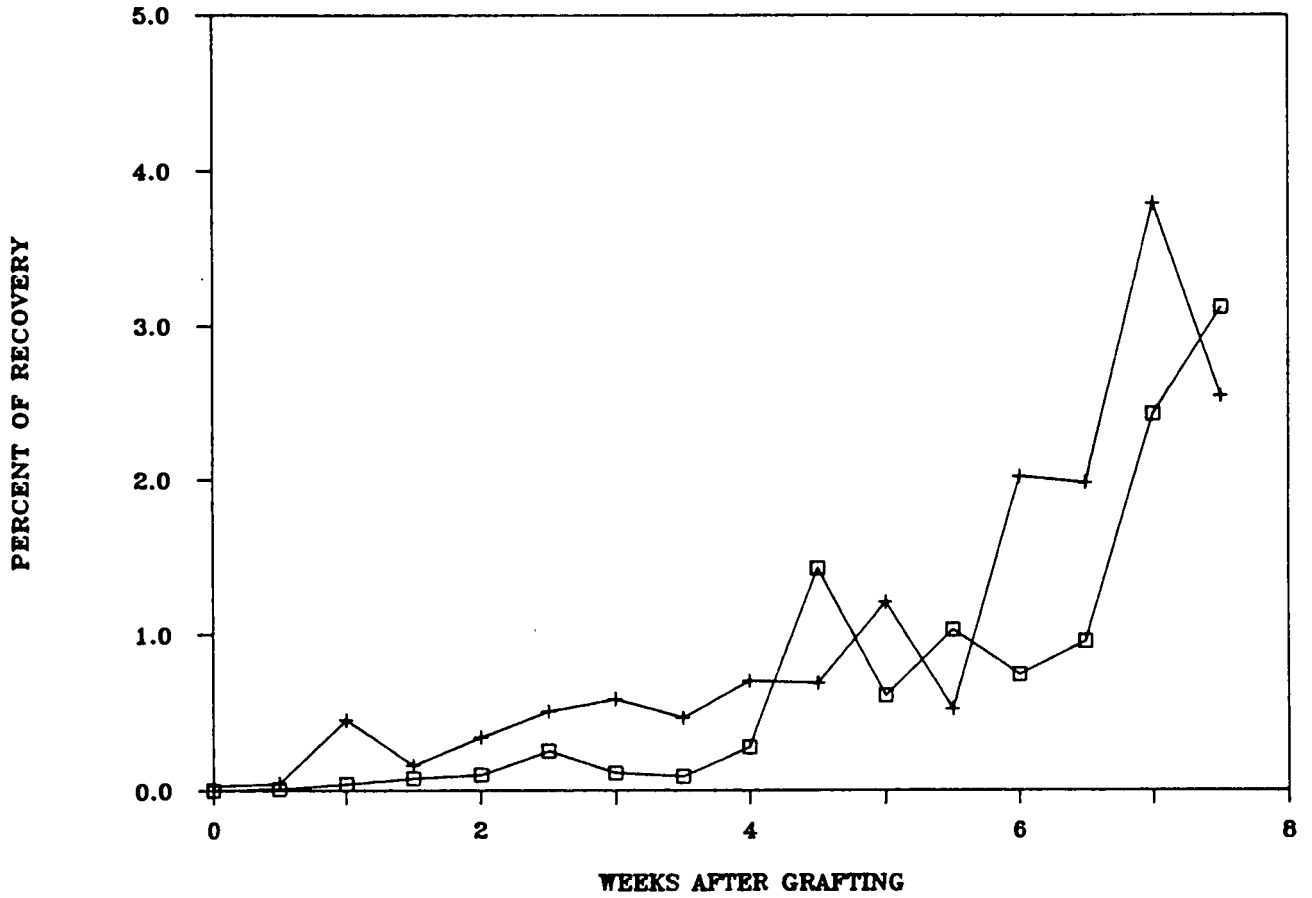

Fig. 31. Percentage of the total recovery of ^{14}C in the graft union the first 7.5 weeks after grafting. Scions were harvested 24 (□) and 48 (+) hr after exposure.

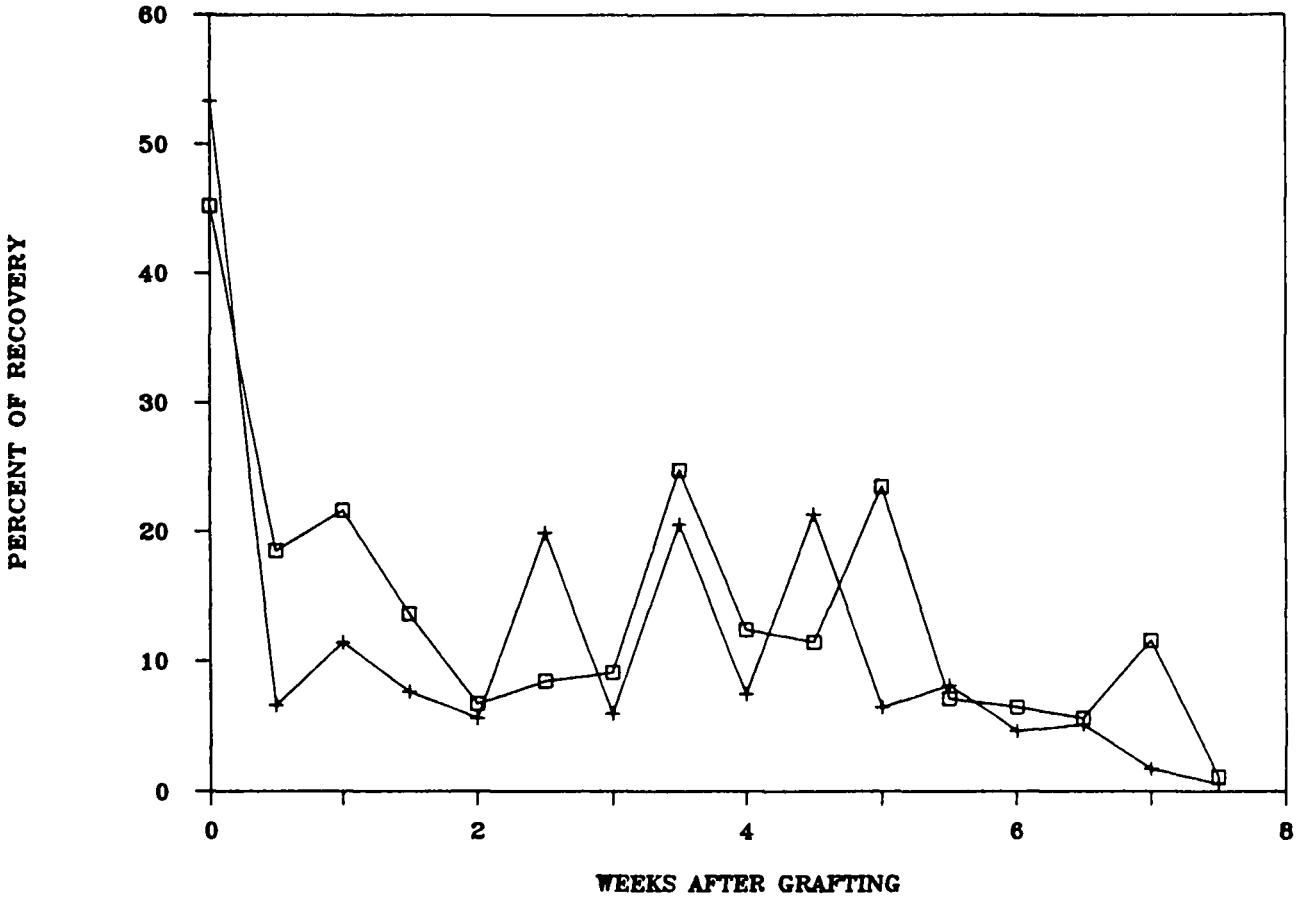

Fig. 32. Percentage of the total recovery of ^{14}C in the needle starch fraction the first 7.5 weeks after grafting. Scions were harvested 24 (□) and 48 (+) hr after exposure.

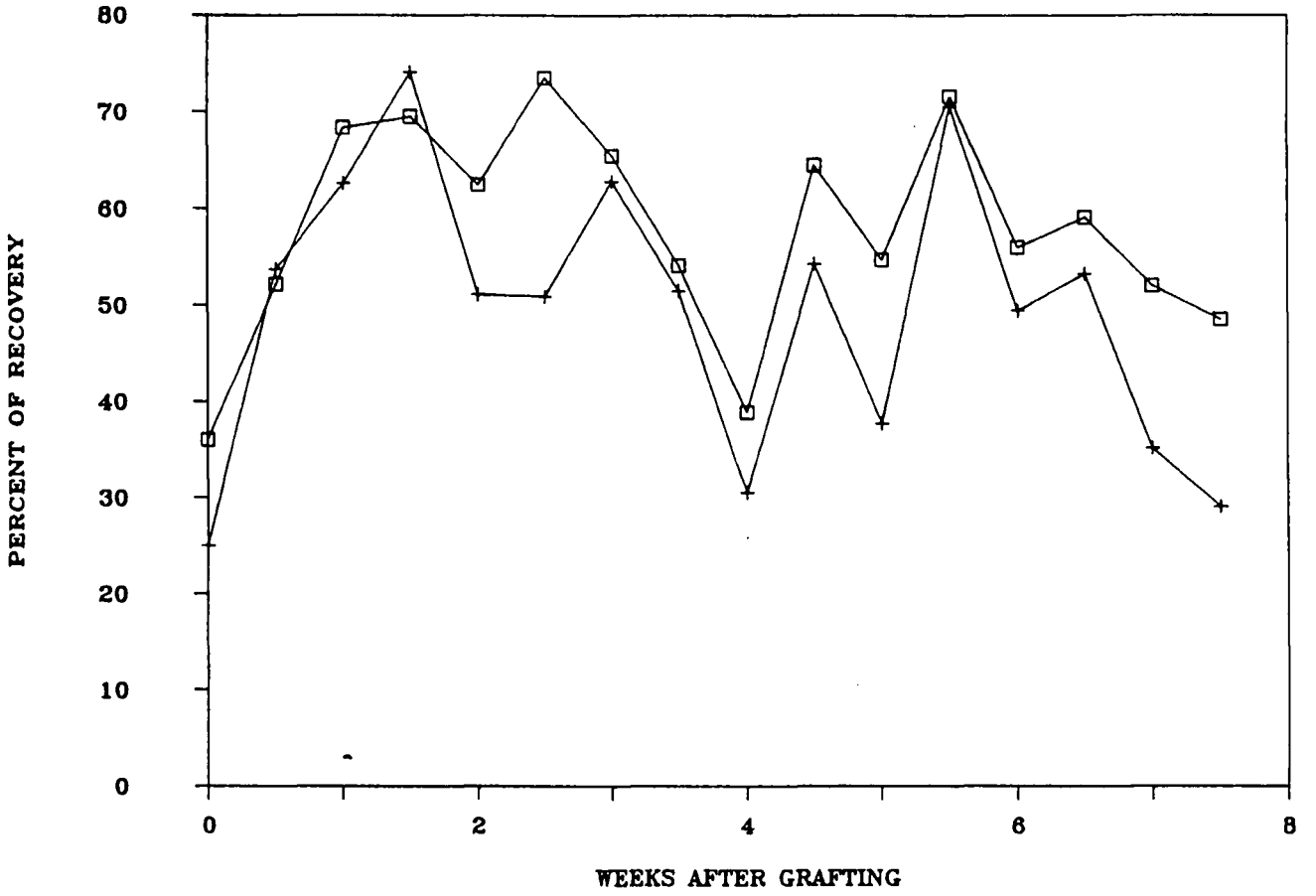

Fig. 33. Percentage of the total recovery of ^{14}C in the needle sugar fraction the first 7.5 weeks after grafting. Scions were harvested 24 (□) and 48 (+) hr after exposure.

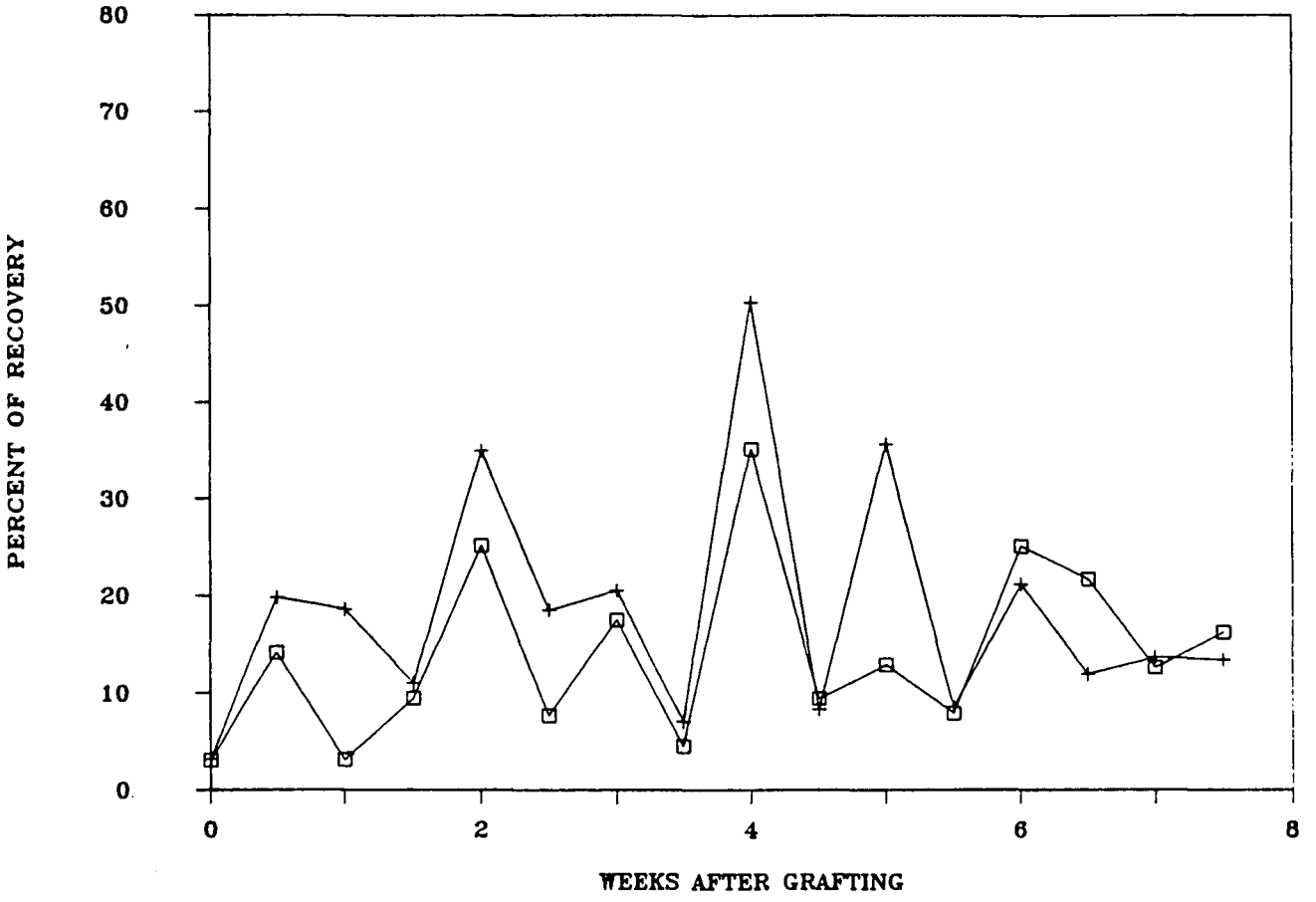

Fig. 34. Percentage of the total recovery of ^{14}C in the bark sugar fraction the first 7.5 weeks after grafting. Scions were harvested 24 (□) and 48 (+) hr after exposure.

total recovery until week 6 (Fig. 35), when the percentage declined.

The decline in all fractions after 6 weeks correlated with the increased label in the bud fractions of both the 24 hr (Fig. 36) and the 48 hr (Fig. 37) harvest. By 7.5 weeks, 52% of the recovered label was found in the elongating buds at the 48 hr harvest. The terminal buds accounted for greater than 50% of the total bud fraction with lower buds contributing the least.

During the first week, substantial recovery of the label was found in the needle lipid fraction (Fig. 38). Only 1 to 2% of the total recovery was found in this fraction. Similar recovery in the lipid needle fraction was observed during the first two weeks of labelling with dormant one year-old established grafts brought into the greenhouse (data not shown). Separation of the needle lipid fraction by TLC revealed that the label was mainly found in about equal amounts in the triglyceride and polar fractions. In contrast to the needle lipids, generally less than 1% of the label was found in the bark lipids (Fig. 39). Recovery in the bark solid fraction was generally less than 2% (Fig. 40), while the needle solid fractions generally ranging from 4 to 10% of the total recovery (Fig. 41).

Figures 42-44 show the visual results of the Rootstock Translocation experiment. The autoradiographs in figures 42 and 43 were made two weeks and six weeks after grafting, respectively, no labelled photosynthate was found in the scions. Label was found in growing scions eight weeks after grafting, with the majority of the label accumulation in the elongating scion shoots (Fig. 44). Similar labelling was noted 10 weeks after grafting when scion growth was three-quarters complete.

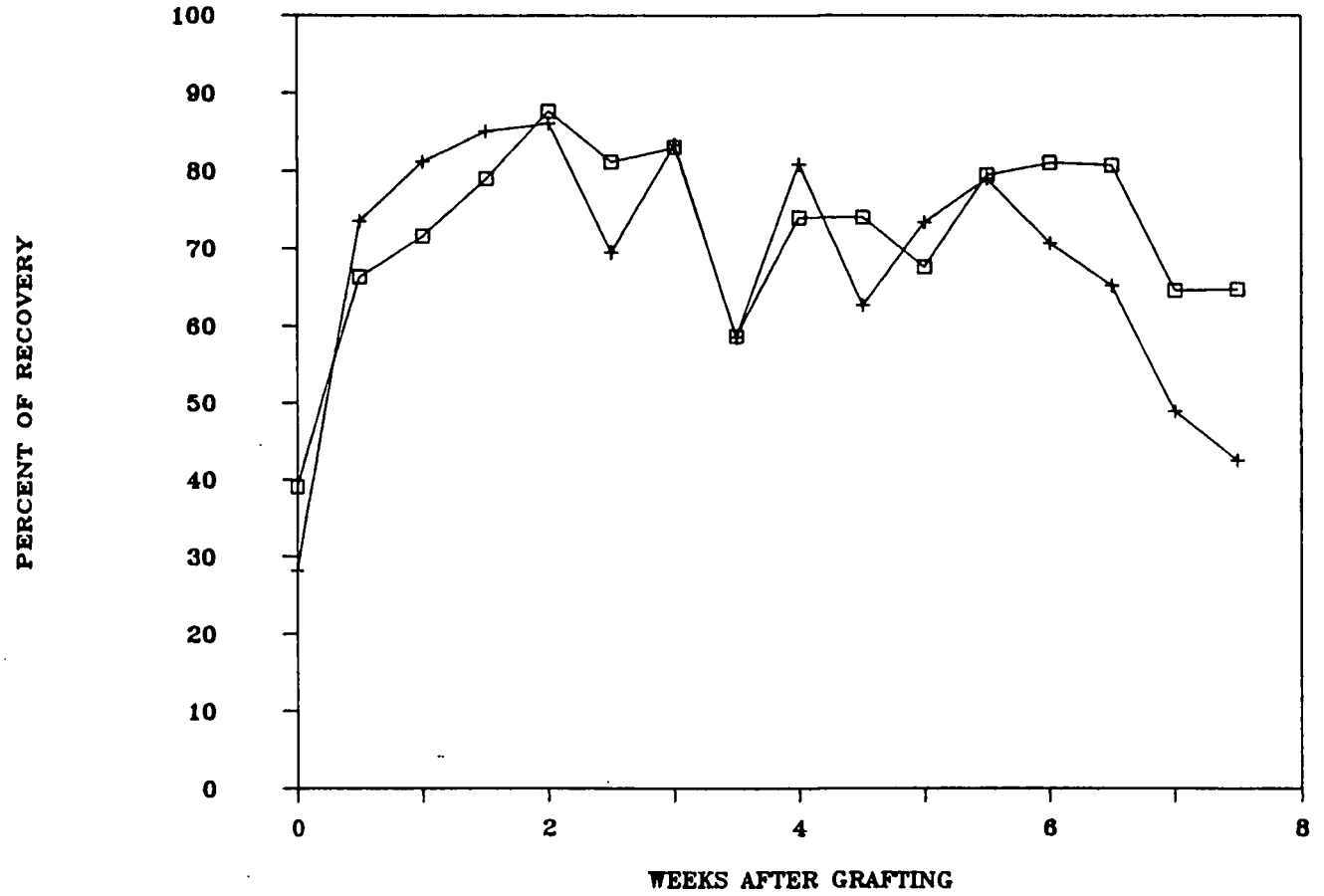

Fig. 35. Percentage of the total recovery of ^{14}C in the combined needle and bark sugar fractions the first 7.5 weeks after grafting. Scions were harvested 24 (□) and 48 (+) hr after exposure.

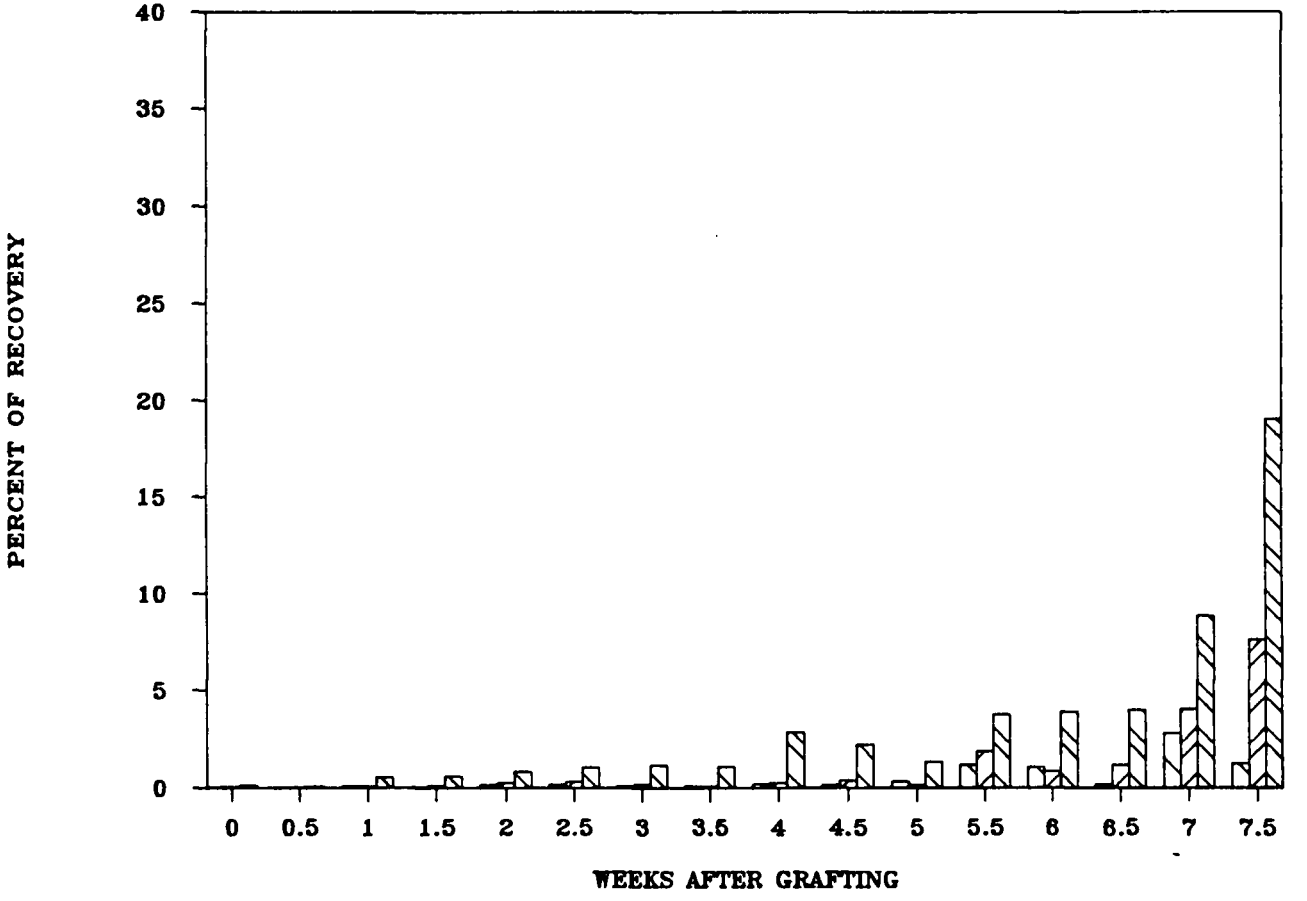

Fig. 36. Percentage of the total recovery of ^{14}C in the three bud fractions at the 24 hr harvest the first 7.5 weeks after grafting. The fractions are represented as ( ) lower buds, ( ) middle buds and ( ) terminal buds.

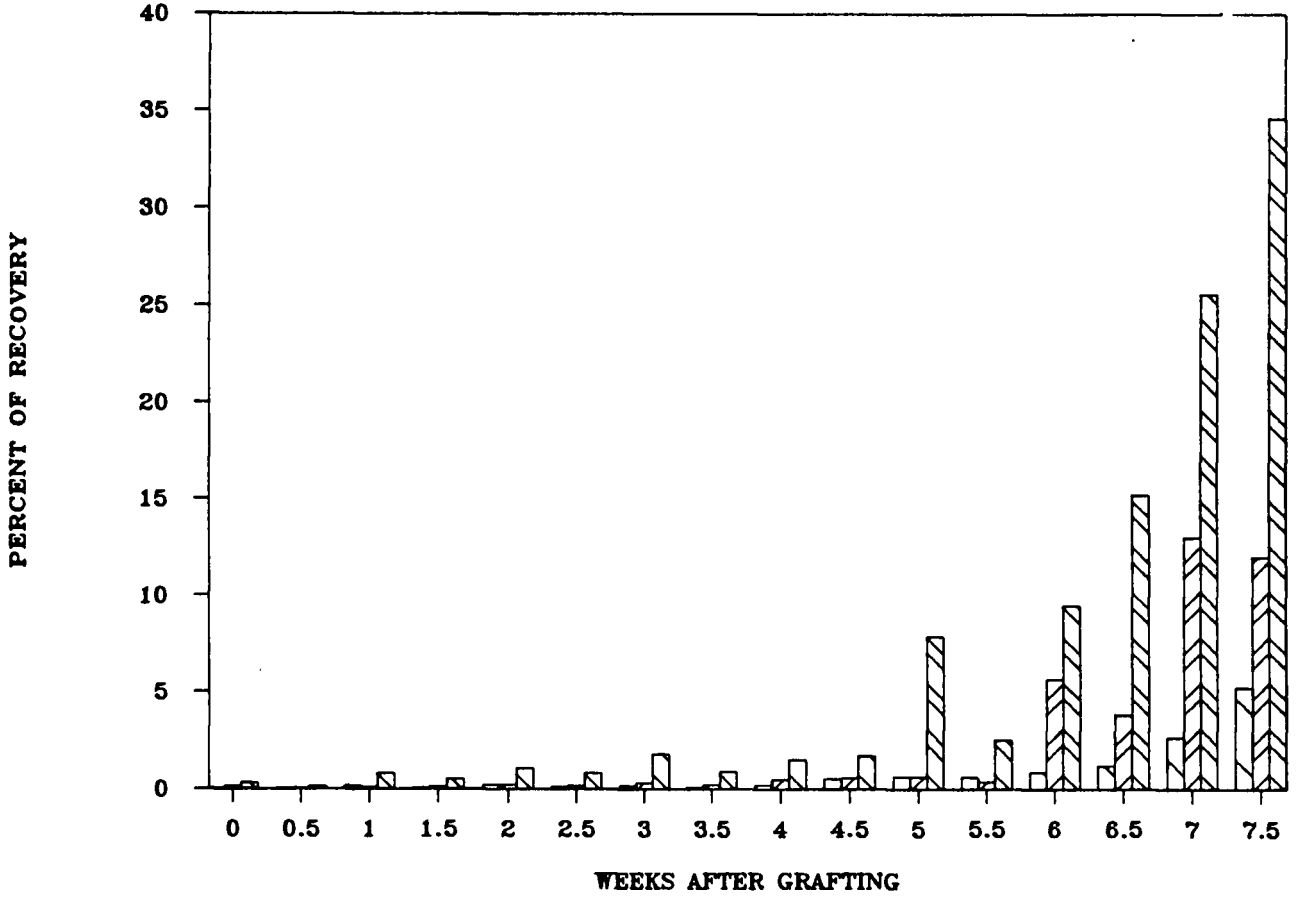

Fig. 37. Percentage of the total recovery of ^{14}C in the three bud fractions at the 48 hr harvest the first 7.5 weeks after grafting. The fractions are represented as (▨) lower buds, (▨) middle buds and (▨) terminal buds.

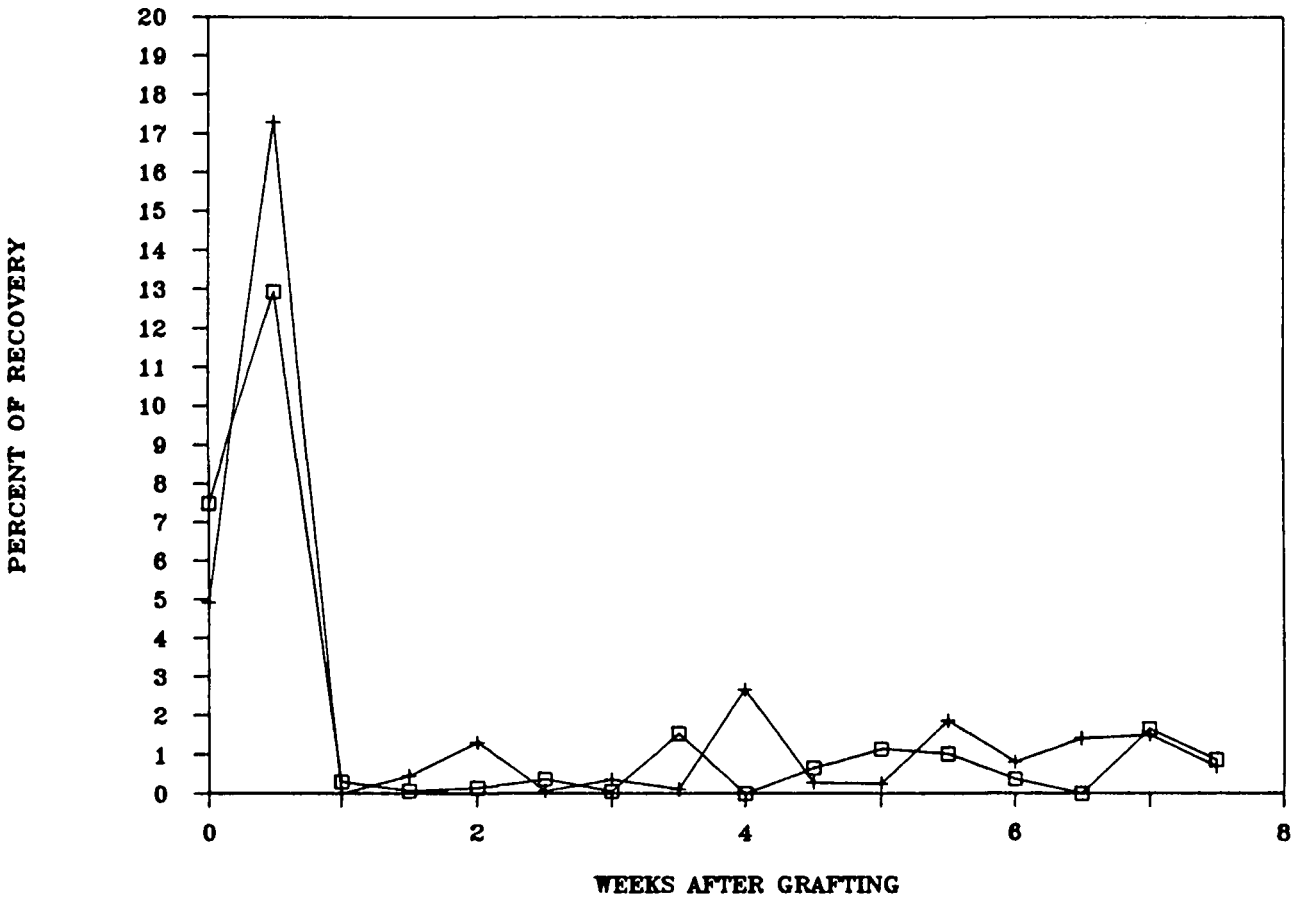

Fig. 38. Percentage of the total recovery of ^{14}C in the needle lipid fraction the first 7.5 weeks after grafting. Scions were harvested 24 (□) and 48 (+) hr after exposure.

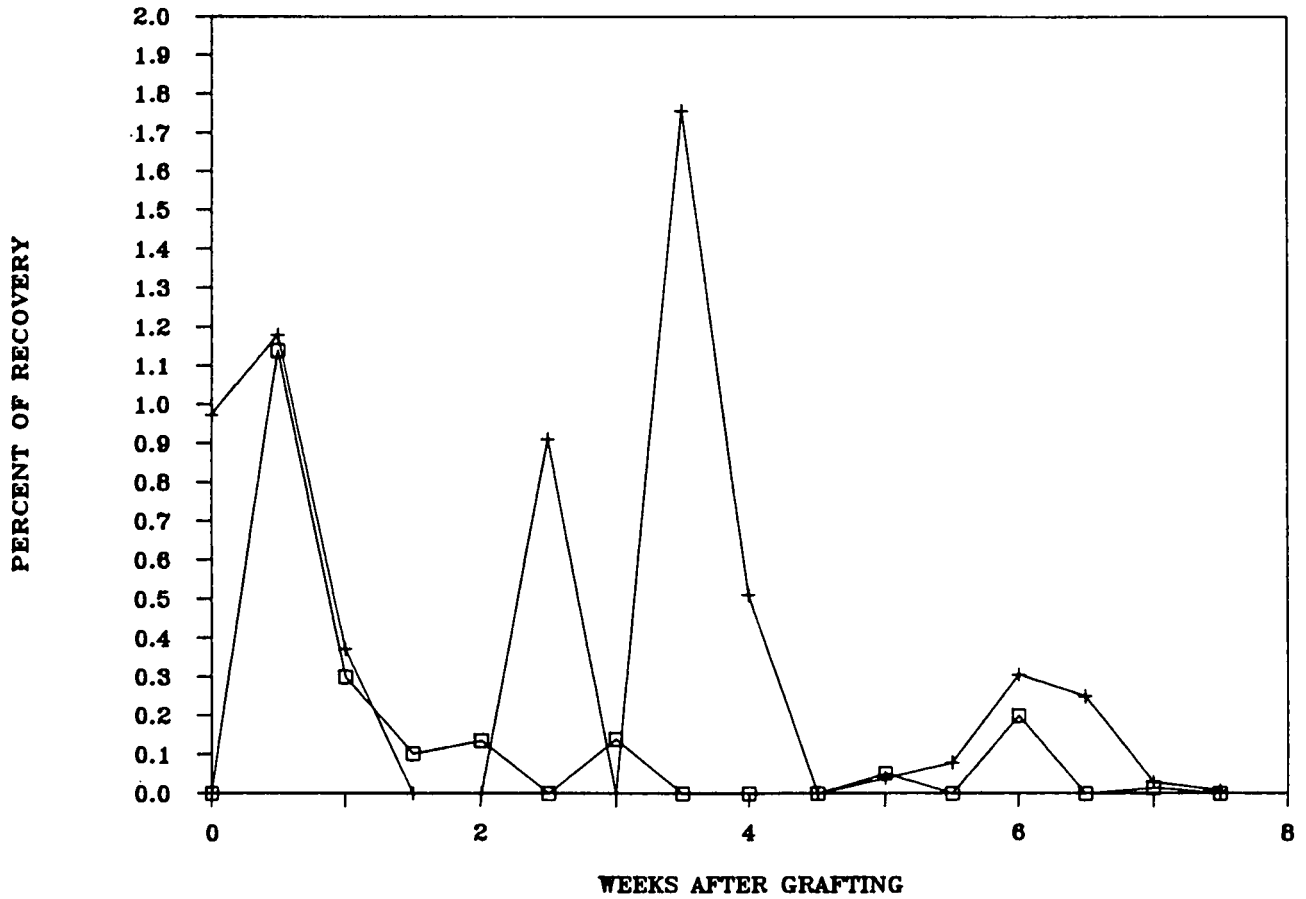

Fig. 39. Percentage of the total recovery of ^{14}C in the bark lipid fraction the first 7.5 weeks after grafting. Scions were harvested 24 (□) and 48 (+) hr after exposure.

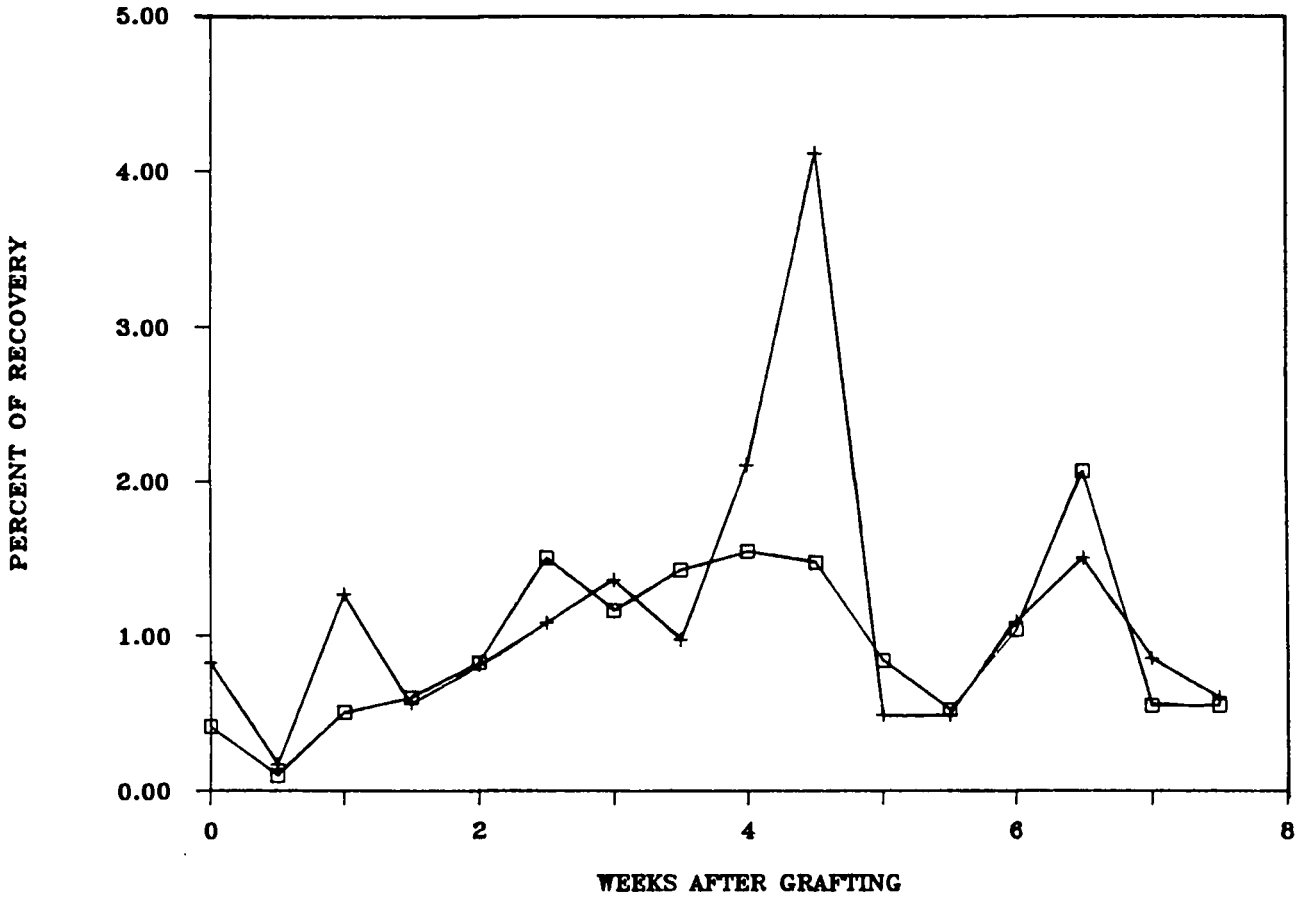

Fig. 40. Percentage of the total recovery of ^{14}C in the bark solid fraction the first 7.5 weeks after grafting. Scions were harvested 24 (□) and 48 (+) hr after exposure.

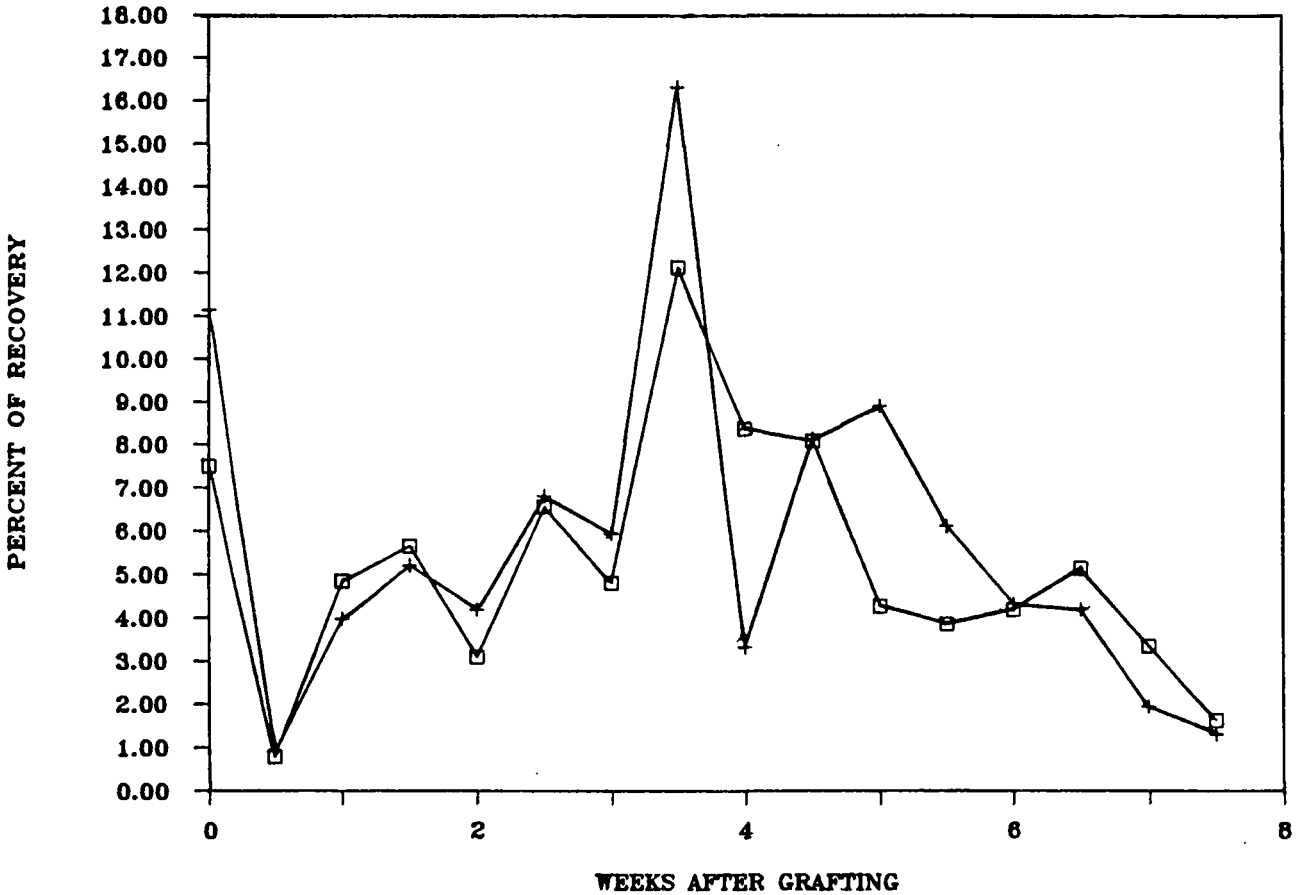

Fig. 41. Percentage of the total recovery of ^{14}C in the needle solid fraction the first 7.5 weeks after grafting. Scions were harvested 24 (□) and 48 (+) hr after exposure.

Fig. 42. Contact print of a representative autoradiograph 2 weeks after grafting from the experiment Rootstock Translocation. The arrow indicates the graft union.

Fig. 43. Contact print of a representative autoradiograph 6 weeks after grafting from the experiment Rootstock Translocation. The arrow indicates the graft union.

Fig. 44. Contact print of a representative autoradiograph 8 weeks after grafting from the experiment Rootstock Translocation. The arrow indicates the graft union.

Bark sugar content of Total Carbohydrate Reserves (TCR) grafts (Fig. 45) doubled the first week to remain relatively constant to week 5. Thereafter, levels declined rapidly through week 8. Mean bud break occurred week 9. There were no significant differences between successful and unsuccessful grafts but differences between weeks were significant (Appendix 2.18). Concurrent with the rapid increase in bark sugar levels the first 2 weeks after grafting, needle sugar levels in TCR scions declined rapidly (Fig. 46). Thereafter, a more gradual decline in the needle sugar content can be discerned. Needle sugar content was not significantly different between successful and unsuccessful grafts but was significantly different between weeks (Appendix 2.19). However, needle sugar levels fluctuated from week to week and between successful and unsuccessful grafts. There were no consistant trends. Starch levels (Fig. 47) increased rapidly the first week, then the rate of increase slowed. After three weeks, starch levels in the successful grafts were significantly higher than in the unsuccessful grafts, with the exception of week 6 (Appendix 18). Starch levels in the successful grafts were still high because bud break had not occurred when the measurements were stopped. The interaction between status (successful and unsuccessful) and week was significant (Appendix 18).

Bark sugars of Total Carbon Reserves - Covered (TCRC) grafts (Fig. 45) also increased rapidly the first week, followed by an increasing rate of decline similar to that of TCR. Except for weeks 2 and 3, there were no significant differences between successful and unsuccessful grafts for TCRC (Appendix 2.19). The needle sugar content of TCRC grafts is shown in figure 46. The initial decline in

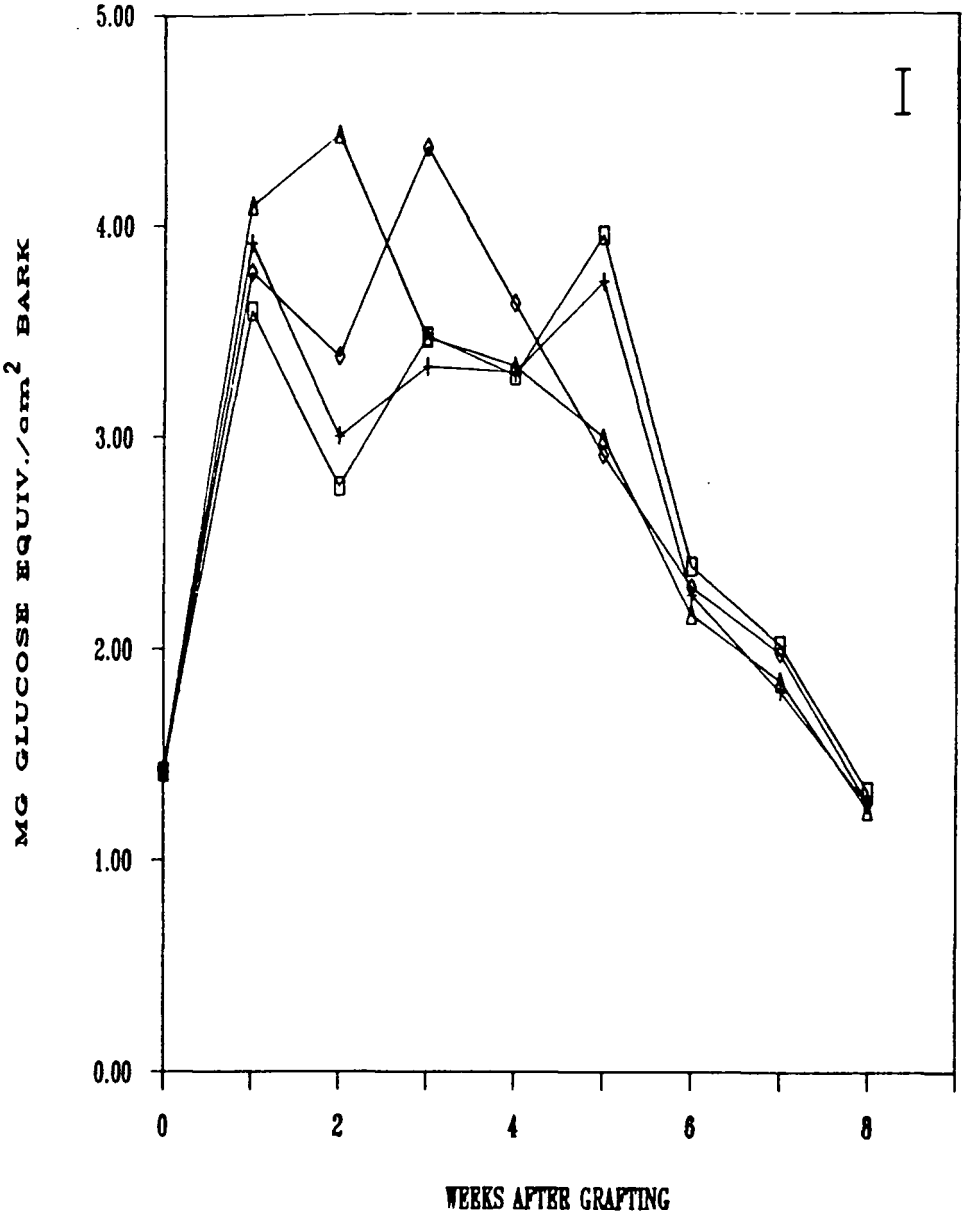

Fig. 45. Mean total bark sugars for 8 weeks for the experiments Total Carbohydrate Reserves and Total Carbon Reserves - Covered. Scions were partitioned into successful (□, ◇) and unsuccessful (+, △) grafts for TCR and TCRC, respectively. The vertical bar indicates the standard error.

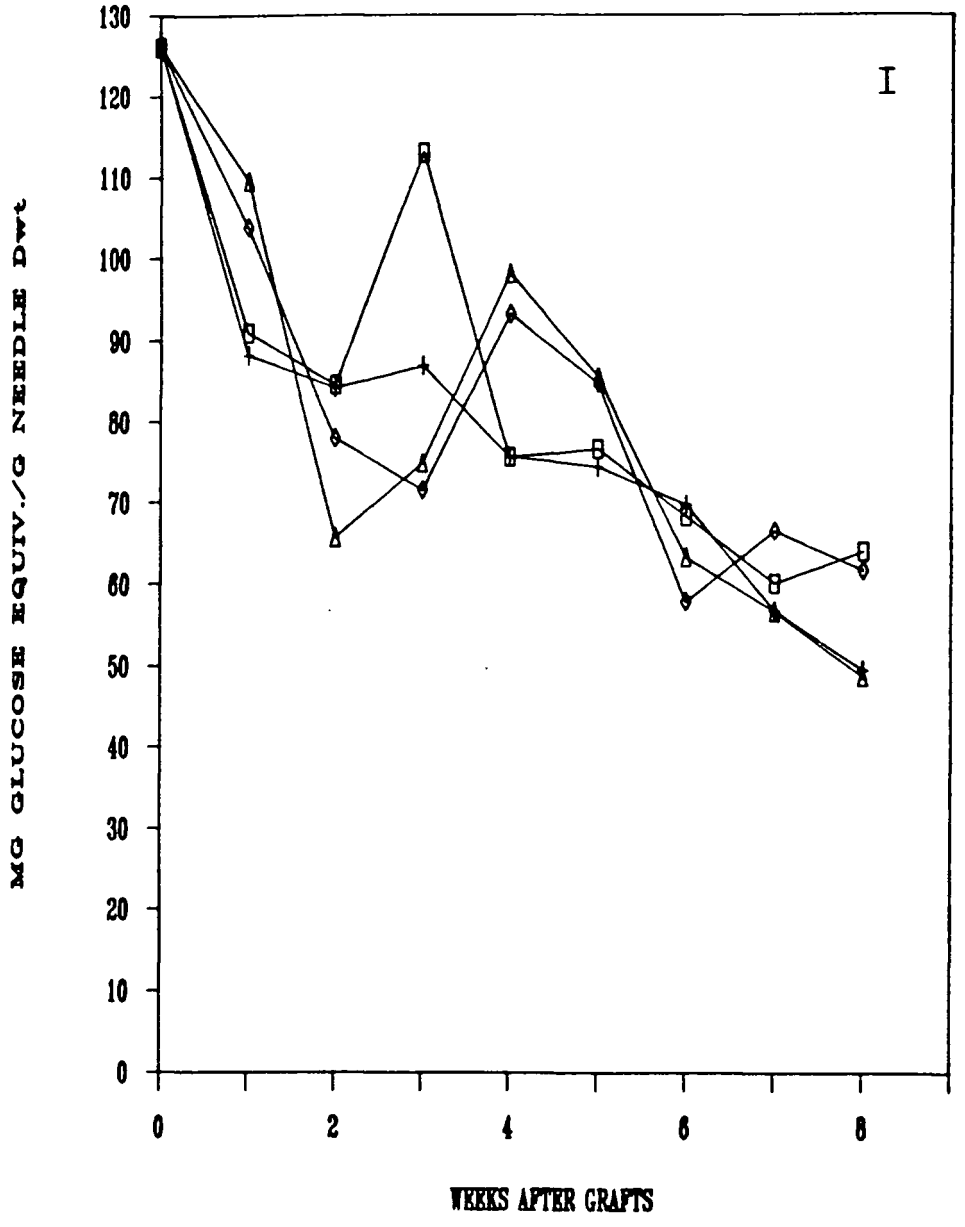

Fig. 46. Mean total needle sugars for 8 weeks for the experiments Total Carbohydrate Reserves and Total Carbon Reserves - Covered. Scions were partitioned into successful (□, ◇) and unsuccessful (+, △) grafts for TCR and TCRC, respectively. The vertical bar indicates the standard error.

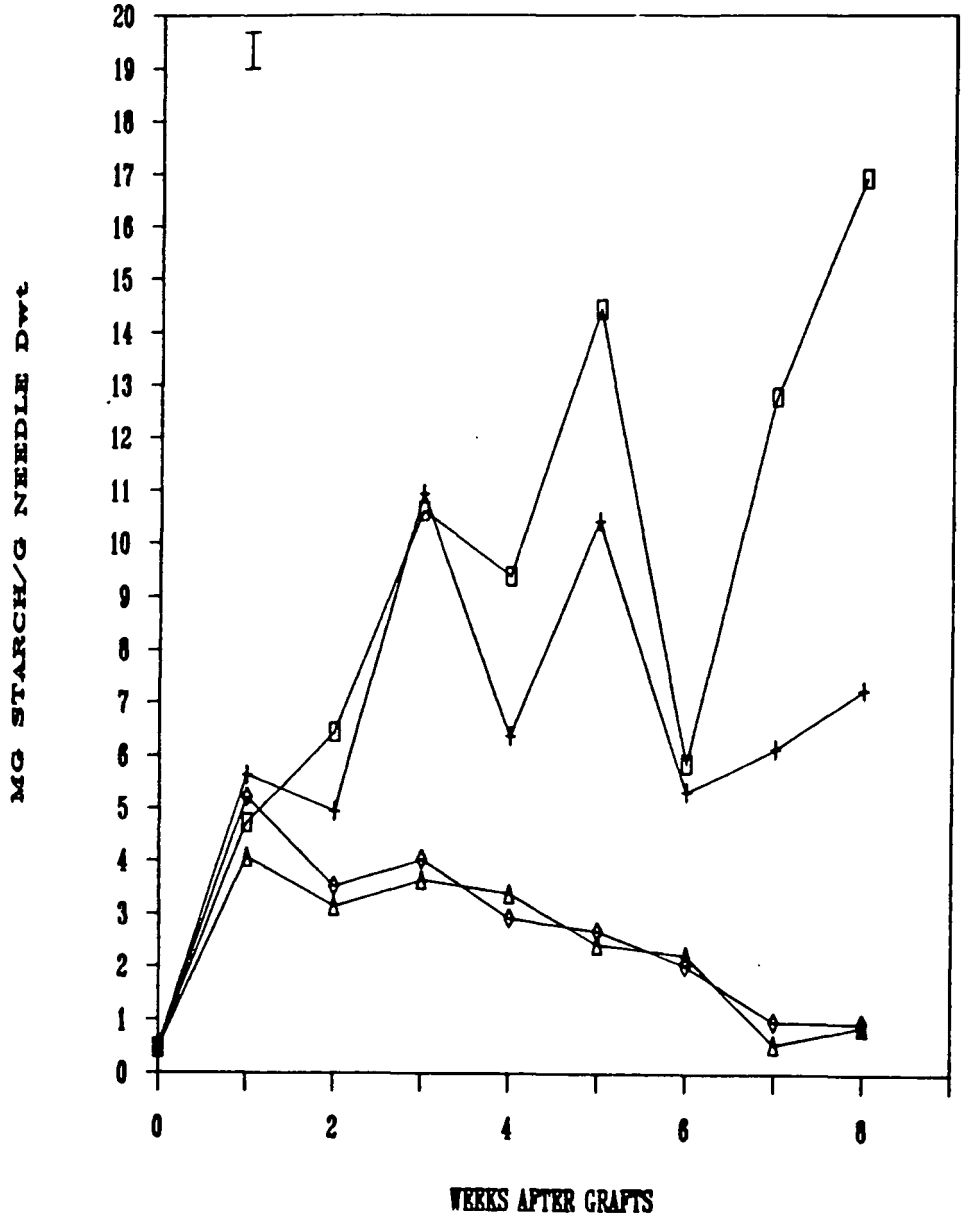

Fig. 47. Mean needle starch content for 8 weeks for the experiments Total Carbohydrate Reserves and Total Carbon Reserves - Covered. Scions were partitioned into successful (□, ◇) and unsuccessful (+, △) grafts for TCR and TCRC, respectively. The vertical bar indicates the standard error.

needle sugar was significantly less than that of the TRC grafts (Appendix 2.21). Rapid declines the second week resulted in lower needle sugar. TCRC needle sugars then increased through the fourth week before declining further. Needle sugar contents of the successful and unsuccessful TCRC grafts were not significantly different until the last two weeks (Appendix 2.19). Needle starch content for the TCRC grafts (Fig. 47) increased substantially the first week then gradually declined thereafter. Starch content was not significantly different between successful and unsuccessful grafts but was between weeks (Appendix 2.20).

Growth parameters for TCR and TCRC are listed in Tables 6 and 7 respectively. There were no significant differences among the parameters for TCR, but there were significant differences in TCRC grafts for total branch growth, growth/cm scion length and number of branches, but these differences do not appear to be correlated with weeks. Comparisons between the growth parameters of TCR and TCRC were not significant for total branch growth, but TCRC scions had significantly longer branches and a longer time to bud break was required. Differences in the success rates were not significant but the number of branches per scion was (Table 8).

Quantification of the non-polar lipid fractions from the TCRC experiment yielded 12 distinct peaks, of which five were identified. Four of the identified peaks: the triglycerides, diglycerides, monoglycerides and the free fatty acids, are shown in figure 48. Of these, only the free fatty acids exhibited a consistent decline with time. However, the decline the first two weeks accounted for 86% of the total decline (0.51 mg/cm^2 bark) over the eight weeks. Figures 49

Table 6. Means of growth parameters of Total Carbohydrate Reserves. Means with the same letter are not significantly different at the 5% level as separated by Protected LSDs. Growth parameters are given in cm, bud break as days after grafting.

Treatment	Bud Break	Branch Number	Total Growth	Average Branch	Growth/ cm Scion
Week 1	58.1±4.0	5.1±4.0	27.7±16.6	5.4±1.0	2.8±20.
Week 2	60.4±9.1	4.4±1.8	26.2±11.1	6.1±1.5	2.3±0.8
Week 3	61.9±8.4	5.1±2.1	27.0±10.6	5.5±1.1	2.3±0.9
Week 4	61.2±5.0	4.4±1.6	24.2±9.8	5.6±1.1	2.2±0.9
Week 5	60.3±8.4	6.0±3.4	31.7±20.7	5.2±1.0	2.7±1.6
Week 6	64.2±5.6	4.7±1.8	24.7±9.2	5.4±0.9	2.1±0.9
Week 7	59.9±5.6	5.7±3.0	29.3±18.4	5.1±0.9	2.4±1.4
Week 8	59.9±1.2	5.8±3.2	30.5±15.8	5.2±1.0	2.5±1.2

Table 7. Means of growth parameters of Total Carbon Reserves - Covered. Means with the same letter are not significant at the 5% level as separated by Protected LSDs. Growth parameters are given in cm, bud break as days after grafting.

Treatment	Bud Break	Branch Number	Total Growth	Average Branch	Growth/ cm Scion
Week 1	64.9±8.1	5.8±2.2 a	38.3±19.2 a	6.4±1.3	3.3±1.8 a
Week 2	64.2±5.5	4.4±2.0 abcd	27.8±12.7 abc	6.4±1.4	2.5±1.3 abcd
Week 3	61.3±7.4	4.5±2.6 abcd	25.0±13.8 bc	5.6±0.9	2.3±1.2 abcd
Week 4	65.1±8.3	3.8±2.1 cd	22.5±15.7 bc	5.6±1.4	2.0±1.3 bc
Week 5	62.2±6.5	3.0±2.1 d	19.8±16.0 c	6.1±2.0	1.9±1.4 bc
Week 6	61.1±5.4	4.7±2.7 abc	27.9±17.0 abc	5.8±1.6	2.6±1.5 ab
Week 7	62.2±5.2	5.1±2.4 ab	32.4±15.5 ab	6.4±1.0	2.7±1.1 ab
Week 8	63.4±6.7	3.3±1.1 cd	17.7±9.4 c	5.3±0.9	1.5±0.9 c

Table 8. Means of growth parameters of Total Carbohydrate Reserves verse Total Carbon Reserves - Covered. Means with the same letter are not significantly different at the 5% level as separated by Protected LSDs. Growth parameters are given in cm, bud break as days after grafting.

	Branch Number		% Success	
Weeks	TCR	TCRC	TCR	TCRC
1	5.1±2.5 ab	5.8±2.2 a	60	64
2	4.4±1.8 abc	4.4±2.0 abc	56	68
3	5.1±2.1 ab	4.5±2.6 abc	68	52
4	4.4±1.6 abc	3.8±2.1 bc	68	80
5	6.0±3.4 a	3.0±2.1 c	68	60
6	4.7±1.8 abc	4.7±2.7 abc	56	72
7	5.7±3.0 a	5.1±2.4 ab	60	60
8	5.8±3.2 a	3.3±1.6 c	65	67
Average Branch			5.4±1.1 b	6.0±1.4 a
Branch : Bud			61.8±6.7 b	63.1±6.7 a

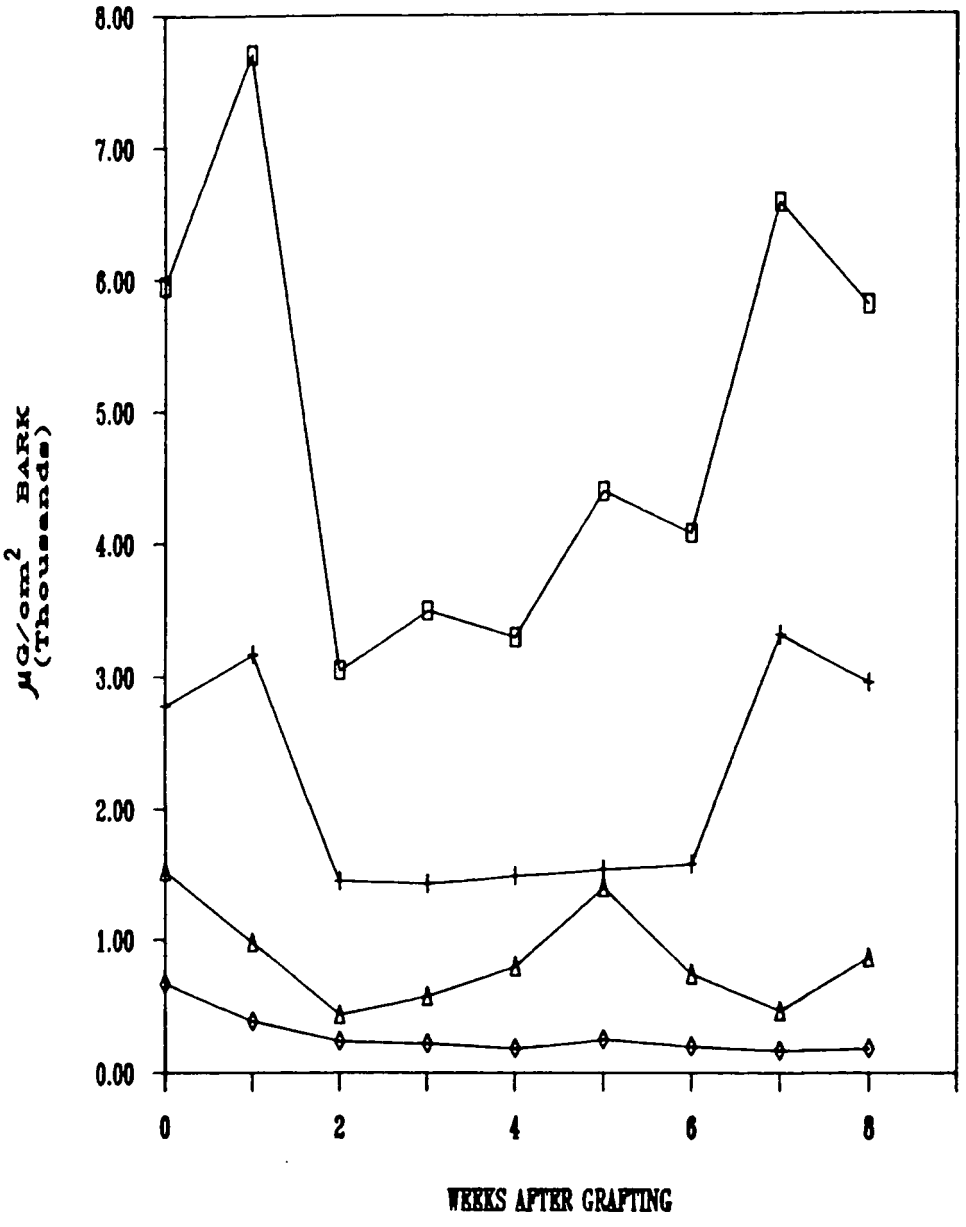

Fig. 48. Changes in the quantity of the identified lipid classes during union development of the experiment Total Carbon Reserves - Covered. The identified classes shown are the triglycerides (□), diglycerides (+), monoglycerides (△) and free fatty acids (◇).

and 50 show the changes in the seven unidentified peaks during union development. The peaks in figure 49 may be wax esters and/or cholesterol esters. This was determined by their short retention times relative to the triglyceride peak and co-chromatography with standards by TLC. The peaks in figure 50, with the exception of Beta, had retention times longer than those of the monoglycerides, the last identified peak to elute. The possibility exist that these peaks are sterols or their derivatives. Of these, both Delta-P and Omega show a gradual decline with time. Preliminary TLC analysis, using bark extracts from dormant and actively-growing branches, indicated a substantial change in the long chain alcohol (COH) fraction. Further analysis revealed an unknown, complex compound that migrated with the COH during TLC. Preliminary gas chromatographic separation of the acetylated COH fraction confirmed that the unknown compound comprised 90% of the COH fraction. The infrared spectrum of the unknown compound is shown in figure 51. Neither NMR nor mass spectra could be obtained. Quantification of the unknown by HPLC is shown in figure 52. Means and standard deviations for these peaks are listed in Appendix 2.22.

Dark respiration (Fig. 53) gradually increased over the first three weeks then declined, becoming constant after week 4. Concurrently, total water potential (Fig. 54) declined the first three weeks, then slowly increased to stabilize through bud break (week 7). Osmotic potentials showed little change from the first week until bud break. Turgor pressures declined the first three weeks, then increased slightly at week 4, and then declined through bud break. There appears to be a strong negative correlation between both

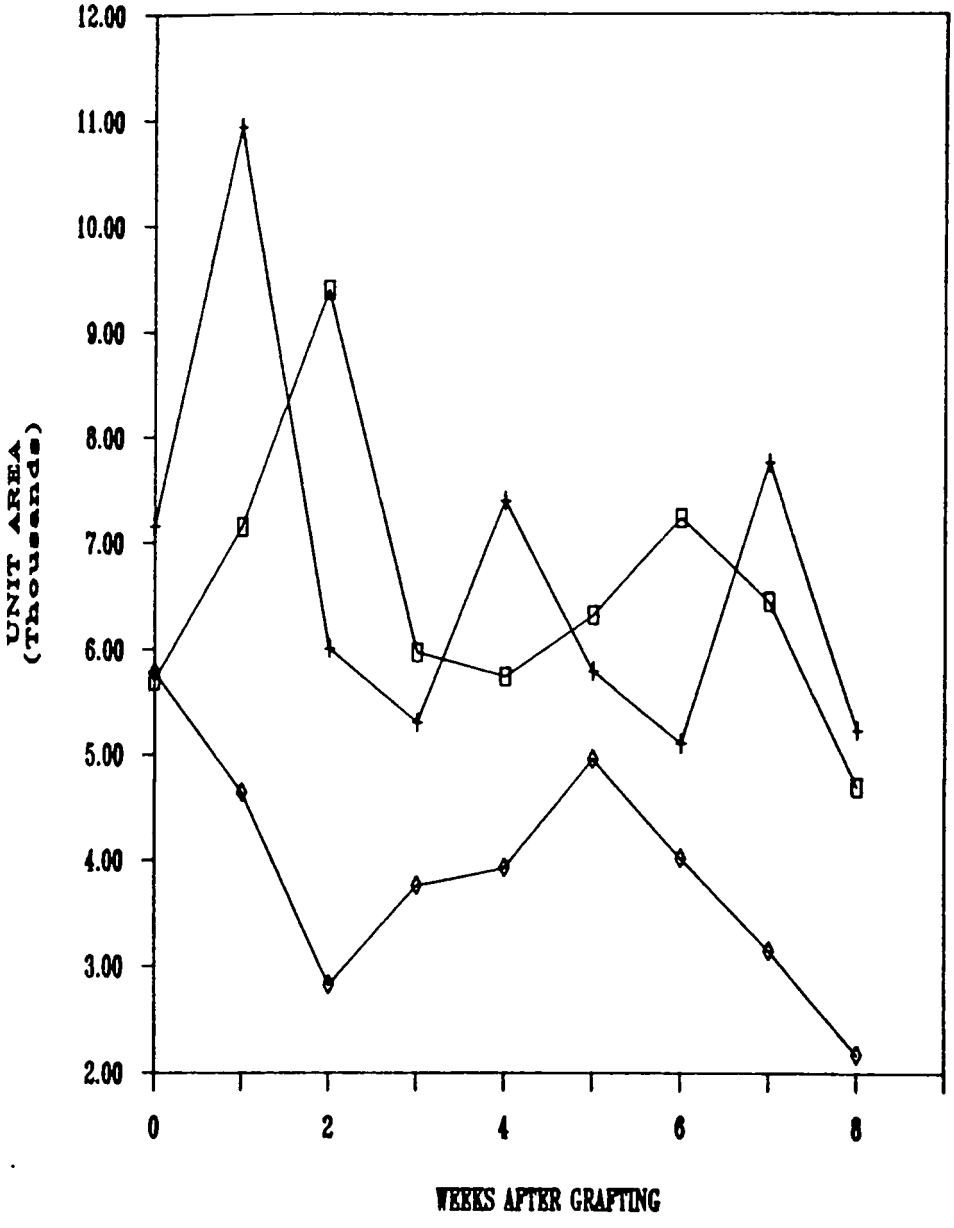

Fig. 49. Changes during union development in quantity of three of the unidentified lipid peaks of the experiment Total Carbon Reserves - Covered. The peaks were designated as Lamba (□), Delta (+) and Alpha (◇).

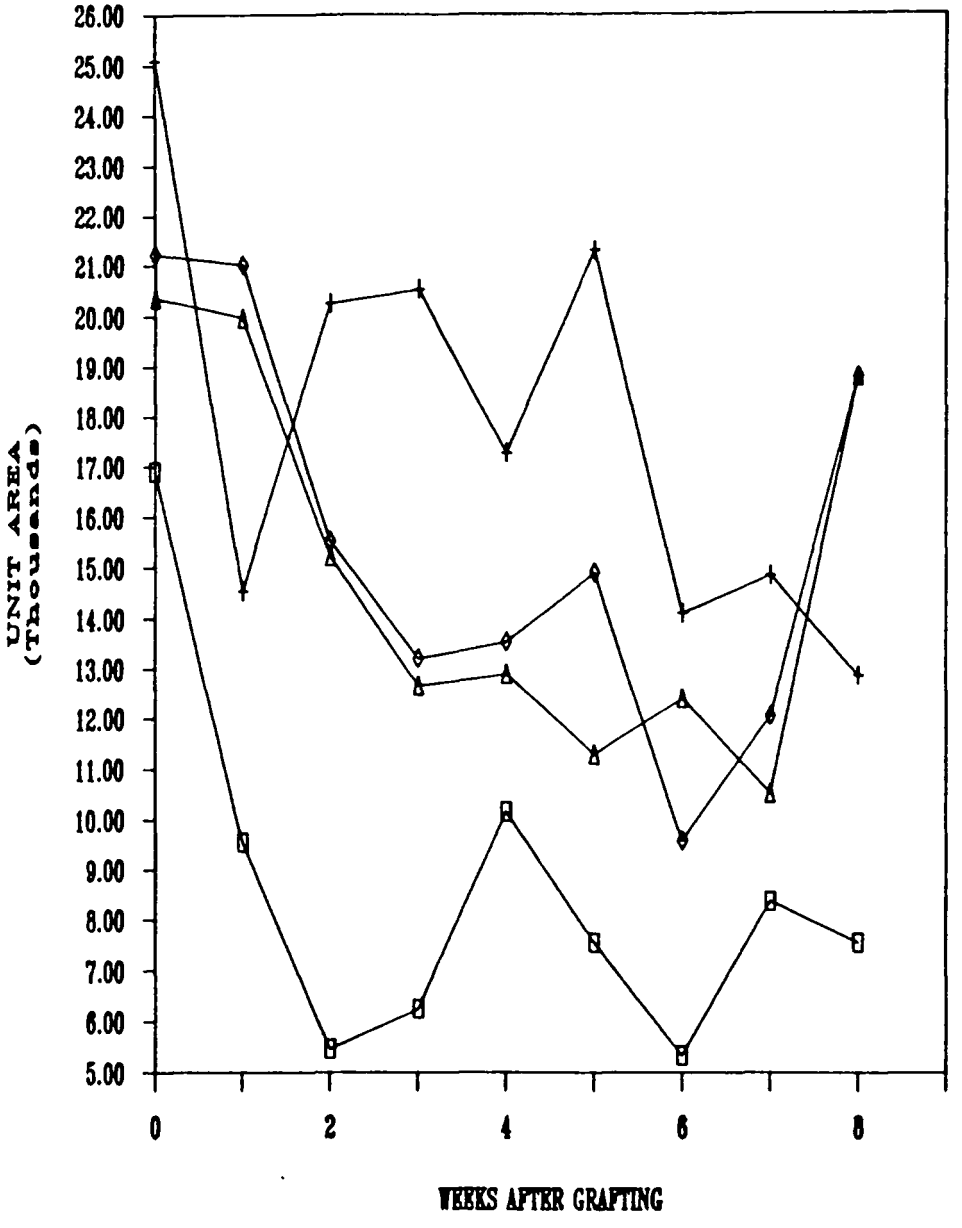

Fig. 50. Changes during union development in quantity of four of the unidentified lipid peaks of the experiment Total Carbon Reserves - Covered. The peaks were designated as Beta (□), Rho (+), Delta-P (◇) and Omega (△).

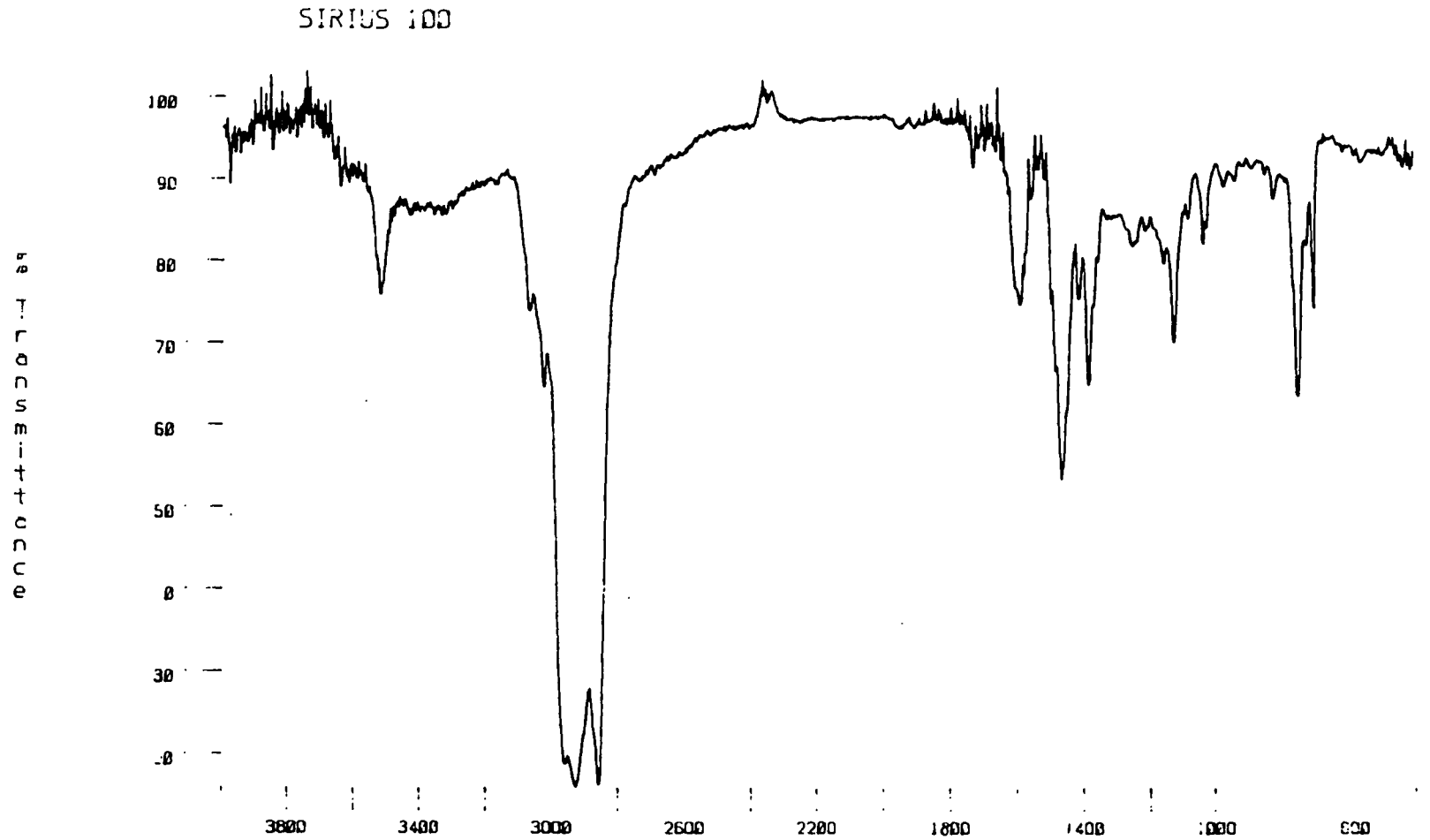

Fig. 51. Infrared spectrum of the Unknown lipid peak.

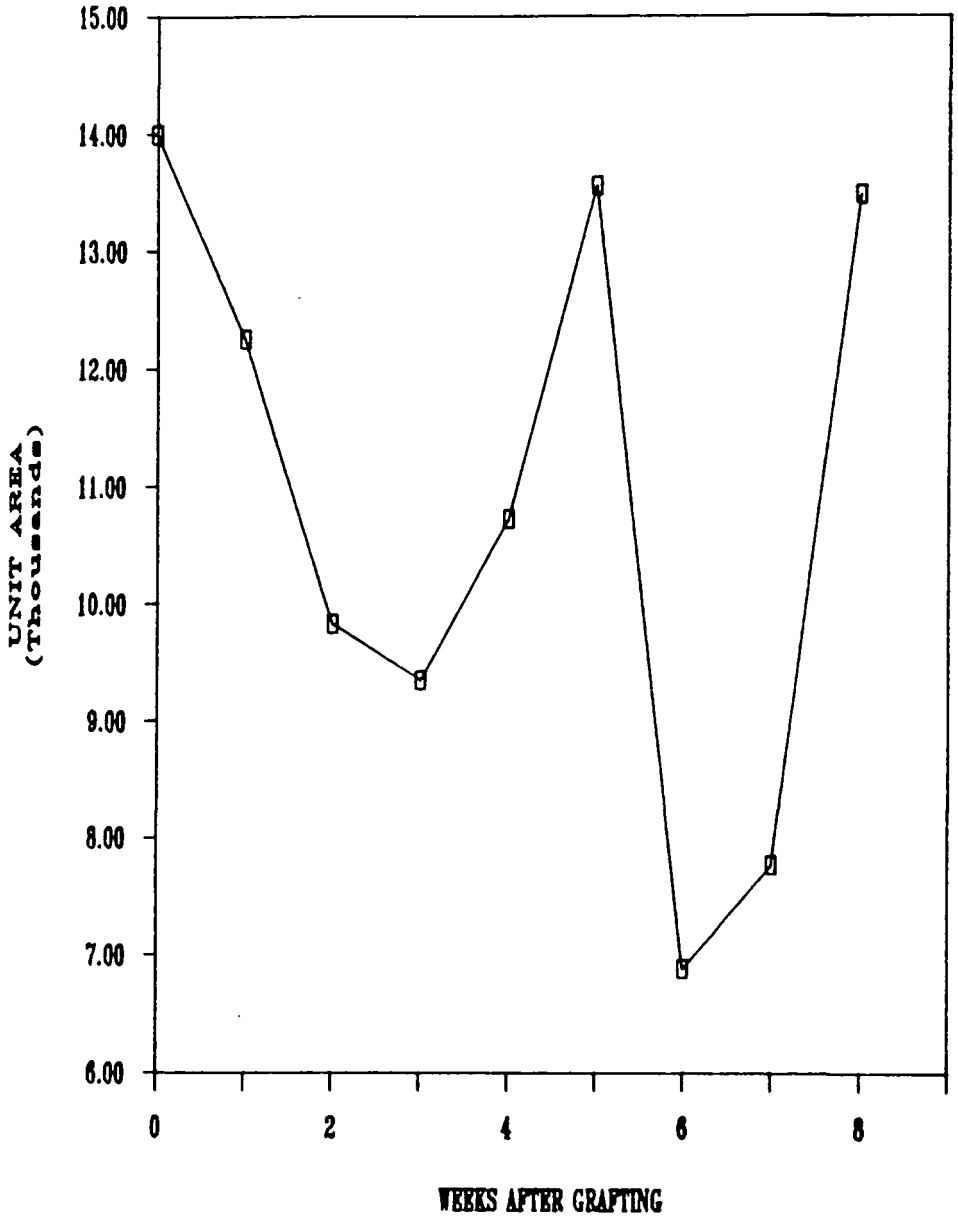

Fig. 52. Changes in the means of the Unknown lipid fraction during union development.

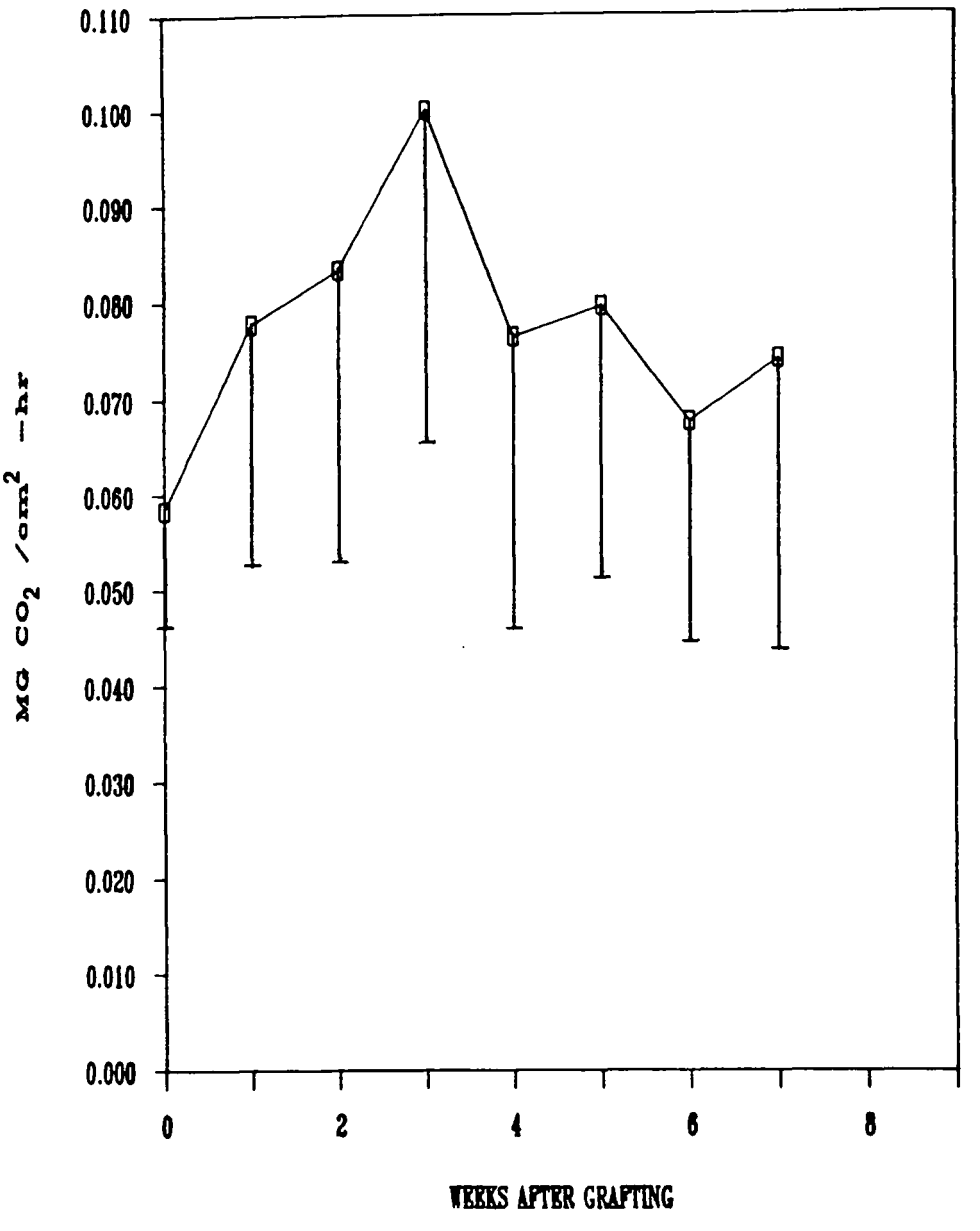

Fig. 53. Dark respiration rates of scion segment during union development. Vertical bars indicate standard errors.

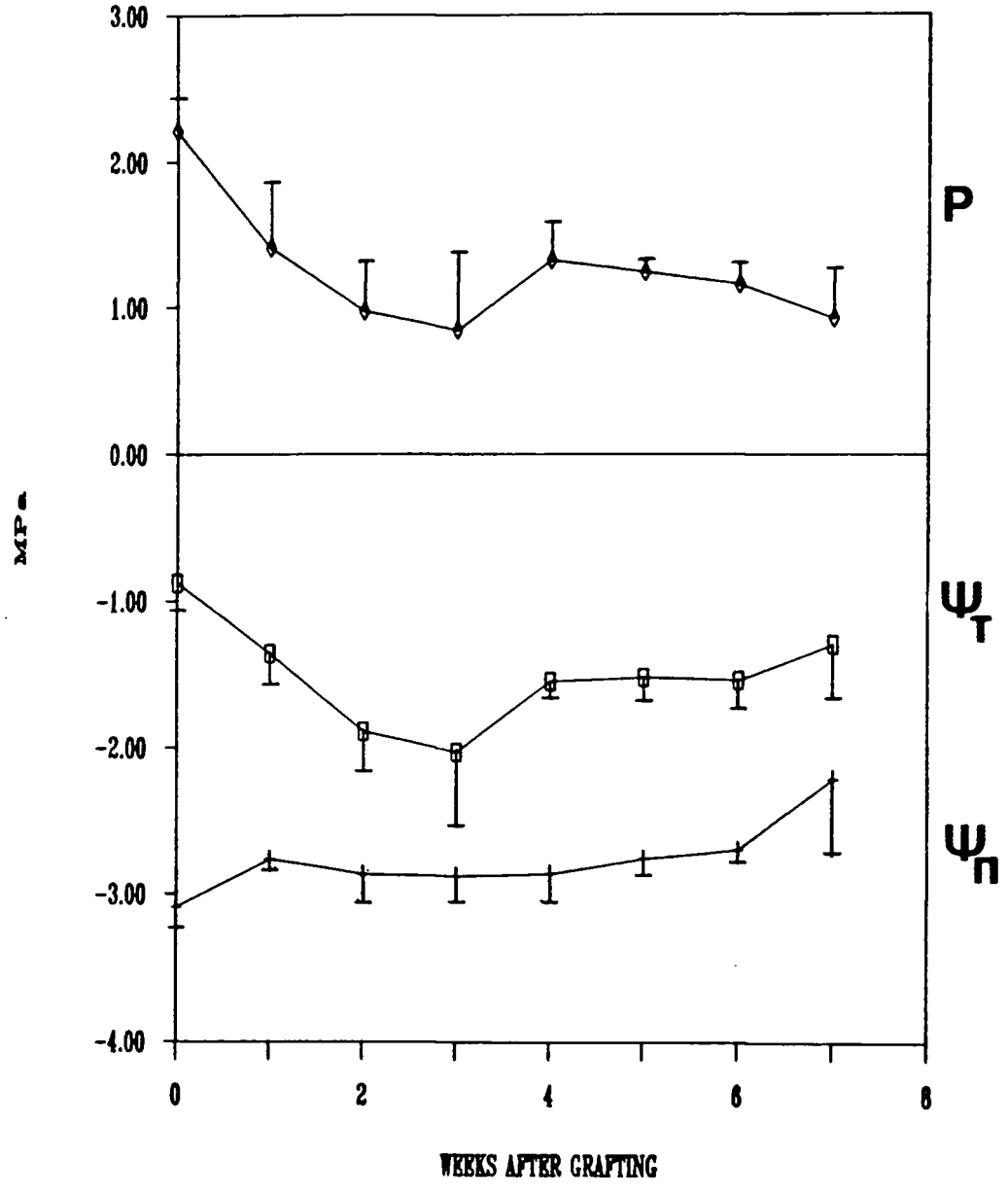

Fig. 54. Mean total water potentials, osmotic potentials and turgor pressures for the grafts used for respiration measurements. Vertical bars indicate standard errors.

Ψ_T and P with respiration rates; as Ψ_T and P declined, respiration rates increased, while a decrease in respiration rates occurred with increasing Ψ_T and P.

Carbon budgets during union formation were developed for successful (Table 9) and unsuccessful (Table 10) grafts of the TCRC experiment. Values for needle sugar and starch were transformed to glucose equivalents per cm^2 bark. Respiration rates were calculated as mg CO_2 per cm^2 bark-week. Three assumptions were made to simplify the calculations of CO_2 loss by respiration: one, respiration rates were assumed to be constant over the course of a day; two, rates were assumed to be constant 3.5 days both prior and after the day respiration was measured; and three, rates were assumed to be equivalent for successful and unsuccessful grafts. Based on these assumptions, the total flux in measured carbohydrates in the successful and unsuccessful grafts account for only 9.8% and 11.7% respectively, of the estimated CO_2 loss during the first 8 weeks of union development. The maximum change in carbohydrates (maximum measured value - measured value at week 8) is given in the parentheses. Still, the carbohydrate change accounts for only 14.6% and 16.1% of the estimated CO_2 loss for successful and unsuccessful scions respectively.

Techniques

There were no differences in success among the treatments of Environment 1984, all were greater than 90% successful (Table 11).

Table 9. Carbon balance sheet per week of Total Carbon Reserves - Covered (TCRC) successful scions. Sign indicates gain (+) or loss (-) from previous week. Respiration rates are estimated as CO_2/cm^2 bark-week. Carbohydrates are in glucose equivalents/cm^2 bark.

Week	Respiration	Bark Starch	Needle Sugar	Needle Starch
1	11.438	+2.361	-2.381	+0.505
2	13.534	-0.400	-2.758	-0.180
3	15.401	+0.992	-0.691	+0.055
4	14.828	-0.742	+2.321	-0.119
5	13.111	-0.718	-0.913	-0.026
6	12.368	-0.623	-2.876	-0.069
7	12.368	-0.313	+0.917	-0.112
8	11.905	-0.709	-0.514	-0.002
Totals	104.954	-0.152	-6.885	+0.051
	(104.954)	(-3.105)	(-6.885)	(-0.454)

104.954 mg CO_2 - 6.985 mg Glucose equiv.
(104.954 mg CO_2 - 10.444 mg Glucose equiv.)

2.385 mmoles CO_2 - 0.233 mmoles Glu. equiv. = -2.152 mmoles (90.2%)

2.385 mmoles CO_2 - 0.348 mmoles Glu. equiv. = -2.037 mmoles (85.4%)

Table 10. Carbon balance sheet per week of Total Carbon Reserves - Covered (TCRC) unsuccessful scions. Sign indicates gain (+) or loss (-) from previous week. Respiration rates are estimated as CO_2/cm^2 bark-week. Carbohydrates are in glucose equivalents/cm^2 bark.

Week	Respiration	Bark Sugar	Needle Sugar	Needle Starch
1	11.438	+2.679	-1.763	+0.384
2	13.534	+0.338	-4.684	-0.099
3	15.401	-0.970	+0.984	+0.052
4	14.828	-0.131	+2.481	-0.027
5	13.111	-0.341	-1.346	-0.101
6	12.368	-0.840	-2.379	-0.023
7	12.368	-0.310	-0.714	-0.176
8	11.905	-0.610	-0.844	+0.036
Totals	104.954	0.185	8.264	-0.045
	(104.954)	(2.864)	(8.264)	(0.374)

104.954 mg CO_2 - 8.404 mg Glucose equiv.
(104.954 mg CO_2 - 11.502 mg Glucose equiv.)

2.385 mmoles CO_2 - 0.280 mmoles Glu. equiv. = -2.105 mmoles (88.3%)

2.385 mmoles CO_2 - 0.383 mmoles Glu. equiv. = -2.002 mmoles (83.9%)

Table 11. Means of growth parameters of Environment During Union Development (Environ). Means with the same letter are not significantly different at the 5% level as separated by Protected LSDs. Growth parameters are given in cm, bud break as days after grafting.

Parmeter	Greenhouse	Lath House	Outdoors
% Success	97.5	100	90
Branch Number	5.3±2.1 ab	4.9±1.9 a	6.2±1.9 a
Terminal Length	7.1±2.8 a	5.9±2.8 b	5.6±2.1 b
Total Growth	37.8±15.8 a	31.6±12.1 b	29.6±10.7 b
Average Branch	7.2±1.5 a	6.1±1.3 b	5.1±1.0 c
Growth/cm_3Scion	2.5±0.9 a	2.0±0.8 b	2.4±0.8 a
Growth/cm^3 Scion	7.04±2.83 a	5.80±2.71 b	7.01±2.9 a
Branch : Bud	57.8±18.5 b	59.9±22.8 b	77.1±21.2 a

Greenhouse grafts had significantly more total scion growth and average branch length. Grafts grown outdoors had significantly higher branch to bud ratios. Lath house scions had significantly lower branch growth/cm scion length; but on a growth /cm^3 scion basis, there were no significant differences.

In the PGS-82 experiment, the 3 min soak proved superior to the 3 sec quick-dip for graft success. The success rate increased with increasing IBA concentrations, with 100% at the 1.0 mM concentration (Fig. 56). In contrast, percent success declined with increasing GA_3 concentrations. The 0.01 and 0.1 mM BA treatments had no affect on graft success while 1.0 mM lowered success compared with the control. All three compounds were detrimental to graft success when applied as a 3 sec quick-dip (Fig. 55).

In 1983 (PGS-83, Table 12) the applications of IBA at 0.1 and 1.0 mM, BA at 0.1 mM and NAA at 0.1 mM resulted in significantly higher success rates than the control (with the exclusion of the detrimental concentrations of IBA and NAA at 10 mM). Higher concentrations of all compounds decreased success. There were no differences in total branch growth or total growth/cm scion among treatments. However, there appear to be less post-union scion growth with the PGS treatments. Most treatments slowed bud break and decreased the mean branch length and number of branches over the control.

In Plant Growth Substances 1984, all grafts in the 27 min soak treatments were unsuccessful. The 3 min treatments were less detrimental to graft success than the 9 min treatments, but still were harmful compared to untreated grafts (Table 13). Among the growth parameters, only bud break was significantly different, with the 9 min

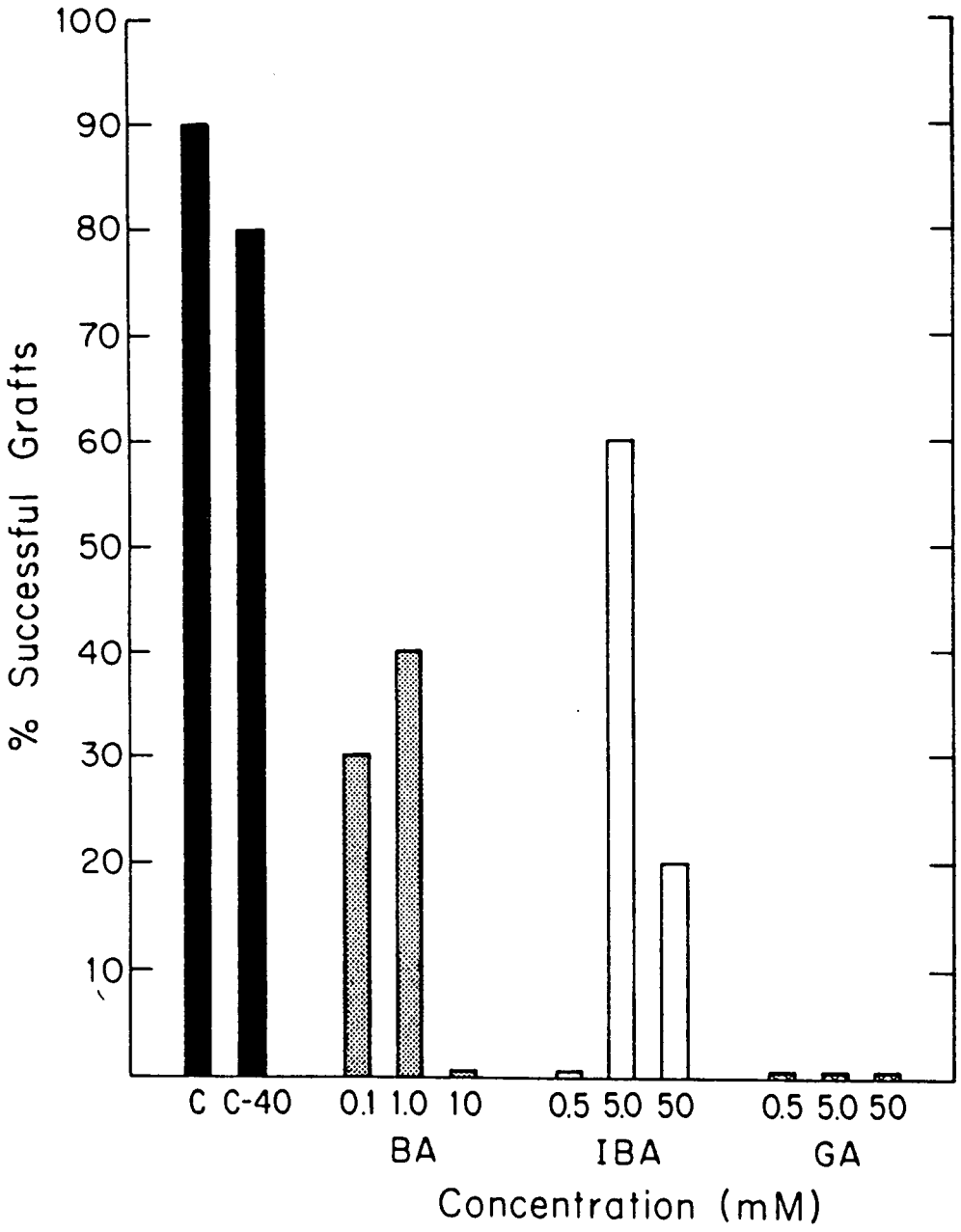

Fig. 55. Percent success per treatment using the 3 sec quick-dip application of the experiment Plant Growth Substances 1982.

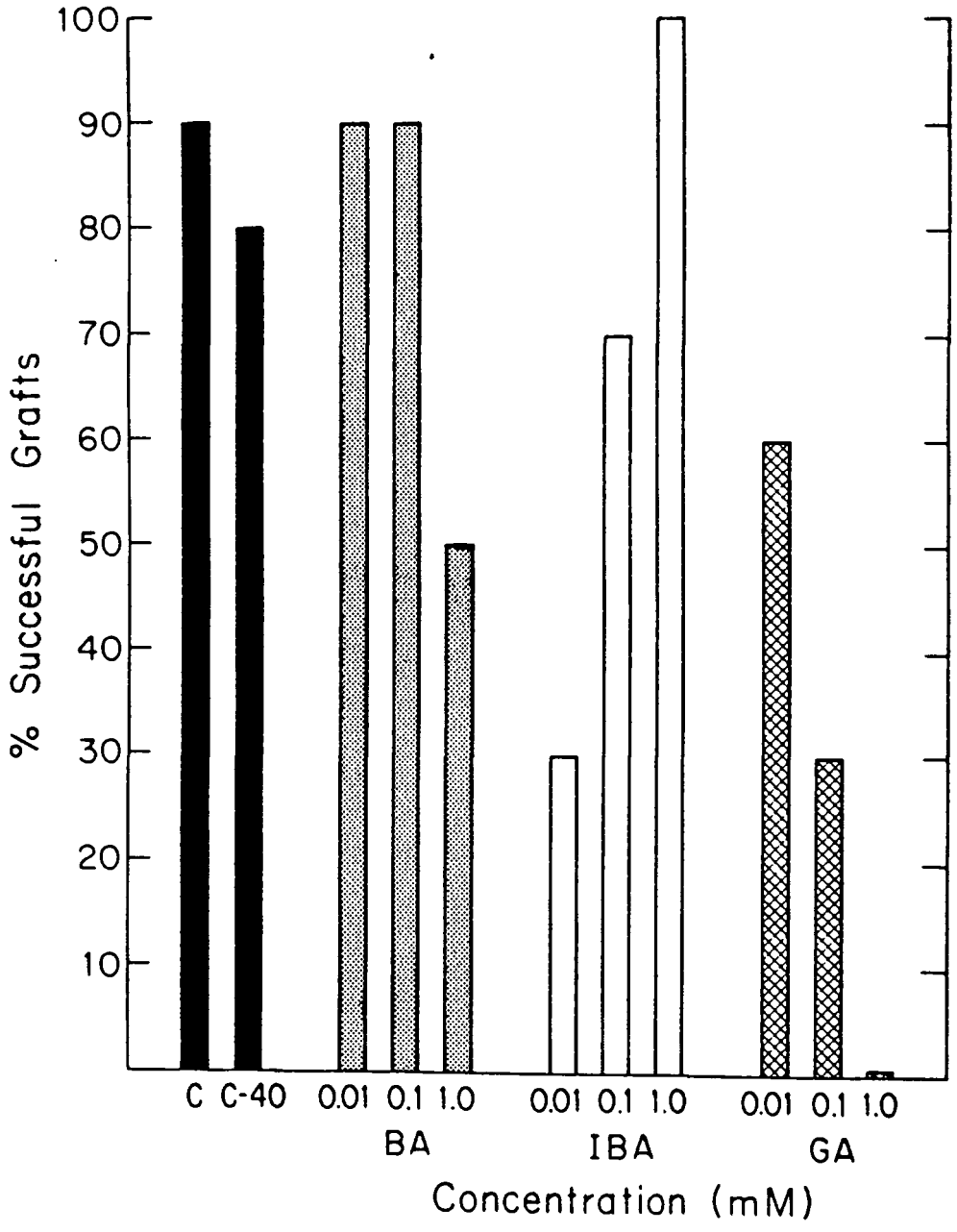

Fig. 56. Percent success per treatment using the 3 min dilute soak application of the experiment Plant Growth Substances 1982.

Table 12. Means of growth parameters of Plant Growth Substances 1983. Means with the same letter are not significantly different at the 5% level as separated by Protected LSDs. Growth parameters are given in cm, bud break as days after grafting.

Conc.	% Success	Bud Break	Branch Number	Total Growth	Terminal Length	Average Branch	Growth/ cm Scion
IBA 0.1	90 ab	57.6±6.8 ab	2.7±1.8	14.3±8.7	5.3±2.0 bc	5.4±1.4 abc	1.3±0.7
1.0	93 a	55.3±4.2 a	2.9±1.8	15.0±9.3	5.8±2.0 ab	5.1±1.0 bcd	1.4±0.7
10.0	20 66.	0±12.6 d	3.2±1.6	12.6±8.7	3.8±3.2 d	3.6±1.8 e	0.9±0.6
BA 0.01	87 abc	59.7±5.3 abc	3.5±1.8	17.0±10.2	5.4±2.1 abc	4.7±1.2 bcd	1.5±0.7
0.1	93 a	58.7±8.6 abc	2.7±1.9	12.8±9.5	5.0±1.7 bcd	4.7±0.8 bcd	1.3±0.7
1.0	72 d	62.3±8.2 cd	3.0±1.5	14.2±9.7	4.3±2.6 cd	4.4±1.7 d	1.4±0.8
NAA 0.1	90 ab	61.1±9.4 bc	2.9±2.0	14.4±11.4	5.5±2.6 abc	4.6±1.2 cd	1.2±0.8
1.0	83 bc	57.1±4.0 ab	3.7±2.1	21.1±12.0	6.7±1.4 a	5.9±1.2 a	1.7±0.7
10.0	0	-------	------	-------	------	-------	------
Control	80 cd	56.1±6.6 a	3.6±1.9	20.2±11.7	5.9±2.4 ab	5.5±1.1 ab	1.8±1.1

Table 13. Means of growth parameters of Plant Growth Substances 1984. Means with the same letter are not significantly different at the 5% level as separated by Protected LSDs. Growth parameters are given in cm, bud break as days after grafting.

Treatment		% Success	Bud Break	Branch Number	Terminal Length	Total Growth	Average Branch	Growth/ cm Scion
CNTL	3 min	56.7	56.5±6.8	3.9±1.8	3.3±1.6	22.6±13.3	5.8±1.6	1.5±0.9
	9 min	33.3	57.8±6.7	4.2±1.9	3.4±2.4	21.6±10.5	5.1±1.5	1.5±0.6
IBA	3 min	43.3	59.2±5.6	4.7±1.8	3.8±1.9	25.5±11.6	5.4±1.1	1.6±0.7
	9 min	6.7	74.0±19.8	4.0±4.2	6.0±2.5	21.9±25.0	4.9±1.0	1.6±1.8
BA	3 min	50	55.2±4.7	3.5±1.3	4.0±2.7	21.1±9.8	5.9±1.3	1.3±0.6
	9 min	16.7	63.8±13.9	3.2±2.2	2.3±2.3	17.0±12.5	5.3±1.0	1.1±1.0

treatments requiring longer for bud break than the 3 min treatments. It is evident the ethanol had an inhibitory effect on graft success and scion growth.

The application of 1000 ppm of both Atrinal and maleic hydrazide (RSReduct-83, Table 14) produced 100% success in the lath house. Of the treatments, only the Atrinal 2000 ppm treatment had significantly lower success. The application of the growth inhibitors significantly increased the time required for bud break. The constant debudding of the rootstocks prevented rootstock shoot growth, yielding a significant increase in scion terminal length, total scion growth and average branch length over the control. The two next most limiting treatments on rootstock shoot growth, 3 debuddings and Atrinal 2000 ppm, also tended to have more scion growth than the the control. In the greenhouse, the application of the growth inhibitors had no significant effect on the growth parameters.

In 1984 (RSReduct-84, Table 15), there were no differences in the success rate, though both debudded and Atrinal-Lath treatments were above 90%. The application of Atrinal to both dormant (lath house) and actively growing rootstocks (greenhouse) significantly decreased total scion growth. The debudded treatment had the highest total scion growth, but was not significantly different from the control. However, on a growth/cm scion basis, the debudded treatment was significantly larger than all other treatments. The use of Atrinal in the greenhouse generally reduced scion growth compared to the control and debudded grafts. Atrinal application in the lath house yielded growth similar to LWR-84 grafts.

Grafts under 20 hr photoperiods during union development (Photo,

Table 14. Means of growth parameter of Rootstock Shoot Reduction 1983. Means with the same letter are not significantly different at the 5% level as separated by Protected LSDs. Growth parameters are given in cm, bud break as days after grafting.

Treatment	% Success	Bud Break	Branch Number	Terminal Length	Total Growth	Average Branch
Lath House						
Control	95.8 a	99.2±9.1 d	3.6±1.8	4.6±1.7 d	14.4±7.5 c	4.0±0.9 c
Const. Debud	83.3 a	101.2±9.9 cd	4.5±1.5	6.3±1.4 a	23.9±9.5 a	5.2±1.1 a
3 Debud	87.0 a	99.8±8.1 d	3.9±1.7	5.6±2.0 abc	19.7±11.1 bc	4.7±1.5 ab
2 Debud	91.7 a	100.6±10.3 cd	4.0±1.4	5.0±1.3 cd	16.5±5.9 bc	4.2±1.0 bc
Int. Debud	87.5 a	106.1±10.6 bc	4.3±2.3	4.6±1.7 d	17.5±11.7 bc	3.9±1.9 c
MH 1000	100.0 a	109.7±11.1	3.4±1.9	5.2±1.5 bcd	14.7±8.6 bc	4.2±1.0 bc
Atrinal 2000	56.7 a	123.6±9.8 a	4.0±1.8	6.0±1.7 abcd	19.9±11.6 bc	4.8±1.2 bc
Atrinal 1000	100.0 a	107.3±11.7 b	4.2±1.3	5.5±12 ab	17.2±6.2 b	4.3±1.3 ab
Greenhouse						
Control	80.0	56.1±5.9	3.6±13	5.9±1.6	20.2±12.2	5.7±1.5
MH 1000	78.6	54.9±9.4	3.6±1.2	5.6±1.5	17.2±7.8	4.7±1.4
Atrinal 1000	87.7	57.5±9.4	3.3±1.7	5.4±2.5	16.4±9.3	4.9±1.4
Atrinal 2000	70.0	59.0±6.9	4.4±1.6	5.1±1.4	19.1±7.8	4.3±0.8

Table 15. Means of growth parameter of Rootstock Shoot Reduction 1984. Means with the same letter are not significantly different at the 5% level as separated by Protected LSDs. Growth parameters are given in cm, bud break as days after grafting.

Parameter	Control	Atrinal-GH	Debudded	Atrinal-Lath
% Success	86.7	80	93.3	96.7
Bud Break	54.0±11.6	56.3±6.0	51.4±10.8	109.0±8.2
Branch Number	7.2±2.2	3.9±3.4	6.2±3.0	4.3±2.5
Terminal Length	8.1±1.7 a	5.3±3.7 c	7.3±3.0 ab	6.3±1.6 bc
Total Growth	33.8±15.8 a	24.1±14.5 b	41.1±17.3 a	23.3±9.1 b
Average Branch	6.8±1.7 a	6.2±1.6 ab	6.8±1.4 a	5.9±1.2 b
Growth/cm Scion	2.5±1.0 b	1.8±1.1 c	3.0±0.9 a	2.0±0.8 c
Branch : Bud	85.7±20.3	44.3±18.8	75.2±20.7	55.5±24.2

Table 16) had earlier union formation and significantly more shoot growth per cm^3 scion (Growth/cm^3 scion) than the control. Grafts under the 20 hr photoperiod after bud break had significantly more branches than the control and a strong tendency toward more total scion branch growth. However, longer photoperiods did not increase graft success.

There were significant differences in all growth parameters for the scions in the Extended Scion Dormancy (Dorm) experiment (Table 17). Scion storage for 6 weeks significantly decreased graft success. Union development in the 10 week storage and the two ABA treatments was significantly earlier (Bud Break) than the control or 6 weeks scion storage. Both the control and 10 week storage scions grew significantly more than the 6 week storage or the ABA treatments. The ABA treatments appear to have reduced scion growth by inhibiting shoot elongation, exemplified by the low Branch : Bud ratio.

Neither the application of antitranspirants to the scions (Antitrans) nor enclosing the grafts in a polyethylene sheet (Tent) increased graft success over the control (Table 18). However, the use of antitranspirants did reduce the time required for union development (Bud Break). Tent scions had significantly more total branches, total branch growth and growth/cm scion length, but had significantly less growth based on growth/cm^3 scion and less average growth/branch.

The growth parameters measured for Rootstock Warming (Table 19), were generally only significant between treatments (control vs. rooting box). The control was superior for all parameters. The experienced grafter had significantly earlier union development as evidenced by scion bud break, while the success rate was dependent on

Table 16. Means of growth parameters of Scion Photoperiod Response. Means with the same letter are not significantly different at the 5% level as separated by Protected LSDs. Growth parameters are given in cm, bud break as days after grafting.

	Treatments		
Parameter	20 Hr	1/2 Bud Break	Control
% Success	93.1	86.2	100
Bud Break	51.1±6.3 b	57.0±5.3 a	57.4±5.1
Branch Number	7.1±2.2 a	6.9±2.4 a	5.3±2.2 b
Terminal Length	7.2±2.8	8.1±2.0	7.8±2.4
Total Growth	46.5±14.0	45.3±17.3	37.2±15.5
Average Branch	6.6±1.0	6.6±1.0	7.1±1.2
Growth/cm_3 Scion	3.1±0.9	2.9±1.0	2.7±1.0
Growth/cm^3 Scion	7.99±1.44 a	7.36±8.6 ab	6.48±1.30 b
Branch : Bud	78.3±17.9 a	77.4±19.7 a	65.7±22.3 b

Table 17. Means of growth parameters of Extended Scion Dormancy. Means with the same letter are not significantly different at the 5% level as separated by Protected LSDs. Growth parameters are given in cm, bud break as days after grafting.

Parameter	Control	6 Weeks	10 Weeks	10-4 ABA	10-5 ABA
% Success	80.0 a	36.7 b	88.5 a	72.4 a	86.7 a
Bud Break	53.9±5.9 b	54.5±9.7 b	43.2±4.3 a	41.0±3.9 a	42.2±5.0 a
Branch Number	7.3±1.9 a	4.9±1.4 bc	5.1±1.3 b	3.9±2.2 c	3.9±2.2 c
Terminal Length	4.2±2.4 bc	2.5±2.1 bc	6.2±2.1 a	4.7±2.5 b	4.4±2.6 b
Total Growth	40.4±16.9 a	25.6±8.9 bc	32.8±7.7 b	21.8±11.3 c	23.7±13.8 c
Average Branch	5.5±1.5 b	5.2±0.9 b	6.5±0.8 a	5.8±1.3 ab	5.8±1.5 a
Growth/cm Scion	2.4±1.4 a	1.8±0.8 bc	2.4±0.5 ab	1.5±0.7 c	1.6±0.9 c
Branch : Bud	72.1±13.0 a	54.6±22.3 bc	63.4±22.8 ab	44.5±25.6 c	47.2±22.1 c

Table 18. Means of growth parameters of Scion Antitranspiration Application (Antitrans) and Polyethylene Enclosed Grafts (Tent). Means with the same letter are not significantly different at the 5% level as separated by Protected LSDs. Growth parameters are given in cm, bud break as days after grafting.

Parmeter	Control	Tent	Antitrans
% Success	82.5	85.2	83.0
Bud Break	45.2±5.1 a	45.1±4.9 a	39.5±2.9 b
Branch Number	4.5±1.6 b	7.3±2.6 a	4.8±1.7 b
Terminal Length	5.5±3.3	4.0±2.7	5.4±3.2
Total Growth	29.7±12.7 b	42.3±16.5 a	33.6±11.1 b
Average Branch	7.2±1.5 a	5.8±1.4 b	7.2±1.4 a
Growth/cm_3 Scion	2.2±0.8	2.1±0.9	2.2±0.7
Growth/cm^3 Scion	5.91±2.23 a	3.30±1.51 b	5.48±1.73 a
Branch : Bud	54.4±18.1	62.6±21.1	59.9±16.0

Table 19. Means for growth parameters of Root-Warming. Means with the same letter are not significantly different at the 5% level as separated by Protected LSDs.

Treatment	Bud Break	Branch Number	Total Growth	Average Branch	Growth/ cm Scion
Lath	99.0±9.8 a	5.6±2.0	30.4±12.0 a	5.5±1.4 a	2.8±1.0 a
Box	108.7±10.5 b	4.8±1.9	23.8±14.7 b	4.6±1.4 b	2.0±1.0 b

Grafter	Bud Break	% Success
Inexperience	109.2±11.5 a	36 b
Experience	98.0±11.1 b	74 a

	Exp.-Lath	Inexp.-Lath	Exp.-Box	Inexp.-Box
% Success	96 a	56 b	52 b	16 c

both grafter and treatment.

The analysis of the growth parameters for Unestablished Rootstocks are compiled in Table 20. Since the success rate in the rooting box treatment was only 15%, the growth parameters of this treatment were not included in the analysis. Greenhouse grafted trees were superior to lath house grafts for all the growth parameters and the success rate. Only the ratio of branches to buds and number of branches were not significant.

Table 20. Means of growth parameters of Unestablished Rootstocks (Lifted-84). Means with the same letter are not significantly different at the 5% level as separated by Protected LSDs. Growth parameters are given in cm, bud break as days after grafting.

Treatment	% Success	Bud Break	Branch Number	Total Growth	Average Branch	Growth/ cm Scion	Branch : Bud
Greenhouse	75 a	68±6.3	4.3±2.3	28.5±14.8 a	6.8±1.3 a	2.6±1.3 a	47.8±25.5
Lath House	45 b	124±8.7	2.8±1.3	14.4±8.7 a	5.0±1.5 b	1.2±0.6 b	30.4±14.0

Chapter 5

DISCUSSION

The development of a successful conifer graft and the speed at which development occurs are dependent on three key, interrelated factors: proper alignment of the cambia of the stock and scion, the post-grafting environment and the physiological state of the rootstock. The interactions of these govern the water status of the scion through their influence on union development, but each can also affect graft success independent of union development. It is with this concept, that the results are interpreted.

The range of scion bud break was thought to be due to variations in union development. Changes in Ψ_T also appeared to correlate with bud break. Therefore it was theorized that bud break could be a good indicator of the maturation of vascular connections between the rootstock and scion. The procedure using acid fuchsin, a xylem translocated dye, established the relationship between vascular connections, the changes in Ψ_T and scion bud break. Acid fuchsin was first used by Vite (1959) and Vite and Rudinsky (1959) to trace the water conducting systems in conifers. Since then, it has been used by Tainter and French (1973) and Kozlowski et al (1967) for similar purposes. Movement of the dye is confined to mature xylem with little movement through the cell walls. Maturation of the connecting tracheids in spruce grafts was characterized by dye in the scions one to two weeks prior to bud break in greenhouse grafts (Fig. 19), and

four to five weeks prior to bud break in lath house grafts (Fig. 23). Marked increases in Ψ_T occurred at bud break (Fig. 20), one week after mature tracheid connections were well-established in greenhouse grafts (Fig. 19). In lath house grafts, the marked increase in Ψ_T (Fig. 22) occurred two to three weeks after tracheid connections had matured (Fig. 23), with bud break occurring two weeks thereafter. Hence, bud break was established as an indicator of vascular connections and was used for separating the grafts into groups, in order to aid interpretation of the results.

The water relations among the groups of the successful grafts generally followed the same pattern as the Group 1 scions for GWR-83 (Fig. 1), GWR-84 (Fig. 2), and LWR-84 (Fig. 3). The later changes in the water parameters of the other successful Groups (2 and 3) are correlated with later bud break and slower union development resulting from less precise cambial alignment. To distingish more easily the physiology of successful and unsuccessful grafts, most of the discussion will center on Group 1 grafts (successful) and unsuccessful grafts.

In all greenhouse grafts, Ψ_T declined rapidly the first two weeks. For the successful grafts (Fig. 1, 2), measured Ψ_T was the lowest during the initial one and a half to two weeks when the scions were essentially detached branches. Anatomical studies of conifer have shown that a callus bridge does not develop until 10 to 15 days after grafting (Copes, 1967; Dormling, 1964). The slow decline in Ψ_T during week 2 indicates water conservation by the scion and the formation of callus bridges in the union. For most conifer species, stomatal closure appears to occur between -1.3 to -1.7 MPa for both

detached branches and water stressed seedlings (Coutts, 1980, 1981; Watts & Neilson, 1978; Lopushinsky, 1969; Drew & Ferrell, 1979). After the first week, the RWC of scions and detached but ungrafted twigs were the same (Fig. 17). By two weeks, the RWC of ungrafted twigs (72.6%) were 15% lower than those of successful grafts. This corresponded with Ψ_T of -2.36 MPa, which were below the Ψ_T at which scions remain viable. No scions with measured Ψ_T below -2.0 MPa were successful. Transpiration rates observed by others generally declined rapidly below the Ψ_T threshold for stomatal closure (Coutts, 1980, 1981; Watts & Neilson, 1978; Lopushinsky, 1969; Drew & Ferrell, 1979; Beadle et al, 1979).

After the second week, Ψ_T increased in all greenhouse grafts with precise cambial alignment (Fig. 1, 2, 9, 18). If the mis-alignment was exaggerated, more than two weeks were required for callus bridge formation (MGWR, Fig. 8). By this time, Ψ_T had declined below -2.0 MPa and the scions were no longer viable. Total water potentials this low (-2.47 MPa) (Fig. 17), correspond to RWC of about 65%. With the development of callus bridges in poorly aligned grafts Ψ_T (Fig. 1, 2, 8, 18) and RWC (Fig. 17) were stable or decreased slowly. If callus bridges did not form, Ψ_T (Fig. 18) and RWC (Fig. 17) continued to decline rapidly, as in the case of the ungrafted twigs (SWL).

After five weeks, the Ψ_T (Fig. 1, 8, 18) and RWC (Fig. 17) of the unsuccessful greenhouse grafts declined, indicating scion death. The decline occurred approximately when connecting tracheids began to mature in successful grafts (Fig. 19). The "walling off" of the scion and the loss of stomatal regulation probably caused the decline in Ψ_T. Wound callus forms several cells deep around the edge

of the wound. As the callus develops, it rolls inward, suberized both adjacent to the wound and the exterior environment (Pirone, 1978). It appears probable that in the unsuccessful grafts, wound callus from the rootstock forms between the rootstock and the scion, disrupting the callus bridges. Apparently, the formation of cambial connections prevents this in successful grafts. However, graft failure in GWR-84 (Fig. 2) was due to poor union quality. All four unsuccessful grafts initiated bud elongation before 44 days, but were determined to be unsuccessful by week 10. The decline in Ψ_T began after bud break. The water loss from the scion during shoot elongation was greater than the amount of water that could cross the union zone. Placing similar grafts in the acid fuchsin dye solution revealed no mature tracheid connections.

In the successful grafts, the maturation of the connecting tracheids resulted in increases in RWC (Fig. 17) and increases in Ψ_T to -1.0 MPa within 2 weeks (Fig. 1, 2, 18). With sufficient water, scion buds began elongation, six to nine weeks after grafting. In contrast to the greenhouse grafts, the Ψ_T of established grafted trees (Fig. 4) increased to -0.8 MPa, and was maintained until bud break, which occurred after 4.5 weeks in the greenhouse.

Osmotic potentials in the greenhouse grafts generally remained constant or increased slowly during the first few weeks after grafting (Fig. 1, 2, 18). However two to three weeks prior to bud break, Ψ_π increased rapidly in successful grafts (Fig. 1, 2), but in established grafts, Ψ_π remained low and did not increase until after bud elongation (EGWR, Fig 4). At approximately the time Ψ_π increased in the successful grafts, Ψ_π began to decline in unsuccessful grafts

(Fig. 1, 18). The degree of mis-alignment of the cambia influenced the decline in Ψ_π. In the MGWR grafts, Ψ_π declined continuously after grafting (Fig. 8). Similar results after the first week were found for grafts in the growth chambers (T&T) at 21.1° C. (Fig. 9) and 26.7° C. (Fig. 13) and for ungrafted twigs (Fig. 18). In the unsuccessful grafts, Ψ_π usually declined earlier and more rapidly than Ψ_T.

The relative changes in Ψ_T and Ψ_π were reflected in P. In the established grafts, P increased approximately 20% the first week and remained near 2.2 MPa before declining during shoot elongation to 1.1 MPa or less (fig. 4). However in greenhouse grafts, P generally declined rapidly the first week, then more gradually to stabilize between 0.5 to 1.0 MPa during scion bud elongation (Fig. 1, 2, 18). Unsuccessful grafts osmotically adjusted, maintaining high P even when graft failure was evident (Fig. 1, 8, 9, 13). Only in the Scion Water Loss experiment were P lower than successful grafts when measurements were stopped. Ungrafted twigs maintained P above 0.0 MPa down to Ψ_T of -4.73 MPa. It can be concluded therefore, that graft failure did not result from low P.

The Ψ_T of successful lath house grafts (LWR-84) declined slowly to a low of -1.4 MPa, generally after six weeks, before gradually increasing (Fig. 3). This was in contrast to the rapid decline the first two weeks followed by the slow increase of Ψ_T in the successful greenhouse grafts. Mature tracheid connections were evident 3.5 to 4.5 weeks prior to bud break (Dye-85, Fig. 23), thus it can be concluded that functional xylem connections were present in G-1 grafts 10.5 to 11.5 weeks after grafting, when Ψ_T increased above -1.0 MPa.

Osmotic potentials of lath house grafts were constant from week 3 until two to three weeks prior to bud break, and thereby maintained P above 1.3 MPa. When Ψ_π increased, P declined. The unsuccessful grafts also initiated shoot elongation but later failed. Like the unsuccessful grafts of GWR-84, both Ψ_T and Ψ_π declined after bud break. Therefore, the major difference between the water relations of lath house and greenhouse grafts is that, although union formation in the lath house was slower, scion water stress was much lower, accounting for the generally higher success rates. It can be concluded that the lower water stress of the scion during union development allows less precision in cambial alignment.

Transpiration rates of plants in uncontrolled environments are highly variable due to the many factors that affect transpiration (Hinckley et al, 1978). In order to assess the role of scion transpiration , transpiration rates were measured on scions grafted on actively growing rootstocks in growth chambers (T&T). The effect of two different temperatures on conifer grafting was also observed. The 21.1° C treatment (T&T) transpiration rates (Fig. 10) declined with Ψ_T over the first two weeks (Fig. 9). This can be interpreted as control of stomatal aperture by Ψ_T. Since mean bud break occurred at week 6, some functional tracheid connections would have been expected by week 4. The increased transpiration rates with increased Ψ_T confirmed this in the successful grafts. However, in the unsuccessful grafts, decreasing Ψ_T after week 2 and the lack of bud break indicate that functional tracheid connections did not form. Yet transpiration rates of the unsuccessful grafts increased after week 2 (Fig. 12), suggesting a loss of stomatal sensitivity to low Ψ_T.

Similar events occurred in the 26.7° treatment (T&T), even though transpiration rates were 10-fold higher. Total water potential appears to have controlled stomatal aperture during the first two weeks. Based on bud break, functional tracheid connections did not occur before the third week, when increases in transpiration and Ψ_T occurred. Declining Ψ_T and the lack of bud break in the unsuccessful grafts indicated no tracheid connections, though transpiration rates increased (Fig. 16).

The formation of callus bridges could account for some of the increase in the transpiration rates of the unsuccessful scions, but the continuous decline in Ψ_T indicates that the transpiration rates were maintained at the expense of decreased RWC of the scion. The SWL experiment established that as Ψ_T declines, so too does the RWC. The decline in Ψ_T implies that transpiration losses exceeded the water passed across the callus bridges. Transpiration rates of the unsuccessful grafts increased in both treatments when Ψ_T decreased below -2.0 MPa. Due to rootstock transpiration and the position of the scion on the rootstock, it is possible that the vapor pressure deficit of the scion may have been low enough for the stomata to open at the low Ψ_T in accord with the observations of Slatyer (1964), Kaufmann (1976) and Dixon et al (1984). An alternative hypothesis is based on the consistent osmotic adjustment observed in the unsuccessful scions. Above some critical Ψ_T value, changes in moderate stomatal aperture. Below the critical Ψ_T value, -2.0 MPa, stomata are less sensitive to Ψ_T and open in response to the high P (Fig. 12, 16) maintained by the osmotic adjustment (Fig. 1, 8, 9, 13). The decline in transpiration rates could be linked to the

decline in RWC. Similar loss of stomatal sensitivity to Ψ_T was recorded in 1983 in the GTT (Fig. 6, 7).

It is unrealistic to compare directly the transpiration rates recorded in the growth chambers with the rates recorded for the established grafts (EGWR, Fig. 5). Yet it is evident that scion transpiration rates of greenhouse grafts were very low during union development.

Prior to bud break, slow translocation and high photosynthetic rates in the spring, (Ericsson, 1979; Webb & Kerchesy, 1977; Little, 1970c), result in accumulation of high levels of carbohydrates conifer needles, mainly as starch. The values range from 10 to 20% of needle dry weight (Little & Loach, 1973; Ritchie, 1982; Fry & Phillip, 1977; Ronco, 1977; Little, 1970c, 1970d; Ericsson, 1978, 1979; Webb & Kerchesky, 1977; Krueger & Trappe, 1967; Kimura, 1969; Pomeroy & Siminovitch, 1969). The needle starch content of established grafts in the greenhouse (EGWR) peaked at 4.8% needle Dwt before declining rapidly with shoot elongation. The starch accumulations were lower than previously reported values of 6 to 25% (Kimura, 1969; Little & Loach, 1973; Little, 1970d; Winjun, 1963; Pomeroy & Siminovitch, 1969; Ericsson, 1979) because of low light levels in the greenhouse and the short time (4.5 weeks) of optimum conditions for photosynthesis before bud break.

Starch levels and transpiration rates in greenhouse grafts (Fig. 24, 25, 29) were much lower than the established grafts. Levels in greenhouse grafts were generally one-third of the established grafts, even though starch accumulated one to two weeks longer before declining. Starch levels generally peaked at weeks 5 or 6, one to

three weeks prior to bud break, but the rapid decline did not occur until around bud break. Starch accumulation rates were highest the first week (Fig. 24, 25, 28) when declined rapidly (Fig. 1., 2, 8). Thereafter, the rate of increase slowed, more in the unsuccessful than the successful grafts, with contents much higher in the successful grafts.

Translocation of the starch from conifer needles to the developing shoot occurs during bud break (Little & Loach, 1973; Ritchie, 1982; Fry & Phillip, 1977; Ronco, 1977; Little, 1970c, 1970d; Ericsson, 1978, 1979; Webb & Kerchesy, 1977; Krueger & Trappe, 1967; Kimura, 1969; Pomeroy & Siminovitch, 1969; Meyer & Spittstoesser, 1971). Similar translocation occurs in the successful scions. However, in the unsuccessful scions, starch levels also decline rapidly, but without bud elongation (Fig. 24, 28). This decline in starch levels of the unsuccessful grafts (Fig. 24, 28) is associated with the declines in both bark (Fig. 45) and needle (Fig. 46) sugars and in the Ψ_π (Fig. 1, 8). It is proposed that the decline in starch content of the unsuccessful scions occurs because of increased demands for sugars to decrease Ψ_π and fuel higher respiration rates. In contrast, the decline in starch content of the unsuccessful grafts for both GWR-84 and LWR-84 is attributed primarily to bud break, though the decline in Ψ_π was probably an additional factor.

In 1983, it appeared that needle starch accumulation might be a factor limiting graft success. A gradient of needle starch content was developed (SSAR, Fig. 29), but there were no consistent differences in the graft success of the covered scions or in most growth parameters (Table 5). It was concluded that photosynthesis

during union development was not required for graft success and that starch accumulation had no effect on post-union scion growth. Greater starch accumulation merely reflected lower water stress in the scions and higher light levels during union development. Earlier, it was shown that photosynthates from the rootstock are not translocated into the scion until after bud break (RSTrans, Fig. 42-44). Therefore, at grafting scions must have enough stored carbon compounds to supply respiration until after bud break. Stored carbon compounds, accumulated the previous year, are important for spring growth of conifers (Mooney, 1972; Kozlowski, 1963, 1966; Larson, 1964; Kozlowski & Keller, 1966; Clausen & Kozlowski, 1967; Olofiboba & Kozlowski, 1973; Kimura, 1969; Allen, 1964). Generally, most of the stored carbon has been reported to be carbohydrates accumulated as starch in the roots (Glerum & Balatinecz, 1980; Olofiboba & Kozlowski, 1973; Ursino et al, 1968; Ritchie, 1982; Parker, 1959; Shiroya et al, 1966). Parker (1959) and Olofibobla and Kozlowski (1973) reported some storage carbon compounds in twigs, but only identified them as non-sugars. However, several studies have implicated neutral lipids in the phloem as storage compounds in conifer twigs (Sinnott, 1918; Zieger, 1964; Kimura, 1969; Ronco, 1973; Holl, 1985).

In order to estimate the role of various storage compounds in spruce scions, an energy budget using the results from the TCR and TCRC grafts was developed. Total bark sugars (Fig 45) and starch levels (Fig. 47) for both uncovered (TCR) and covered (TCRC) grafts increased substantially one week after grafting. Declines in needle sugars at this time could account for the increase in bark sugars and starch in covered scions, but do not account for the estimated

respiration rates (Tables 9, 10). After the first week, bark sugars were highly variable but generally declined after four weeks. The insignificance difference between uncovered and covered scions for bark sugars indicates little net translocation of sugars from the needles. Needle sugar content was also variable but tended to decline slowly after two weeks (Fig. 46). The general decline in needle sugars in uncovered and covered scions was the same. It appears then, that most of the photosynthates produced in the uncovered scions were accumulated as needle starch, assuming that respiration rates were equivalent. Translocation from the needles is low until the buds become active.

Respiration rates (Fig. 53) appear to be negatively correlated with Ψ_T (Fig. 54). As Ψ_T declined to a low the third week, respiration rates peaked. Thereafter, when Ψ_T increased respiration rates decreased. This negative correlation is opposite to the effects of water stress on respiration rates in Abies (Puritch, 1973) and Pinus halepensis (Melzack et al, 1985), but it does agree with the results of Brix (1962) for loblolly pine.

Maximum changes in the measured carbohydrates of covered scions accounted for only 14.6% and 16.1% (successful and unsuccessful, respectively) of the total estimated CO_2 loss by respiration. The anthrone technique has been criticized as a poor method for estimating total sugars (Johanson, 1953). However Yemn and Willis (1954) modified the procedure and demonstrated its accuracy for quantitating hexoses but not pentoses. With the modified anthrone procedure, the quantification of non-reducing sugars is equal to the sum of the component sugars (Yemn & Willis, 1954). However, due to differences

in absorbance of the different anthrone complexes, the use of glucose standards underestimates sucrose concentrations by 5% and fructose concentrations by 10%. In spruce, raffinose, pungenin, and sucrose constitute the majority of bark and needle total sugars in the winter, with raffinose and pungenin contributing approximately 50% of the total sugar (Neish, 1958). Underestimates of raffinose and pungenin would be higher, therefore, the percentage that carbohydrates contribute to respirational CO_2 loss can not be determined. However, errors in the total sugar would still not account for the estimated CO_2 loss.

Declines in free fatty acids of the quantitated neutral lipids were observed both in preliminary TLC separations and by HPLC (fig. 48, Appendix 2.22), but quantities were low. Of the other peaks (Fig. 48-50, 52), only the Unknown (Fig. 52) appears to have the potential to be identified as the major storage compound in the bark. Unfortunately, the evidence to support the identification of the unknown compound as the storage lipid is not strong.

Since the environmental factors were constant during labelling, the percentage of recovery of $^{14}CO_2$ in the scions can be used as a relative measure of photosynthesis (Fig. 30). Both the photosynthetic rates and drop rapidly the first week. Recovery was lowest weeks 2 and 3, when was also at low levels. Photosynthetic rates remained very low during union development. This was an effect of the water stress imposed on the scion and concurred with the low transpiration rates observed prior to the maturation of tracheid connections (T&T, Fig. 10, week 3). It also agrees with the observations reported by others on the effects of water stress

(Dykstra, 1974; Beadle & Jarvis, 1977; Beadle et al 1981; Melzack et al, 1985; Kaufmann 1968; Puritch, 1973; Watts and Neilson, 1978). Recovery remained low until after 4.5 weeks. Little difference in total recovery was noted between 24 hr and 48 hr, so it can be assumed that minimum label was lost by respiration. For the 24 hr harvest, it appears that scions with some mature tracheid connections were selected for weeks 5 and 5.5, but not for weeks 6 and 6.5. At that point, differences in the rate of union development would become evident in both the water relations and photosynthetic rates, therefore scion selection played a major role in the results until bud break (week 7). With the beginning of bud break, and a much higher probability of functional tracheid connections, recovery rates increased as Ψ_T increased.

Recovery in the starch fraction was high at grafting, but decreased by 50% three days later. This suggest that most of the starch accumulation the first week occurred during the first few days as Ψ_T rapidly declined. The percentage of recovery in the starch fraction corresponds to the lower rate of accumulation of starch in the needles. After 5.5 weeks the low recovery of the label in the starch fraction occurs in conjunction with the rapid decline in total starch that occurs one to two weeks prior to bud break.

After week 0.5, most of the label was recovered primarily in the needle sugar fractions (Fig. 35), except for week 4. The amount of label found in the bark sugars was unexpected, considering the limited water flow in the xylem and the lack of photosynthetic sinks. Despite the levels in the bark, it was not until week 6 that label was recovered in significant quantity in either the buds or the graft

union, at which time functional tracheids were established. It can be concluded then, that there is little phloem translocation in the twig until the connecting tracheids mature. The low percentage of label found in the graft union before six weeks indicates the developing union is not a sink for scion photosynthates until after vascular connections are established. A similar conclusion can be made for the scion buds.

To summarize briefly, after grafting most of the photosynthates remain in the needles, mainly in the form of sugars, until tracheid connections have matured. When water stress is reduced, bud activity begins and the sugars are transported from the needles into the developing buds, mainly the terminal whorl.

The interrelationships between the water relations and carbon metabolism of the greenhouse scions is proposed as follows. After grafting, Ψ_T declines rapidly generally reaching a low in two weeks, when callus bridges are formed. Water translocation across the parenchyma cells in the callus bridges maintain both the RWC and Ψ_T of the scions, in balance with transpirational losses. Starch accumulates in the needles rapidly at first, then slows due to lower photosynthetic and higher respiration rates caused jointly by water stress and stomatal closure. Gradually, as the callus bridges expand, the Ψ_T, RWC and transpiration rates of successful grafts increase. Mature tracheid connections develop approximately two weeks prior to bud break. Stomatal conductance then increases, but is still less than in established grafts. Bud break occurs when the water stress of the scion declines, to a Ψ_T of about -1.0 MPa. Imprecise alignment of the cambia slows the development of the callus bridges, delaying the

formation of functional tracheid connections, which results in lower Ψ_T during union development. This lower Ψ_T results in further decreases of stomatal conductance and higher respiration rates, resulting in turn in lower photosynthetic rates and starch accumulation. Lower Ψ_T also result in low Ψ_π in order to maintain P, thereby diverting more photosynthates as osmoticum.

Most of the scion photosynthates remain in the needles as sugar. The sugar translocated into the stem the first few weeks, apparently balances respiration rates of the stem. Little photosynthate translocation occurs in the stem until approximately a week before bud break, when functional tracheids have developed. Respiration rates appear to be higher than photosynthetic rates, resulting in approximately linear declines in needle sugar. However the decline in the measured total sugars does not correspond with the increase in Ψ_π. Therefore it is proposed that most of the decline in sugars is due to the metabolism of raffinose and pungenin; with the decline induced by the high respiration rates and warm temperatures. These two sugars are osmotically equivalent to sucrose (Neish, 1959) and would supply more energy per increase in Ψ_π. The metabolism of storage lipids would result in a slower reduction in sugar content, sustaining Ψ_π.

Several factors, therefore, increase the probability of graft failure in imprecisely aligned grafts. First, slower callus bridge formation result in lower Ψ_T, thereby increasing respiration rates, decreasing photosynthesis and retaining more photosynthates as osmoticum in order to maintain Ψ_π, and sustain P. At some critical point, Ψ_T starts to decline. It is proposed that this decline occurs due to a decrease in the water flow through the callus bridges. At

constant transpiration rates, this restricted water supply would further reduce the Ψ_T and RWC of the scion. Below a critical Ψ_T, stomata appear to be insensitive to Ψ_T and may open in response to the high P, resulting in higher transpiration rates. Accumulated starch would be metabolized to supply the increased needs for respiration and osmotic adjustment. Once the stomata lose sensitivity to Ψ_T, they rapidly deplete their usable water reserves and die. Therefore, the alignment of the cambia is the most critical factor for graft success. Techniques to shorten the time required for union formation or to reduce scion water stress, would result in higher graft success by allowing for less precision in cambial alignment. Several stategies were pursued in this study and are described below. In addition, scion quality was also evaluated by measuring various aspects of growth.

Generally, researchers and Pacific Northwest nurserymen have grafted conifers onto rootstocks actively growing in the greenhouse (Orr-Ewing & Prideaux, 1959; Holst, 1956a; Holst et al, 1956; Dorsman, 1966). This practice increases production costs and increases water stress on the scion during union formation. The results from Environ (Table 11) demonstrate that in the Pacific Northwest, spruce can be grafted on dormant rootstocks and either overwintered in a shaded cold frame or outdoors with a windbreak, without decreasing the success rate. Due to the moderate temperatures and high humidity of a Northwest winter, scion water stress is reduced, compensating for the slower development of the union. The high success rates of outdoor grafts indicate that scions can survive occassionally moderately low temperatures and occasional warm, windy days. Greenhouse grafts

produced more total growth, but standardized growth by scion length or cm^3 scion were not significantly different between outdoor and greenhouse grafts. If the lath house grafts had been removed from the shade at bud break, scion growth probably would have been greater.

The use of plant growth substances (PGS) to improve graft success has previously produced mixed results (Smith et al, 1972; Jackson & Zak, 1949; Davis, 1949; McQuilkin, 1950). Compared to many angiosperms, callus development and union formation in many conifers is slow. Therefore it was hypothesized that pretreating scions with PGS would improve graft success. In 1982, PGS were applied as a 3 min soak or a 3 sec quick-dip. Quick-dip application proved to be detrimental to union formation (Fig. 55). However the results from applications of 0.1 mM BA and 1.0 mM IBA as a three min soak (Fig. 56) suggested further study.

In the 1983 study (PGS-83, Table 12), both of these concentrations plus 0.1 mM IBA and NAA had significantly higher success rates than the control. However, the use of these compounds tended to decrease scion growth, though not significantly. The NAA concentrations were near toxic levels and blue spruce sensitivity to BA was also high. The optimum concentrations from 1982 were again the best. In 1984, two concentrations, 0.1 mM BA and 1.0 mM IBA, were evaluated to determine the length of time of which scions could be soaked without detrimental effects (Table 13). Unfortunately, the high alcohol concentration required to keep the compounds in solution was toxic for all soak times, so the results are inconclusive. However, with the 3 min soak, there was no significant difference in the growth parameters between the control and the PGS treatments.

The application of 0.1 mM BA or 1.0 mM IBA will improve graft success as a 3 min soak; but the optimun time of soaking is undetermined. The use of PGS on conifer grafts as a comman practice is not recommended because scion quality tended to be lower and success can be more easily acheived by less time-consuming techniques.

In a preliminary study, repeated debudding of the rootstocks in the greenhouse increased graft success and scion quality. The results can be explained by the Photosynthate Allocation Theory (Loomis, 1953; Gordan & Larson, 1968; Ursino et al, 1968; Larson & Gordan, 1969; Balakinecz et al, 1966; Zieme, 1971; Shiroya et al, 1962b). Preventing rootstock shoot growth results in more photosynthates being available to the cambia for union development and sequential scion growth. Rootstock debudding was repeated in the greenhouse (RSReduct-84, Table 15) and in the lath house treatments of RSReduct-83 (Table 14). The treatments that most severely limited rootstock growth increased scion shoot growth, with continuous debudding resulting in significantly more scion growth then the control for all growth parameters in the lath house, and on a growth/cm scion basis in the greenhouse. Less severe debudding treatments were not significantly different from control grafts, though the three debuddings treatment tended to have higher scion quality. Regrowth of rootstock buds during union development and scion growth competed with the scion for photosynthates, which reduced the effects acheived by rootstock debudding. Of the lath house treatments only 2000 ppm Atrinal reduced success and increased the time required for bud break. Yet, 2000 ppm Atrinal improved scion quality. In the lath house, 1000 ppm Atrinal resulted in 100% success

and increased scion quality over the control. In 1983, there was no significant difference among the greenhouse treatments, but Atrinal significantly reduced the scion quality in 1984. The same treatment produced similar success and scion quality as LWR-84 grafts.

Repeated debudding of the rootstocks improved scion quality for both lath house and greenhouse grafts and increased graft success in the greenhouse. There appeared to be a stronger graft union formed in repeated debudded treatments and more scion growth the second year, but these were not measured. Since debudding is a very labor intensive technique, the practice is not economically feasible on a large scale; but may be useful where only a few grafts are to be made, and higher success and scion quality are the main concern. The growth retardants were ineffective at the concentrations tested.

The extension of daylength and the increased light quantity did not improve graft success (Photo, Table 16). However, long days decreased the time required for union formation. The cambial activity of the rootstocks apparently was increased by the additional photosynthesis in the 20 hr photoperiod treatment. This treatment had no significant effect on scion growth compared with the 1/2 bud break treatment. This result is expected since scion photosynthesis is low and photosynthate translocation from the rootstock to the scion does not occur until after scion bud break. Extended photoperiods increase scion growth, but are not benefical until after bud break of the scion.

Blake (1983) reported that cold storage of white spruce seedlings reduced transpiration rates upon outplanting, possibly due to high ABA content. None of the treatments to increase ABA levels in the scion

improved graft success over the control (Table 17). But 10 weeks of scion storage prior to grafting and the ABA treatments significantly decreased the time until bud break. However, there was no difference in bud break between these treatments and GWR-84 scions. All of the treatments significantly reduced total scion growth compared to the control, but on the total growth/cm scion basis, only the 10 week storage treatment was as good as the control.

The application of film-forming antitranspirants to scions has been a question of several nurserymen. A recent trial of many of these film-forming compounds on cut Christmas trees (Montano & Proebsting, 1986) found Vapor Guard to be the most effective in reducing water loss. The Antitrans treatment significantly reduced the time required for bud break (Table 18), but it did not improve scion quality over the control. Covering grafts with a polyethylene sheet is cited in Plant Propagation (Hartman & Kester, 1975), but is rarely practiced by nurserymen. Covering the grafts in the present study with the polyethylene sheet resulted in significantly more growth compared to the control, but on a growth/cm scion basis, there was no significant difference (Table 18). Neither treatment improved graft success over normal greenhouse grafting (Table 18). The polyethylene tent study was on an open-bottomed bench. The use of a rooting bench would have increased the relative humidity inside the enclosure and may have affected the results. The use of the antitranspirant may be benefical in environments where water stress is more pronounced, but it was not benefical under the conditions tested.

Warming the root-zone of roses has been shown to increase both the quality and number of blooms (Moss & Dalgleish, 1984). It was

hypothesized that increasing root activity while the shoot and scion were still dormant might result in earlier union development and increased graft success. However the results, with blue spruce were just the opposite (Rootwarm, Table 19). root-zone warming significantly increased the time required for bud break while lowering the success rate and most growth parameters, compared to untreated lath house grafts.

Fordham and Spraker (1977) suggested that rootstocks should be potted one growing season before grafting. However, some nurserymen have expressed interest in grafting onto rootstocks that are lifted and potted the previous fall. Budbreak of scions on recently potted rootstocks (Lifted-84, Table 20), was much slower, indicating that union development was also delayed. Greenhouse grafts had significantly more growth than lath house grafts, but still much less than that normally found for greenhouse grafts. The success rate in the greenhouse was slightly less than average, while that in the lath house grafts was much lower. The differences in graft success and late bud break suggest the importance of at least partial regeneration of the roots of the rootstock prior to grafting. While it appears possible to graft unto unestablished rootstocks once they have been brought into active growth, the long period required for union development requires greater precision in cambial alignment and increases the chance of scion failure.

Chapter 6

BIBLIOGRAPHY

Ackerson, R. C. 1981. Osmoregulation in cotton in response to water stress. II Leaf carbohydrate status in relation to osmotic adjustment. Plant Physiol. 67:489-499.

Allen, R. M. 1964. Contributions of roots stems and leaves to height growth of longleaf pine. For. Sci. 10:14-16.

Aronsson, A., Ingesten, T. and Loof, L. 1976. Carbohydrate metabolism and frost hardiness in pine and spruce seedlings grown at different photoperiods and themoperiods. Physiol. Plant.36:127-132.

Aspinall, D. 1980. Role of abscisic acid and other hormones in adapation to water stress. In: "Adaptation of Plants to Water and High Temperature Stress". (N. C. Turner and P. J. Kramer, eds). pp. 154-172. John Wiley & Sons, Inc. New York. ISBN 0-471-05372-4.

Arnott, J. T. 1974. Growth response of white and Engelmann spruce provenances to extended photoperiod using continuous and intermitten light. Can. J. For. Res. 4:69-78.

Balatiecz, J. J., Forward, D. F. and Bidwell, R. G. S. 1966. Distribution of photoassimilated $^{14}CO_2$ in young jack pine seedlings. Can. J. Bot. 44:362-365.

Beadle, C. L. and Jarvis, P. G. 1977. The effect of shoot water status on some photosynthetic partial processes in Sitka spruce. Physiol. Plant. 41:7-13.

Beadle, C. L., Jarvis, P. G. and Neilson, R. E. 1979. Leaf conductance as related to xylem water potential and carbon dioxide concentration in Sitka spruce. Physiol. Plant. 45:158-166.

Beadle, C. L., Neilson, R. E., Jarvis, P. G. and Talbot, H. 1981. Photosynthesis as related to xylem water potential and carbon dioxide concentration in Sitka spruce. Physiol. Plant. 52:391-400.

Beardsell, M. F., Jarvis, P. G. and Davidson, B. 1972. A null-balance diffusion porometer suitable for use with leaves of many shapes. J. App. Ecol. 9:677-690.

Beeson Jr., R. C., Montano, J. M. and Proebsting, W. M. 1986. A Method for determining the apoplastic water volume of conifer needles. Physiol. Plant. 66:129-133.

Bellandi, D. M. and Dorffling, K. 1974. Transport of Abscisic acid-2-C^{14} in intact pea seedlings. Physiol. Plant. 32:365-368.

Blackman, P. G. and Davies, W. J. 1983. The effects of cytokinins and ABA on stomata behavior of maize and commelina. J. Exp. Bot. 34:1619-1626.

Blake, J. and Ferrell, W. K. 1977. The association between soil and xylem water potential, leaf resistance, and abscisic acid content in droughted seedlings of Douglas-fir (Pseudotusuga menziesii). Physiol. Plant. 39:106-109.

Blake, J. 1983. Transplanting shock in white spruce: Effect of cold-storage and root pruning on water relations and stomata conditioning. Physiol. Plant. 57:210-216.

Bligh, E. G. and Dyers, W. J. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. & Physiol. 37:911-917.

Boersig, M. and Negm, F. B. 1985. A one-step clean-up for HPLC analysis of carbohydrate. HortSci. 20:555.

Booth, T. H. and Sanders, T. C. 1979. Air temperature and the growth of grafted radiata pine. Aust. For. Res. 9:91-99.

Boyer, J. S. 1967. Matrix potentials of Leaves. Plant. Physiol. 42:213-217.

Boyer, J. S. 1985. Water transport. Ann. Rev. Plant Physiol. 36:473-516.

Brix, H. 1962. The effects of water stress on the rates of photosynthesis and respiration in tomato plants and loblolly pine. Physiol. Plant. 15:10-20.

Campbell, E. S., Papendick, R. I., Rabie, E. and Shayo-Ngowi, A. J. 1979. A comparison of osmotic potential, elastic modulus and apoplastic water in leaves of dryland winter wheat. Agron. J. 71:31-36.

Cheung, Y. N. S., Tyree, M. T. and Dainty, J. 1975. Water relations parameters of single leaves obtained in a pressure bomb and some ecological interpretations. Can. J. Bot. 53:1342-1346.

Christersson, L. 1972. The transpiration rate of unhardened, hardened, and dehardened seedlings of spruce and pine. Physiol. Plant. 26:258-263.

Clausen, J. J. and Kozlowski, T. T. 1967. Food sources for growth of Pinus resinosa shoots. Adv. Fronteirs Plant Sci. 18:23-32.

Copes, D. 1967a. Influence of cambial contact lenght on graft survival and leader elongation in Douglas-fir. U. S. Forest. Serv., Pac. NW Forest Range Exp. Sta. Res. Note 69.

Copes, D. 1967b. Graft union formation in Douglas-fir. Am. J. Bot. 53:285-289.

Cornish, K. and Zeevaart, J. A. D. 1985. Movement of abscisic acid into the apoplast in response to water stress in Xanthium strumarium L. Plant Physiol. 78:623-626.

Coutts, M. P. 1980. Control of water loss by actively growing Sitka spruce seedlings after transplanting. J. Exp. Bot. 31:1587-1597.

Coutts, M. P. 1981. Leaf water potential and control of water loss in droughted Sitka spruce seedlings. J. Exp. Bot. 32:1193-1201.

Cowan, J. R. 1977. Stomatal behavior and environment. Adv. Bot. Res. 4:117-228.

Cram, W. H. and Lindquish, C. H. 1963. Germination and growth of three tree species under four photoperiods. For. Sci. 9:279-282.

Davies, C. R., Seth, A. K. and Wareing, P. F. 1966. Auxin and kinetin interaction in apical dominance. Science. 151:468-469.

Davies, F. S. and Lakso, A. N. 1978. Diurnal and seasonal changes in leaf water potential components and elastic properties in response to water stress in apple trees. Physiol. Plant. 46:109-114.

Davies, J. and Kozlowski, T. T. 1974. Stomata responses of five woody angiosperms to light intensity and humidity. Can. J. Bot. 52:1525-1534.

Davis, E. A. 1949. Effects of several plant growth-regulators on wound-healing of sugar maple. Bot. Gaz. 111:69-77.

Demaggio, A. E. 1966. Phloem differentiation: Induced stimulation by gibberellin. Science. 152:370-372.

Denne, M. P. and Wilson, J. E. 1977. Some quantitative effects of IAA on the wood production and tracheid dimensions of Picea. Planta. 134:223-228.

Digby, J. and Wareing, P. F. 1966a. The effect of applied growth hormones on cambial division and the differentiation of the cambial activity. Ann. Bot. 30:539-548.

Digby, J. and Wareing, P. F. 1966b. Endogenous hormone levels and cambial activity. Ann. Bot. 30:607-622.

Dixon, M. A., Grace, J. and Tyree, M. T. 1984. Concurrent measurements of stem density, leaf and stem water potential, stomata conductance and cavitation of sapling of Thuja occidentalis L. Plant Cell Envir. 7:615-618.

Doley, P. and Leyton, L. 1970. Effects of growth regulating substances and water potential on the development of wound callus in Fraxinus. New Phyto. 69:87-102.

Dörffling, K., Sanka, B. and Tietz, D. 1971. Variational metabolism of abscisic acid in pea seedlings during and after water stress. Planta. 121:57-66.

Dörffling, K, Streich, J., Kruse, W. and Maxfeldt, B. 1977. Abscisic acid and the after-effect of water stress on stomatal openning potential. Z. Pflenzen. 81:43-56.

Dörffling, K. and Teetz, D. 1985. Abscisic acid in leaf epidermis of Commelina communis L.: Distribution and correlation with stomata closure. J. Plant Physiol. 117:297-305.

Dorsman, D. 1966. Grafting of woody plants in the greenhouse. Proc. XVII Intern. Hort. Cong. 1:366.

Dormling, I. 1964. Anatomy of graft unions. Studia. For. Suecia. 130:1-137.

Downton, W. J. S. 1983. Osmotic adjustment during water stress protects the photosynthetic apparatus against photoinhibition. Plant Sci. Let. 30:137-143.

Drew, H. P. and Ferrell, W. K. 1979. Seasonal changes in the water balance of Douglas-fir (Pseudotsuga menziesii) seedlings grown under different light intensities. Can. J. Bot. 57:666-674.

Dreywood, R. 1946. Quantitative test for carbohydrate material. Ind. Eng. Chem. (Anal) 18:499.

Dykstia, G. F. 1974. Photosynthesis and carbon dioxide transfer resistance of lodge pole pine seedlings in relation to irradiance, temperature, and water potential. Can. J. For. Res. 4:201-206.

Ericsson, A. 1978. Seasonal changes in translocation of ^{14}C from different age-classes of needles on 20 year-old Scots pine trees (Pinus silvestris). Physiol. Plant. 43:351-358.

Ericsson, A. 1979. Effects of fertilization and irrigation on the seasonal changes of carbohydrate reserves in different age-classes of needles on 20 year-old Scots pine (Pinus silvestris). Physiol. Plant. 45:270-280.

Evans, G. E. and Rasmussen, H. P. 1972. Anatomical changes in developing graft unions of Juniperus L. J. Am. Soc. Hort. Sci. 92:221-232.

Farquhar, G. D. 1978. Feed-forward responses of stomata to humidity. Aust. J. Plant Physiol. 5:787-800.

Fiscus, E. L., Klute, A. and Kaufman, M. R. 1983. An interpretation of some whole plant water transport phenomena. Plant Physiol. 71:810-817.

Fordham, A. J. and Spraker, L. J. 1977. Propagation mannual of gymnosperms. Arnoldia. 37:1-88.

Fosket, D. E. 1970. The time course of xylem differentiation and its relation to deoxyribonucleic acid synthesis in cultured coleus stem segments. Plant Physiol. 46:64-68.

Freeland, R. D. 1944. Apparent photosynthesis in some conifers during winter. Plant Physiol. 19:179-185.

Fry, D. J. and Phillips, I. D. J. 1977. Photosynthesis of conifers in relation to annual growth cycles and dry matter production. II. Seasonal photosynthetic capacity and mesophyll ultrastructure in Abies grandis, Picea sitchensis, Tsuga heterophylla and Larix leptlepis growing in S. W. England. Physiol. Plant. 40:300-306.

Gates, D. M. 1966. Transpiration and energy exchange. Quart. Rev. Biol. 41:353-364.

Glerum, C. and Balatinecz, J. J. 1980. Formation and distribution of food reserves during autumn and then subsequent utilization in jack pine. Can. J. Bot. 58:40-54.

Gordon, J. C. and Larson, P. R. 1968. Seasonal course of photosynthesis, respiration, and distribution of ^{14}C in young Pinus resinora trees as related to wood formation. Plant Physiol. 43:1617-1624.

Grigsby, H. C. 1957. What we know about grafting. Proc. Four. South. Conf. For. Tree Imp. 122-125.

Hanover, J. W. 1975. Genetics of blue spruce. USDA For. Ser. Res. Pap. WO. 28.

Hanover, J. W. and Reicosky, D. A. 1972. Accelerated growth for early testing of spruce seedlings. For. Sci. 18:92-94.

Harrison, M. and Klein, R. 1979. Role of growth regulators in initiation of secondary xylem and phloem cells. Bot. Gaz. 140:20-24.

Hartmann, H. T. and Kester, D. 1975. "Plant Propagation", Third ed. Prentice-Hall, Inc. Englewood Cliffs, NJ.

Heide, O. M. 1974. Growth and dormancy in Norway spruce ecotypes (Picea abies). I. Interaction of photoperiod and temperature. Physiol. Plant. 30:1-12.

Hejnowicz, A. and Tomaszewski, M. 1969. Growth regulators and wood formation in Pinus silvestris. Physiol. Plant. 22:904-921.

Hellkvist, J., Richards, G. P. and Jarvis, P. J. 1974. Vertical gradients of water potential and tissue water relations in Sitka spruce trees measured with the pressure chamber. J. Appl. Ecol. 11:637-668.

Henson, I. E. 1984. Effect of atmospheric humidity on abscisic acid accumulation and water status in leaves of rice (Oryza sativa L.). Ann. Bot 54:569-582.

Hinckley, T. M., Lassoce, J. P. and Running, S. W. 1978. Temporal and spatical variations in the water status of forest trees. For. Sci. Monogr. 20.

Hinckley, T. M., Duhme, F., Hinckley, A. R. and Richter, H. 1980. Water relations of drought hardy shurbs: Osmotic potential and stomatal reactivity. Plant Cell Envir. 3:131-140.

Hiron, R. W. P. and Wright, S. T. C. 1983. The role of endogenous abscisic acid in the response of plants to stress. J. Exp. Bot. 24:769-781.

Holl, W. 1985. Seasonal fluctation of reserve material in the trunkwood of spruce (Picea abies (L.) Karst.). J. Plant Physiol. 117:355-362.

Holst, M. J. 1956a. Scion storage and graft protection in the spring grafting of red pines. Canad. For. Bran., For. Res. Div. Tech. Note 29.

Holst, M. J. 1956b. Phenology of rootstocks and grafts in a timing experiment with autumn and winter grafting of Norway and white spruce. Canad. For. Bran., For. Res. Div. Tech. Note 48.

Holst, M. J., Santon, J. A. and Yeatman, C. W. 1956. Greenhouse grafting of spruce and hard pine at the Petawawa Forest Experiment Station. Canad. For. Bran., For. Res. Div. Tech. Note 33.

Hsiao, T. C. 1973. Plant responses to water stress. Ann. Rev. Plant Physiol. 24:519-570.

Hsiao, T. C., Acevedo, E., Fereres, E. and Henderson, D. W. 1976. Stress metabolism. Phil. Trans. R. Soc. Lond. B. 273:479-500.

Jackson, L. W. R. and Zak, B. 1949. Grafting methods used in studies of the little-leaf disease of shortleaf pine. J. For. 47:904-908.

Jacobs, W. P. 1952. The role of auxin in differentiation of xylem around a wound. Am. J. Bot. 39:301-309.

Jacobs, W. P. 1970. Regeneration and differentiation of seive tube elements. Int. Rev. Cyto. 28:239-273.

Jarvis, P. D. 1980. Stomatal response to water stress in conifers. In: "Adapation of Plants to Water and High Temperature Stress". (N. C. Turner and P. S. Kramer, eds). pp. 105-121. John Wiley & Sons. New York. ISBN 0-471-05372-4.

Jeffs, R. A. and Northcote, D. H. 1966. Experiment induction of vascular tissue in an undifferentiated plant callus. Biochem. J. 101:146-152.

Jeffs, R. A. and Northcote, D. H. 1967. The influence of indol-3yl acetic acid and sugar on the pattern of induced differentiation in plant tissue culture. J. Cell. Sci. 2:77-88.

Jenson, K. F. and Gatherum, G. E. 1965. Effect of temperature, photoperiod and provenance on growth and development of Scots pine seedlings. For. Sci. 11:189-200.

Johanson, R. 1953. Interference of pentose in the estimation of hexose sugar with anthrone. Nature (Lond.). 171:176-177.

Johnson, J. D. and Ferrell, W. K. 1982. The relationship of abscisic acid metabolism to stomatal conductance in Douglas-fir during water stress. Physiol. Plant. 55:431-437.

Johnson, J. D. and Ferrell, W. K. 1983. Stomata response to vapor pressure deficit and the effect of plant water stress. Plant Cell Envir. 6:451-456.

Kandike, R. A., Timmins, R. and Worrall, J. 1980. Pressure-volume curves of shoots and roots of normal and drought-conditioned western hemlock seedlings. Can. J. For. Res. 10:10-16.

Kandler, O. and Hopf, H. 1980. Occurrence, metabolism and function of oligosaccharides. In "The Biochemistry of Plants". (T. Akazawa and K. Okamoto, eds.), Vol 3, pps. 221-269. Academic Press Inc. New York. ISBN 0-12-675403-9.

Kaufman, M. R. 1968. Water relation of pine seedlings in relation to root and shoot growth. Plant Physiol. 43:281-288.

Kaufman, M. R. 1976. Stomatal response of Engelmann spruce to humidity, light and water stress. Plant Physiol. 57:898-901.

Kaufman, M. R. 1979. Stomata control and the development of water deficits in Engelmann spruce seedlings during drought. Can. J. For. Res. 9:297-304.

Kawase, M. and Nitsch, J. P. 1958. Growth substances and the photoperiodic control in Betula pubescens. Plant Physiol. 33:XIX.

Kawase, M. and Nitsch, J. P. 1959. Growth substances and the photoperiodic control of growth in Betula pubescens and Betula lutea. Plant Physiol. 34:IV.

Kimura, M. 1969. Ecological and physiological studies on the vegetation of Mt. Shimagare. VII Analysis of production processes of young Abies stand based on the carbohydrate ecomony. Bot. Mag. Tokyo. 82:6-19.

Kostoff, D. 1928. Studies on callus tissue. Am. J. Bot. 25:565-575.

Kozlowski, T. T. 1943. Transpiration rates of some forest tree species during the dormant season. Plant Physiol. 18:252-600.

Kozlowski, T. T. 1963. Growth characteristic of forest trees. J. For. 61:655-662.

Kozlowski, T. T. 1982. Water supply and tree growth. I. Water deficits. Forestry Abs. 43:57-95.

Kozlowski, T. T., Hughes, J. F., Leyton, L. 1967. Movement of injected dyes in gymnosperm stems in relation to tracheid alignment. For. 60:207-219.

Kozlowski, T. T. and Keller, T. 1966. Food relations of woody plants. Bot. Rev. 32:292-382.

Kozlowski, T. T. and Winget, C. H. 1964. The role of reserves in leaves, branches, stems and roots of shoot growth of red pine. Am. J. Bot. 51:522-529.

Kramer, P. J. 1975. Drought tolerance of pine seedlings under various climatic conditions. For. Sci. 21:72-82.

Kriedemann, P. E., Loveys, B. R., Fuller, G. L. and Leopold, A. C. 1972. Abscisic acid and stomata regulation. Plant Physiol. 49:842-847.

Krueger, K. W. and Trappe, J. M. 1967. Food reserves and seasonal growth of Douglas-fir seedlings. For. Sci. 13:192-202.

Krueger, K. W. and Ruth, R. H. 1969. Comparative photosynthesis of red alder, Douglas-fir, Sitka spruce and western hemlock seedlings. Can. J. Bot. 47:519-527.

Kubik, M. and Planka, A. 1984. Abscisic acid induced decay of strawberry transpiration. Physiol. Plant. 60:539-542.

Kuraishi, S. and Muir, R. 1964. The relationship of gibberellin and auxin in plant growth. Plant Cell Physiol. 5:61-69.

Kyriakopoulos, E. and Richter, H. 1977. A comparison of methods for the determination of water status in Quercus ilex L. Z. Pflanzenphysiol. 82:14-27.

Lange, O. L., Losch, R., Schulze, E. D. and Kappen, L. 1971. Response of stomata to changes in humidity. Planta. 100:76-86.

Larson, P. R. 1964. Contribution of different-aged needles to growth and wood formation of young red pines. For. Sci. 10:224-238.

Lenton, J. R., Perry, V. M. and Saunders, P. F. 1972. Endogenous abscisic acid in relation to photoperiodically induced bud dormancy. Planta (Berl.). 106:13-22.

Leverenz, J. W. and Jarvis, P. G. 1979. Photosynthesis in Sitka spruce. VIII The effect of light flux density and direction on the rate of net photosynthesis and the stomatal conductance of needles. J. App. Ecol. 16:919-932.

Little, C. H. A. 1970a. Derivation of springtime starch increase in balsam fir (Abies balsamea). Can. J. Bot. 48:1995-1999.

Little, C. H. A. 1970b. Seasonal changes in carbohydrate and moisture content in needles of balsam fir (Abies balsamea). Can. J. Bot. 48:2021-2028.

Little, C. H. A. 1974. Relationship between the starch level at budbreak and current shoot growth in Abies balsamea L. Can. J. For. Res. 4:268-273.

Little, C. H. A. 1975. Inhibition of cambial activity in Abies balsamea by internal water stress: Role of abscisic acid. Can. J. Bot. 53:3041-3050.

Little, C. H. A. and Eidt, D. C. 1968. Effect of abscisic acid on budbreak and transpiration in woody species. Nature. 220:498-499.

Little, C. H. A. and Eidt, D. C. 1970. Relationship between transpiration and cambial activity in Abies balsamea. Can. J. Bot. 48:1027-1028.

Little, C. H. A. and Loach, K. 1973. Effect of changes in carbohydrate concentration on the rate of net photosynthesis in mature leaves of Abies balsamea. Can. J. Bot. 51:751-758.

Little, C. H. A. and Wareing, P. F. 1981. Control of cambial activity and dormancy in Picea sitchensis by indol-3yl-acetic and abscisic acid. Can. J. Bot. 59:1480-1493.

Lipetz, J. 1970. Wound-healing in higher plants. Int. Rev. Cyto. 27:1-28.

Loach, K. and Little, C. H. A. 1970. Production, storage and use of photosynthate during shoot elongation in balsam fir (Abies balsamea). Can. J. Bot. 48:1161-1168.

Long, A. 1983. Turgor-regulated translocation. Plant Cell Envir. 6:683-689.

Lopushinsky, W. 1969. tomata closure in conifer seedlings in response to leaf moisture stress. Bot. Gaz. 130:258-263.

Ludlow, M. M. 1980. Adaptative significance of stomatal resposes to water stress. In: "Adaptation of Plants to Water and High Temperature Stress". (N. C. Turner and P. J. Kramer, eds). pp. 123-138. John Wiley & Sons. New York. ISBN 0-471-05372-4.

Maier-Maercker, V. 1979. "Peristomatal transpiration" and stomatal movement: A controversial view. II Observation of stomatal movement under different conditions of water supply and demand. Z. Pflanzen. 91:157-172.

McDonald, B. 1958. Propagation of Picea. Proc. Plant Prop. Soc. 4:123-126.

McQuilkin, W. E. 1950. Effects of some growth regulators and dressing on the healing of tree wounds. J. For. 48:423-428.

Meinzer, F. C. 1982. The effect of vapor pressure on stomata control of gas exchange in Douglas-fir (Pseudotsuga menziesii) saplings. Oecologia 54:236-242.

Melzack, R. N., Bravdo, B. and Riov, J. 1985. The effect of water stress on photosynthesis and related parameters in Pinus halepensis. Physiol. Plant. 64:295-300.

Meyer Jr, M. M. and Spittstoesser, W. E. 1971. The utilization of carbohydrates and nitrogen reserves by Taxus during the spring growth period. Physiol. Plant. 24:306-314.

Montano, J. and Proebsting, W. M. 1986. Stored cut Douglas-fir: Relationship to damage threshold. HortSci. In press.

Mooney, H. A. 1972. The carbon balance of plants. Ann. Rev. Ecol & Systematics 3:315-346.

Morgan, J. M. 1984. Osmoregulation and water stress in higher plants. Ann. Rev. Plant Physiol. 35:299-319.

Moss, G. J. and Dalgleish, R. 1984. Increased returns from roses with root zone warming. J. Am. Soc. Hort. Sci. 109:893-898.

Naqui, S. M. and Enguild, K. C. 1974. Action of abscisic acid on auxin transport and its relation to phototropism. Physiol. Plant. 30:283-287.

Neilson, R. E. and Jarvis, P. G. 1975. Photosynthesis in Sitka spruce (Picea sitchensis (Bong.) Car.). VI Response of stomata to temperature. J. App. Ecol. 12:879-891.

Neish, A. C. 1958. Seasonal changes in metabolism of spruce leaves. Can. J. Bot. 36:649-662.

Nenjuhin, V. H. 1965. Certain physiological features of pine grafts during periods of coalescence. For. Abs. 28(3):526.

Nenjuhin, V. H. 1966. Anatomy of grafts of certain Pinus species. For. Abs. 28(3):3749.

Nienstaedt, H. 1959. The effect of rootstock activity on the success of fall grafting of spruce. J. For. 57:828-831.

Nienstaedt, H., Cech, F. C., Mergen, F., Wang, C. W. and Zak, B. 1958. Vegetative propagation in forest genetic research and practice. J. For. 56:826-839.

Nitsch, J. P. 1956. Light and plant propagation. Proc. Int. Pl. Prop. Soc. 6:122-130.

Nitsch, J. P. 1957a. Growth responses of woody plants to photoperiodic stimuli. Proc. Am. Soc. Hort. Sci. 70:512-525.

Nitsch, J. P. 1957b. Photoperiodism in woody plants. Proc. Am. Soc. Hort. Sci. 70:526-544.

Nobel, P. S. 1983. "Biophysical Plant Physiology and Ecology". W. H. Freeman & Co. New York. ISBN 0-7167-1447-7.

Olofiboba, M. O. and Kozlowski, T. T. 1973. Accumulation and utilization of carbohydrate reserves in shoot growth of Pinus resinosa. Can. J. For. Res. 3:346-353.

Ontario Forest Research Centre. 1979. Grafting and storage of fall-collected spruce and pine scions. For. Res. Note 21.

Orr-Ewing, A. L. and Prideaux, D. C. 1959. Grafting methods for the Douglas-fir. For. Chron. 35:192-202.

Outlaw Jr, W. H. 1983. Current concepts on the role of potassium in stomata movements. Physiol. Plant. 59:302-311.

Overbeek, J. V. 1966. Plant hormones and regulators. Science 152:723-731.

Parker, J. 1959. Seasonal variations in sugars of conifers with some observations on cold resistance. For. Sci. 5:56-63.

Parker, J. 1963. Causes of the winter decline in transpiration and photosynthesis in some evergreens. For. Sci. 9:158-166.

Pawsey, C. K. 1970. Development of grafts of Pinus radiata in relation to age of scion source. Aust. For. Res. 5:15-18.

Perry, T. O. and Hellmers, H. 1973. Effects of abscisic acid on growth and dormancy of two races of red maple. Bot. Gaz. 134:283-289.

Philipson, J. J. and Coutts, M. P. 1980. Effects of growth hormone applications on the secondary growth of roots and stems in Picea sitchensis. Ann. Bot. 46:747-755.

Phillips, I. D. J. and Wareing, P. F. 1959. Studies in dormancy of sycamore. II The effect of daylength on the natural growth-inhibitor content of the shoot. J. Exp. Bot. 10:504-514.

Pierce, M. and Raschke, K. 1980. Correlation between loss of turgor and accumulation of abscisic acid in detached leaves. Planta. 148:179-182.

Pirone, P. P. 1978. "Tree Maintenance, Fifth Ed". Oxford University Press, Inc. ISBN 0-19-502321-8.

Pomeroy, M. K. and Siminovitch, D. 1969. Seasonal biochemical changes in the living bark and needles of red pine (Pinus resinora) in relation to adaptation to freezing. Can. J. Bot. 48:953-967.

Potter, J. R. and Breen, P. J. 1980. Maintenance of high photosynthetic rates during the accumulation of high leaf starch levels in sunflower and soybean. Plant Physiol. 66:528-531.

Powell, L. E. 1976. Effect of photoperiod on endogenous abscisic acid in Malus and Betula. Hortsci. 11:498-499.

Puritch, G. S. 1973. Effect of water stress on photosynthesis, respiration and transpiration of four Abies species. Can. J. For. Res. 3:293-298.

Rast, D., McInnes, A. G. and Neish, A. C. 1963. Synthesis of raffinose in spruce twigs. Can. J. Bot. 41:1681-1686.

Richter, H. 1981. Pressure-volume curves and drought injury. Physiol. Plant. 52:124-128.

Richter, H., Duhme, F., Glatzel, G., Hinckley, T. M. and Karlic, H. 1981. Some limitations and applications of the pressure-volume curve technique in ecophysiological research. In: "Plants and Their Atmospheric Environment". (J. Grace, E. D. Ford and P. G. Jarvis, eds), pp.263-272. Blackwell Scientific Publisher, Oxford. ISBN 0-470-27125-6.

Ritchie, G. A. 1982. Carbohydrate reserves and root growth potential of Douglas-fir seedlings before and after cold storage. Can. J. For. Res. 12:905-912.

Robards, H. W., Davidson, E. and Kidwai, P. 1969. Short-term effect of some chemicals on cambial activity. J. Exp. Bot. 20:912-920.

Roberts, R. H. 1949. Theoretical aspects of graftage. Bot. Rev. 15:423-463.

Roberts, S. W. and Knoerr, K. R. 1977. Components of water potential estimated from xylem pressure measurements in five tree species. Oecologia (Berl.) 28:191-202.

Robinson, N. and Preiss, J. 1985. Biochemical phenomema associated with stomata function. Physiol. Plant. 64:141-146.

Ronco, F. 1972. Overwinter food reserves of potted Engelmann spruce seedlings. Can. J. For. Res. 2:489-492.

Ronco, F. 1973. Food reserves of Engelmann spruce planting stock. For. Sci. 19:213-219.

Schier, G. A. 1970. Seasonal pathways of ^{14}C-photosynthate in red pine labelled in May, July and October. For. Sci. 16:2-13.

Schmidtling, R. C. 1973. Rootstock influences early fruitfulness, growth and survival in loblolly pine grafts. Proc. South. For. Tree Imp. Conf. 12:86-90.

Sheriff, D. W. 1979. Stomatal aperture and the sensing of the environment by guard cells. Plant Cell Envir. 2:15-22.

Shimomura, T. and Fozihara, K. 1977. Physiological study of graft union formation in cactus: II Role of auxin on vascular connection between stock and scion. J. Jap. Soc. Hort. Sci. 45:397-406.

Shiroya, T., Lister, G. R., Slakis, V., Krofkov, G. and Nelson, C. D. 1962. Translocation of the products of photosynthesis to roots of pine seedlings. Can. J. Bot. 40:1125-1135.

Shiroya, T., Lister, G. R., Slankis, V. Krofkov, G. and Nelson, C. D. 1966. Seasonal changes in the respiration, photosynthesis and translocation of the ^{14}C labelled products of photosynthesis in young Pinus strobus L. plants. Ann. Bot. 30:81-91.

Shiroya, T., Slakis, V., Krofkov, G. and Nelson, C. D. 1962. The nature of photosynthate in Pinus strobus seedlings. Can. J. Bot. 40:669-676.

Scholander, P. F., Hammel, H. T., Hemmingsen, E. A. and Bradstreet, E. D. 1964. Hydrostatic pressure and osmotic potentials in leaves of mangrove and some other plants. Proc. Natl. Acad. Sci. 51:119-125.

Scholander, P. F., Hammel, H. T., Bradstreet, E. D. and Hemmingsen, E. A. 1965. Sap pressure in vascular plants. Science. 148:339-349.

Sinclair, T. R. and Ludlow, M. M. 1985. Who taught plants themodynamics? The unfulfilled potential of plant water potential. Aust. J. Plant Physiol. 12:213-217.

Sinnott, E. W. 1918. Factors determining character and distribution of food reserves in woody plants. Bot. Gaz. 66:162-178.

Slatyer, R. O. 1967. "Plant-Water Relationships". Academic Press Inc. New York.

Smit-Spinks, B., Swanson, B. T. and Markhart III, A. H. 1984. Changes in water relations, water flux, and root exudate abscisic acid content with cold acclimation of Pinus sylvestris. Aust. J. Plant Physiol. 11:431-441.

Smith, P. J. and Mansfield, J. A. 1982. Control of the CO_2 responses of stomata by indol-3-yl acetic acid and abscisic acid. J. Exp. Bot. 33:360-365.

Smith, W. H., Goddard, R. E. and Hickman, J. G. 1972. Techniques of improved grafting. U. S. For. Ser. Tree Plant. Notes. 23:13-15.

Talbot, A. J. B., Tyree, M. T. and Dainty, J. 1975. Some notes concerning the measurement of water potentials of leaf tissures with specific reference to Tsuga canadensis and Picea abies. Can. J. Bot. 53:748-788.

Tainter, F. H. and French, D. W. 1972. The movement of Dye solution in dwarf mistletoe-inflected Black spruce trees. Can. J. For. Res. 3:312-315.

Terry, J. G., Fosket, D. E. and Healper, P. K. 1971. Xylem formation: A paradigm of cytodifferentiation in higher plants. Am. Sci. 59:338-352.

Teskey, R. O., Hinckley, T. M. and Grier, C. C. 1983. Effect of interruption of flow path on stomatal conductance of Abies amabilis. J. Exp. Bot. 34:1251-1259.

Teskey, R. O., Hinckley, T. M. and Grier, C. C. 1984. Temperature-induced change in the water relations of Abies amabilis (Dougl.) Forbes. Plant Physiol. 74:77-80.

Thorne, J. H. and Keller, H. R. 1974. Influences of assimilate demand on photosynthesis, diffusive resistances, translocation and carbohydrate levels of soybean leaves. Plant Physiol. 54:201-207.

Tinus, R. W. 1971. Response of Pinus ponderosa Laws, and Picea pungens Englm. seedlings to extension of photoperiod with continuous and intermittent light. Plant Physiol. 46:Supp-133.

Torrey, J. G. and Loomis, R. S. 1967. Auxin-cytokinin control of secondary vascular tissue formation in isolated roots of Raphanus. Am. J. Bot. 54:1098-1106.

Tyree, M. T. and Jarvis, P. G. 1982. Water in tissues and cells.-In "Encyclopedia of Plant Physiology". Vol. 12B. (O. L. Lange, P. S. Nobel, C. B. Osmond and H. Ziegler eds). pps. 35-78. Springer-Verlerg, Berlin-Heiderberg, Germany. ISBN 0-387-10763-0.

Tyree, M. T. and Hammel, H. T. 1972. The measurement of turgor pressure and the water relations of plants by the pressure-bomb technique. J. Exp. Bot. 23:267-282.

Tyree, M. T., Dainty, J. and Benis, M. 1973. The water relations of hemlock (Tsuga canadensis). I: Some equilibrium water relations as measured by the pressure-bomb technique. Can. J. Bot. 51:1471-1480.

Tyree, M. T., Cheung, Y. N. S., MacGregor, M. E. and Talbot, A. J. B. 1978. The characteristics of seasonal and ontogenetic changes in the tissue-water relations of Acer, Populus, Tsuga and Picea. Can. J. Bot. 56:635-647.

Tyree, M. T. and Richter, H. 1982. Alternate methods of analyzing water potential isotherms: Some cautions and clarifications. II: Curvilinearity in water potential isotherms. Can. J. Bot. 60:911-916.

Turner, N. C. and Jones, M. M. 1980. Turgor maintenance by osmotic adjustment: A review and evaluation. In: "Adaptation of Plants to Water and High Temperature Stress". (N. C. Turner and P J. Kramer, eds). pp. 87-103. John Wiley & Sons. New York. ISBN 0-471-05372-4.

Ursino, D. J., Nelson, C. D. and Krotkev, G. 1968. Season changes in the distribution of photoassimilated ^{14}C in young pine plants. Plant Physiol. 43:845-852.

Verduin, J. 1959. Photosynthesis in conifers computed per unit leaf area, dry weight, chlorophyll content and respiratory rate. Ecol. 40:738.

Vité, J. P. 1959. Observations on the movement of injected dyes in Pinus ponderosa and Abies concolor. Contrib. Boyce Thompson Inst. 20:7-26.

Vité, J. P. and Rudinsky, J. A. 1959. The water-conducting systems in conifers and their importance to the distribution of trunk-injected chemicals. Contrib. Boyce Thompson Inst. 20:27-38.

Vreuzdenhil, D. 1983. Abscisic acid inhibits phloem loading of sucrose. Physiol. Plant. 57:463-467.

Wardlaw, I. F. 1968. The control and pattern of movement of carbohydrates in plants. Bot. Rev. 34:79-105.

Wareing, P. F. 1951a. Growth studies in woody species III. Further photoperiodic effects in Pinus sylvestris. Physiol. Plant. 4:41-56.

Wareing, P. F. 1951b. Growth studies in woody species IX. The initiation of cambial activity in ring porous species. Physiol. Plant. 4:546-562.

Watts, W. R. and Neilson. R. E. 1978. Photosynthesis in Sitka spruce (Picea sitchensis (Bong.)(Carr.)). VIII Measurement of stomata conductance and $^{14}CO_2$ uptake in controlled environments. J. App. Ecol. 15:245-255.

Webb, C. D. 1961. Field grafting of Loblolly pine. N. C. State Col. Sch. For. Tech. Rep. 10.

Webb, W. L. and Karchesy, J. K. 1977. Starch content of Douglas-fir defoliated by the tussack moth. Can. J. For. Res. 7:186-188.

Wentmore, R. H. and Rier, J. P. 1963. Experimental induction of vascular tissues in callus of angiosperms. Am. J. Bot. 50:418-430.

Wheeler, N. 1979. Effect of continuous photoperiod on growth and development of Lodgepole pine grafts and seedlings. Can. J. For. Res. 9:276-283.

Whitehead, D., Sheriff, D. W. and Greer, D. H. 1983. The relationship between stomata conductance, transpiration rate and tracheid structures in Pinus radiata clones grown at different water vapor saturation deficits. Plant Cell Envir. 6:703-710.

Willard, F. 1968. Notes on the grafting of Picea pungens 'Kosteriana°. Proc. Int. Plant Prop. Soc. 18:84-87.

Wilson, J. W. 1978. The position of regenerating cambia: auxin/sucrose ratio and the gradient induction hypothesis. Proc. R. Soc. Lond. B. 203:153-176.

Winjum, J. K. 1963. Effect of lifting date and storage on 2+0 Douglas-fir and Noble fir. J. For. 61:648-654.

Wodzicki, T. 1964. Photoperiod control of natural growth substances and wood formation in larch. J. Exp. Bot. 15:584-599.

Wodzicki, T. J. and Wodzicki, A. B. 1980. Seasonal abscisic acid accumulation in stem cambial region of Pinus sylvestris and its contribution to the hypothesis of a late-wood control system in conifers. Physiol. Plant. 48:443-447.

Yemm, E. W. and Willis, A. J. 1954. The estimation of carbohydrates in plant extracts by anthrone. Biochem. J. 57:508-514.

Young, E. and Hanover, J. W. 1976. Accelerating maturity in Picea seedlings. Acta. Hort. 56:105-114.

Young, E. and Hanover, J. W. 1977. Effects of quality, intensity and duration of light breaks during a long night of dormancy in blue spruce (Picea pungens Engelm.) seedlings. Plant Physiol. 60:271-273.

Zahner, R. 1955. Effect of interrupted dark period on height growth of two tree species. For. Sci. 1:193-195.

Zajaczkowski, S. 1973. Auxin stimulation of cambial activity in Pinus silvestris: I The differential cambial response. Physiol. Plant. 29:281-287.

Zakrzewski, J. 1983. Hormonal control of cambial activity and vessel differentiation in Quercus robur. Physiol. Plant. 57:537-542.

Zieger, E. and Field, C. 1982. Photocontrol of the functional coupling between photosynthesis and stomata conductance in the intact leaf. Plant Physiol. 70:370-375.

Zeiger, E., Grivet, C., Assman, S. M., Deitzer, G. F. and Hannegan, M. W. 1985. Stomata limitation to carbon gain in Paphiopedilum sp. (Orchidaceae) and its reversal by blue light. Plant Physiol. 77:456-460.

Ziegler, H. 1964. Storage, mobilization and distribution of reserve material in trees. In Formation of Wood in Forest Trees. (M. H. Zimmerman ed.) pps. 303-320.

Zieme, R. R. 1971. Translocation of ^{14}C in ponderosa pine seedlings. Can. J. Bot. 49:167-171.

APPENDICES

Appendix 1. A method for determining the apoplastic water volume of conifer needles. A methods paper in Physiol. Plant. 66:129-133.

A METHOD FOR DETERMINING THE APOPLASTIC WATER VOLUME OF CONIFER NEEDLES

R.C. Beeson, J.M. Montano and W.M. Proebsting

Beeson, R.C., Montano, J.M. and Proebsting, W.M. A method for determining the apoplastic water volume of conifer needles. - Physiol. Plant. 66 :129-133

ABSTRACT

A method for direct estimation of percent apoplastic water volume (% APO) in conifer needles is described. The method presented here and called the pressure-needle (P-N) method, measures the relative water content of the needles to develop a curve similar to the pressure-volume (P-V) curve. P-V and P-N curves were developed for Picea pungens cv.'Hoopsi', Pinus sylvestris, Abies grandis, and Pseudotsuga menziesii var. menziesii. The % APO estimated by the 2 procedures were found to differ by 2-fold, while other curve parameters were similar. The P-V method generated consistently higher and more variable % APO than the P-N method. This is due to the inclusion of the % apoplastic water of the stem tissue in the P-V method. For conifers, the P-N method offers a more accurate and precise method for the determination of % APO.

Additional key words - Abies, Picea, Pinus, Pseudotsuga, water relations.

R.C. Beeson (reprint requests), J.M. Montano and W.M. Proebsting. Department of Horticulture, Oregon State University, Corvallis, OR 97331 U.S.A.

INTRODUCTION

Pressure-volume (P-V) curves have been generated and analyzed by the methods of Tyree and Hammel (1972) and Cheung (1975) and reviewed by Tyree and Hammel (1972) and by Hellkvist et al. (1974). From P-V generated data using type II transformations (1/balance pressure vs summation of volume expressed (Kyriakopoulos and Richter 1981), various water relations parameters and their relation to water deficts in the plant can be calculated. When osmotic potential (Ψ_π) is measured either by vapor pressure osmometry or cryoscopy, cell sap is diluted by the water contained in the apoplast causing an under estimation of the true Ψ_π and therefore the calculated turgor pressure (P) (eq. 1), unless the measured Ψ_π is corrected for dilution (Talbot et al. 1974); where Ψ_L is the total water potential and ρgh is the height correction term.

$$\Psi_L = P - \Psi_\pi + \rho gh \qquad (1)$$

At moderate water deficts, this error can lead to the calculation of negative turgor pressure which is rarely encountered in living plants (Hellkvist et al. 1974, Talbot et al. 1974 Campbell et al. 1979).

The percent apoplastic water volume (% APO) of a twig calculated from a P-V curve, is an average of stem and leaf apoplastic water (Cheung et al, 1975). In conifers, Ψ_π determined from expressed sap from the needles, but corrected with the % APO of the twig, overestimates P by an amount proportional to the quantity of stem tissue present, because the % APO of the twig and needles differ. Since it is not feasible to develop P-V curves from a single needle, the objectives of this study were: 1) to develop a method to

determine % APO of conifer needles, and 2) to compare that method (P-N) to the P-V method in several conifer species.

MATERIALS AND METHODS

The P-V curves were developed by measuring the volume of sap expressed from the twigs with increasing balance pressure, whereas the relative water content (RWC) of the needles calculated at increasing balance pressures was used to generate the P-N curve.

Eight to 12 cm twigs were collected from Pinus sylvestris (Scots pine), Pseudotsuga menziesii var. menziesii (Douglas-fir), and Abies grandis (grand fir), that were of similar size and sun exposure within species. The samples were collected from January though March, 1984 from trees growing in Corvallis, OR. Twigs of Picea pungens cv. 'Hoopsi' (blue spruce) were collected from a nursery near Salem, OR, in September, 1983 for the development of the P-N method and again in January, 1984 for the generation of the P-V curves.

After collection, the twig bases were recut under water and placed in a container of water, covered with a polyethylene bag and stored at 0.5° C. Prior to measurement, the twig base was again cut under water and placed in a 50 ml beaker in 1 cm of water. This was covered with a polyethylene bag, sealed and stored at 2.5° C for 18 h. This procedure resulted in Ψ_L of less than -1.5 MPa.

In the P-N procedure, sets of needles were removed initially and after periods of overpressure in order to determine RWC of the needles. About 2 cm of bark at the base of the twig was removed and the twig was inserted in a rubber stopper and then inserted on the chamber cap. Then 3 or 4 sets of 5 randomly selected needles were excised and each set immediately weighed to 0.1 mg, then dried at 70°

C for 24 h. and reweighed. The twig and stopper were sealed on the chamber cap with putty (Binney & Smith Co., Easton, PA) and the inital balance pressure determined.

During the generation of the curves, overpressures varied from 0.6 to 1.8 MPa and were maintained for 7 to 20 min for the non-linear portion of the curve; the amount of overpressure and the time held was dependent on both the species and the approach to incipient plasmolysis. Once the curve became linear, overpressures of 1.2 to 2.0 MPa were used depending on species and the time varied from 20 to 40 min to achieve spacings on the curve of 0.30 to 0.35 MPa per point. Maximum overpressures ranged from 1.2 MPa for grand fir and Douglas-fir to 1.5 MPa for blue spruce to 1.8 to 2.0 MPa for Scots pine. The time required increased as the balance pressure increased. Expressed sap was absorbed by tissue paper and discarded. After each period of overpressure, the chamber pressure was decreased at the rate of 0.1 MPa/6 s to atmospheric pressure, and 2 replicate sets of 5 needles randomly selected were quickly removed and weighed to 0.1 mg then oven dried. After needle excision, the twig was sealed in the pressure chamber, the BP determined and the procedure repeated. The P-V method was conducted as described else where (Scholander et al. 1964, Scholander et al. 1965, Tyree and Hammel 1972). Overpressures and times were similar to those those used in the P-N method.

A correction factor, the mean dry weight / fresh weight ratios of the intial excised needle sets (balance pressure < 0.15 MPa), was used to calculate needle weight at full turgor (FW_T; eq. 2) of the subsequent needle sets.

$$DW / CF = FW_T \qquad (2)$$

Where DW is the oven dry weight and CF is the correction factor. The RWC was then calculated for each replication, where FW_P is the fresh weight of a sample at some known balance pressure (eq. 3).

$$(FW_P - DW) / (FW_T - DW) = RWC \qquad (3)$$

The RWC values of the two replications for each balance pressure were averaged and plotted as 1 / balance pressure vs RWC. Linear regression of the points on the straight portion of the lines yielded equations from which the RWC at infinite pressure (= % apoplastic water) and ψ_π at full turgor (100% RWC) were derived. At least four P-V curves and three P-N curves were generated for each species.

Water potential of spruce scions was determined with a wet-ring themocouple psychrometer; that of Douglas-fir using a pressure chamber. Osmotic potential was determined using expressed sap from previously frozen needles in a vapor-pressure osmomter (Wescor, Inc; Logan, UT).

RESULTS

Curves produced by the P-N method were similar to the P-V curves which were converted from the summation of volume expressed to RWC for comparison (Fig. 1 - 4). The % APO was calculated for each species by the P-V and P-N methods (Tab. 1). The r^2 values for the linear portion of the P-N curves were all greater than 0.95, for the P-V curves 0.98.

The % APO calculated by P-N and P-V were significantly different (5%, Tab. 1) for Scots pine and blue spruce, but not for Douglas-fir and grand fir. For each species, the % APO determined by the P-V method was larger than that determined by the P-N method. A comparison of the Ψ_π at 100% RWC indicated that only the values for blue spruce differed significantly (5%) between the 2 methods (Tab. 1).

A relationship was observed between the dry weight of the twig and the % APO calculated. Using linear regression correlations between twig dry weight and % APO were significant (5%) for both pine (0.9587) and spruce (0.9037) but not for Douglas-fir and grand fir (Table 2).

The effects of correcting Ψ_π were illustrated using Douglas-fir and blue spruce. Small, cut Douglas-fir trees were dried in a greenhouse (16/10° $\pm$ 5° C day/night temperature and 50 to 70% RH) for 12 days and then rehydrated by recutting the base of the stem and placing in a reservoir of water (Fig. 5). Using uncorrected Ψ_π, P as low as -1.5 MPa was calculated. Correction for % APO with either the P-V or P-N values indicated that P remained above 0.1 MPa.

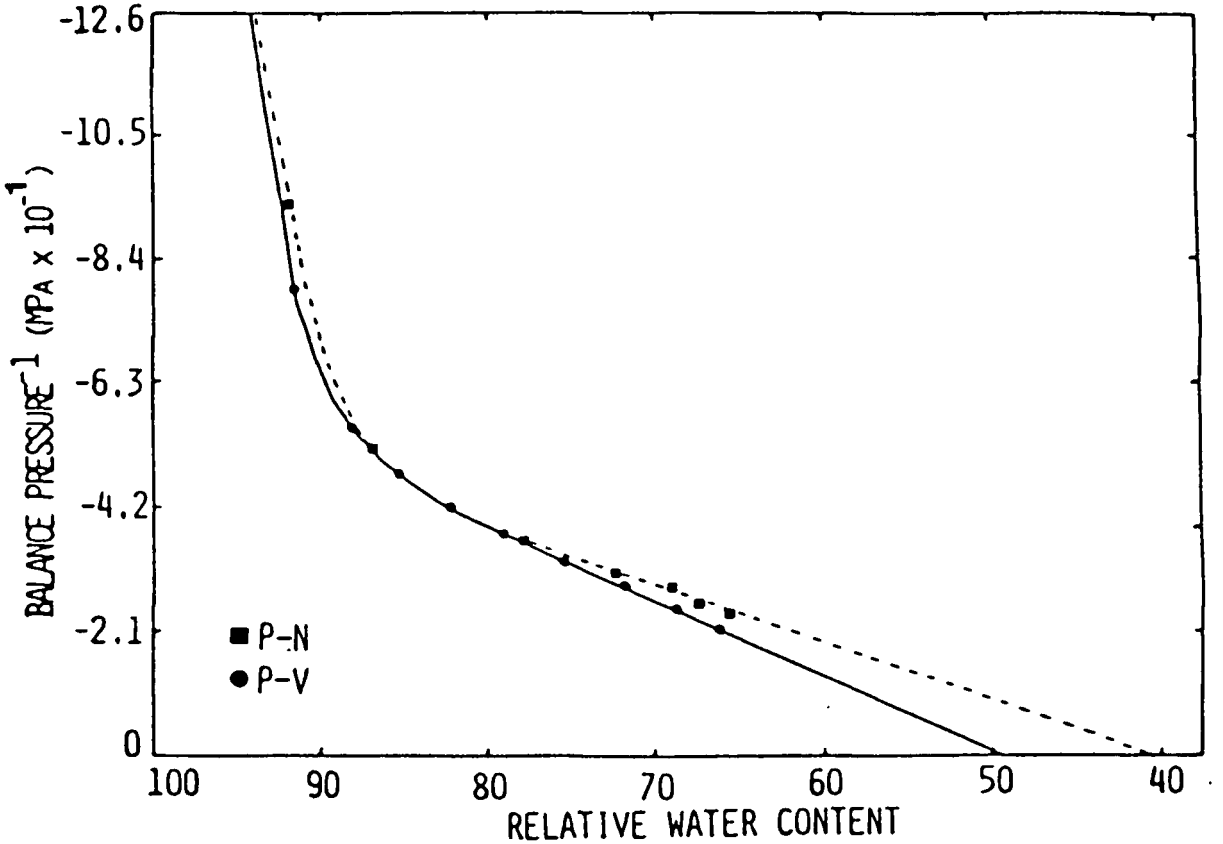

Fig. 1.1. Representative P/V and P/N curves generated from Scots pine twigs.

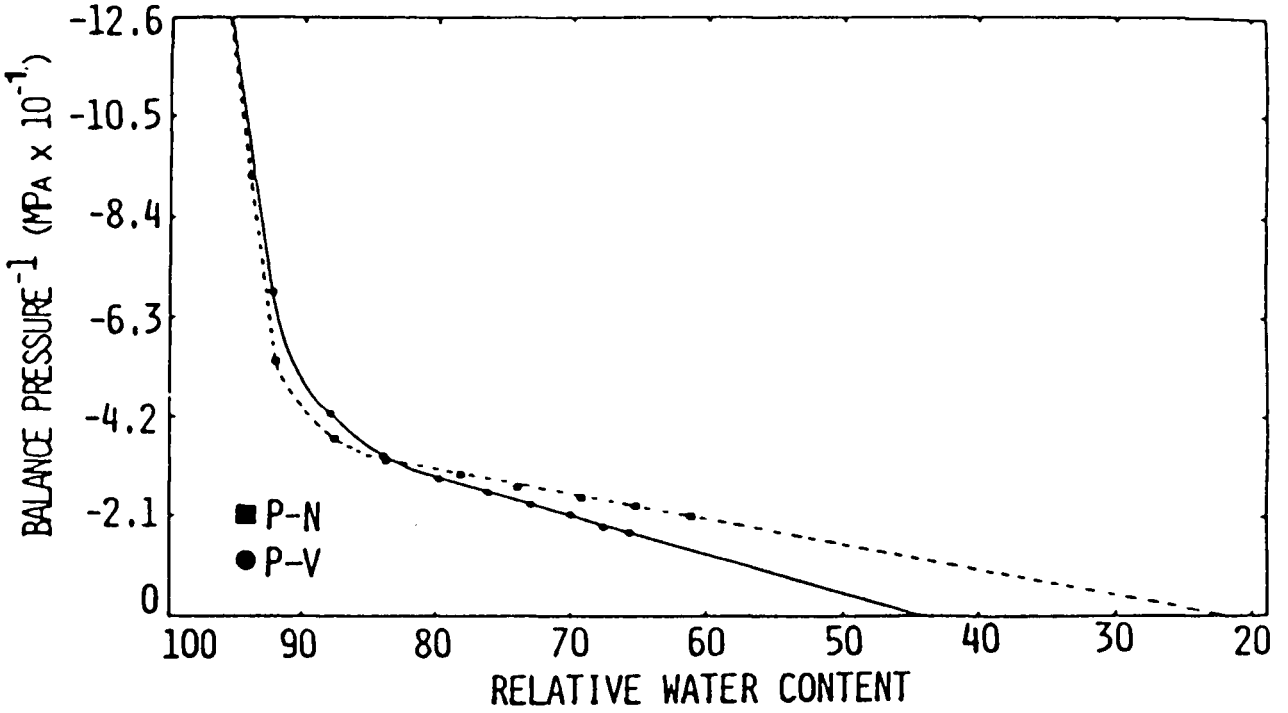

Fig. 1.2. Representative P/V and P/N curves generated from blue spruce twigs.

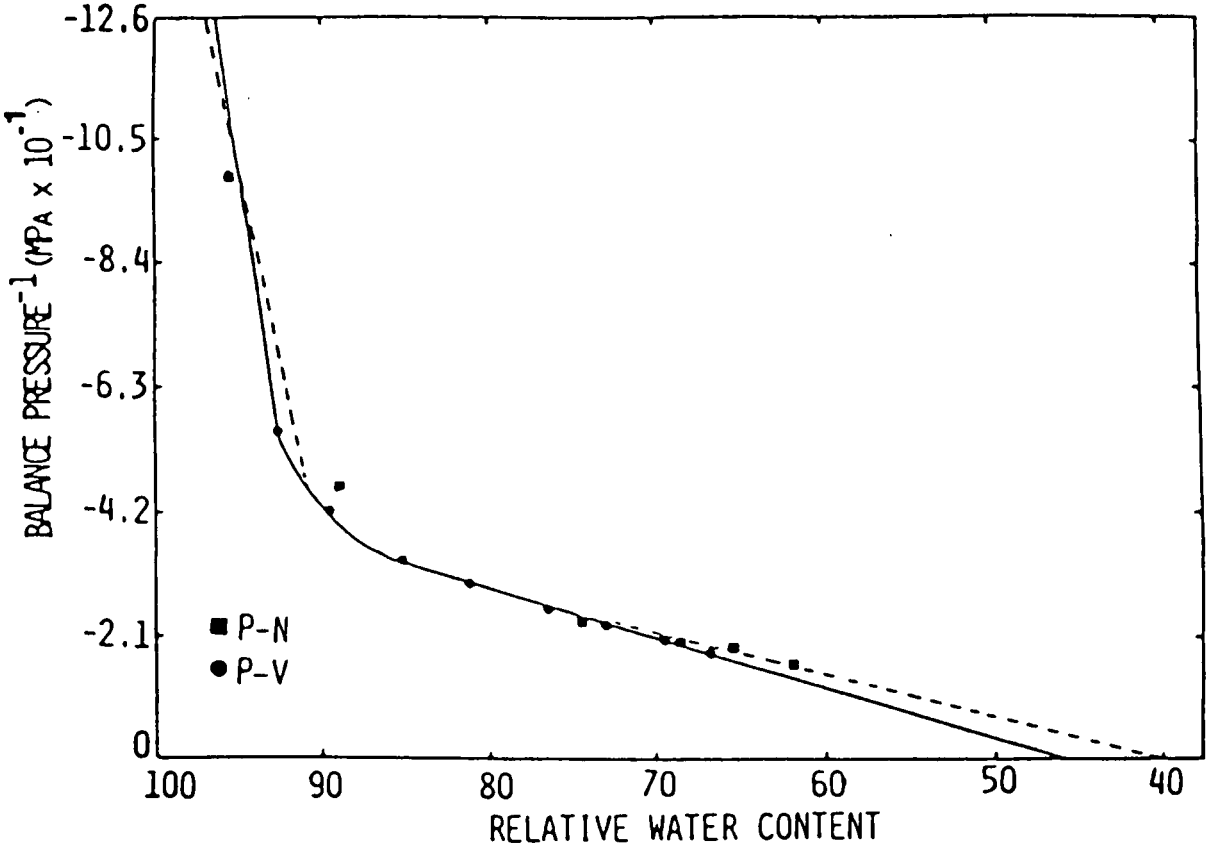

Fig. 1.3. Representative P/V and P/N curves generated from Douglas-fir twigs.

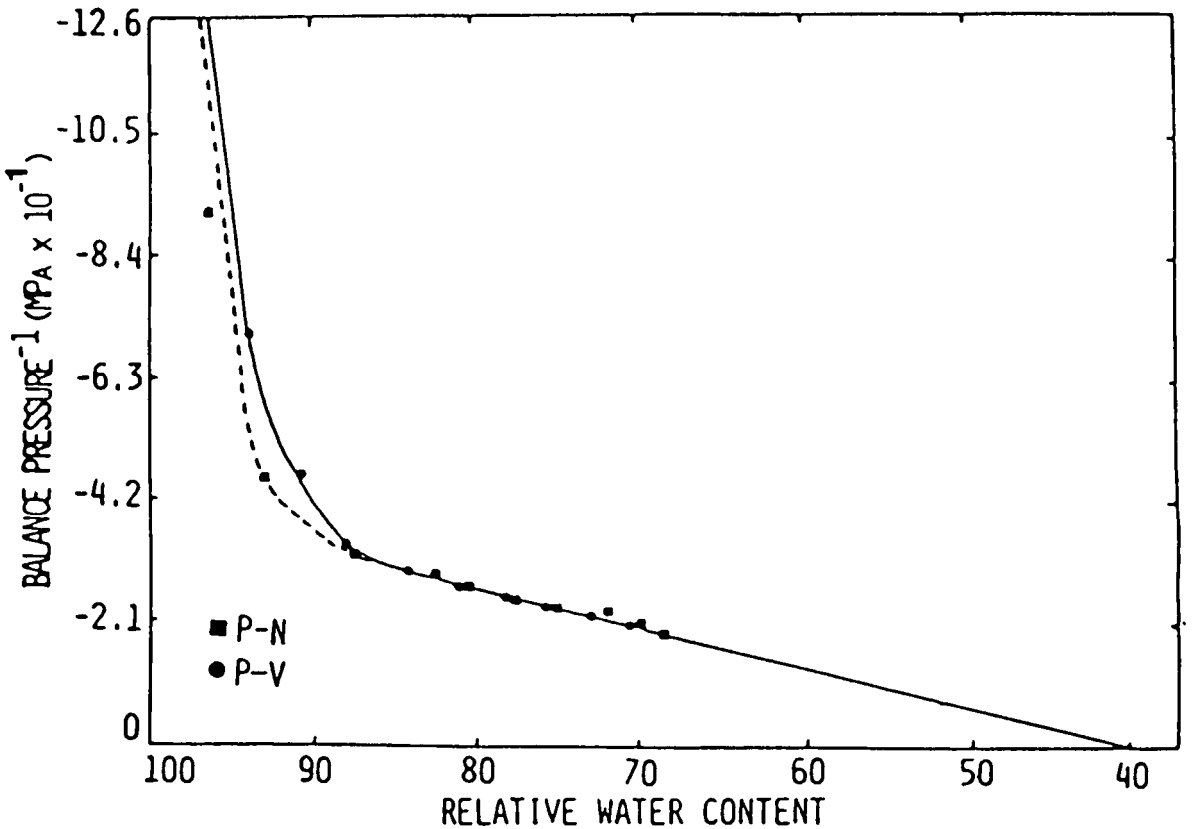

Fig. 1.4. Representative P/V and P/N curves generated from grand fir twigs.

Table 1.1. The mean values of % APO and maximum turgor pressure for each species by the P/V and P/N methods. (a) Values obtained for % APO and their standard deviations for each species by each method and the number of replications (n). (b) Turgor pressure at 100% RWC for each species. *Methods significantly different at 5% level by Protected LSD.

Species	P/V		P/N	
	% APO[a]	MPa[b]	% APO	MPa
	($x \pm s_D$ (n))	($x \pm s_D$)	($x \pm s_D$)	($x \pm s_D$)
Pinus sylvestris	59.1±13.4* (5)	1.66±0.18	40.9±5.2* (5)	1.57±0.19
Picea pungens 'Hoopsi'	44.9±9.9* (5)	2.19±0.11*	19.5±1.3* (3)	2.44±0.04*
Pseudotsuga menziesii	45.0±5.2 (5)	2.25±0.3	41.6±2.2 (3)	2.49±0.18
Abies grandis	41.4±2.5 (4)	2.53±0.12	39.9±1.7 (3)	2.44±0.03

Table 1.2. Correlations (r^2) of twig dry weight with % apoplastic water volume for each species of conifer. The % APO was determined by the P-V method. The r^2 of the pine and spruce were significant at the 5% level, whereas the r^2 of the Douglas-fir and grand fir were not.

	Pine		Spruce		Douglas-fir		Grand fir	
Curve	weight	% APO	weight	% APO	weight	% APO	weight	% APO
1	1.927	57.6	4.548	34.2	1.268	36.7	1.336	39.6
2	5.539	82.5	5.756	43.5	1.171	46.0	1.576	45.0
3	1.874	49.6	5.322	46.4	1.565	48.6	1.825	40.5
4	1.602	51.3	6.703	60.6	1.545	49.9	1.774	40.6
5	2.760	54.6	5.312	40.0	1.549	43.6		
r^2	.959		.904		.510		.007	

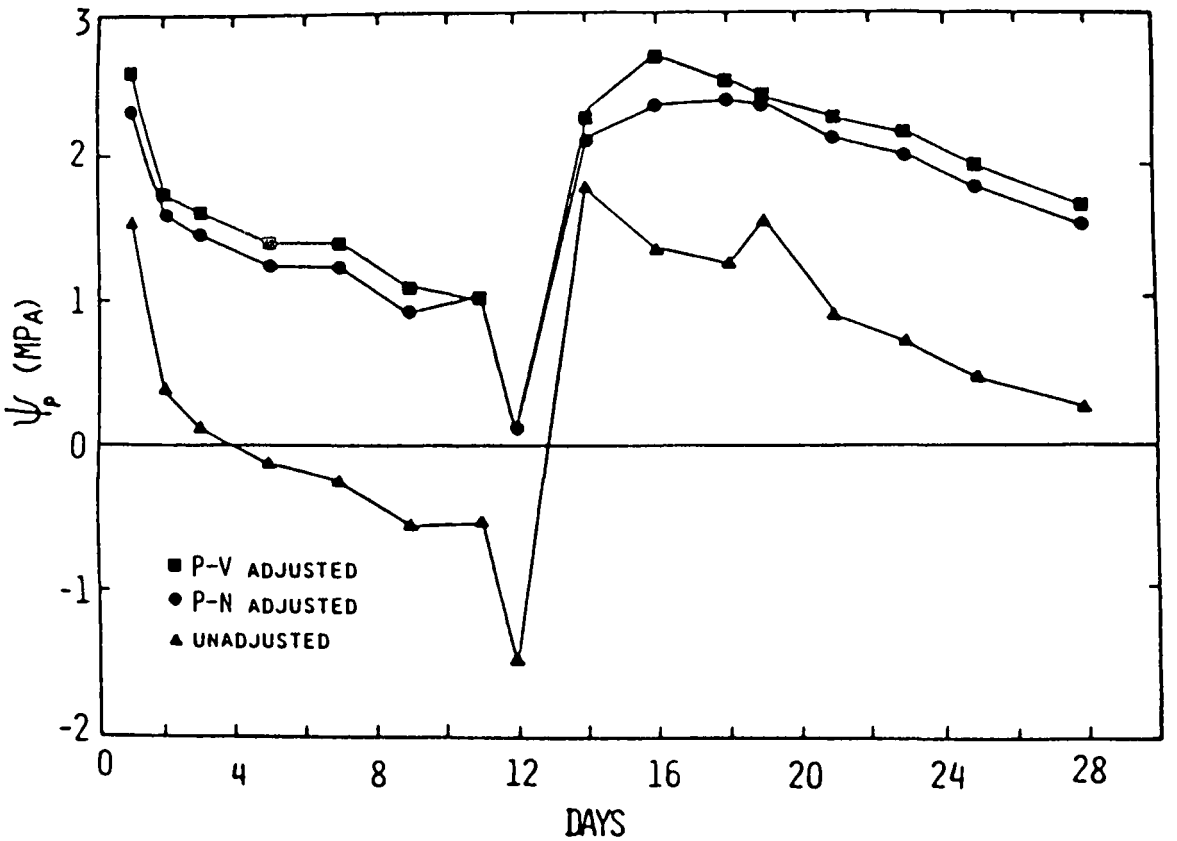

Fig. 1.5. Effect of adjusting turgor pressure using % APO calculated by pressure/volume or by pressure/volume methods. Douglas-fir trees were stored for 12 d at $16^{o}/10^{b} \pm 5^{o}$C day/night temperatures and 50 to 70% RH. The Ψ_L and Ψ_π were measured every 2 days. Each point is the average of 5 trees.

Similarly, during development of blue spruce grafts, P of the scions gradually declined (Fig. 6). Calculated P was lower and more accurate when corrected by P-N than by P-V.

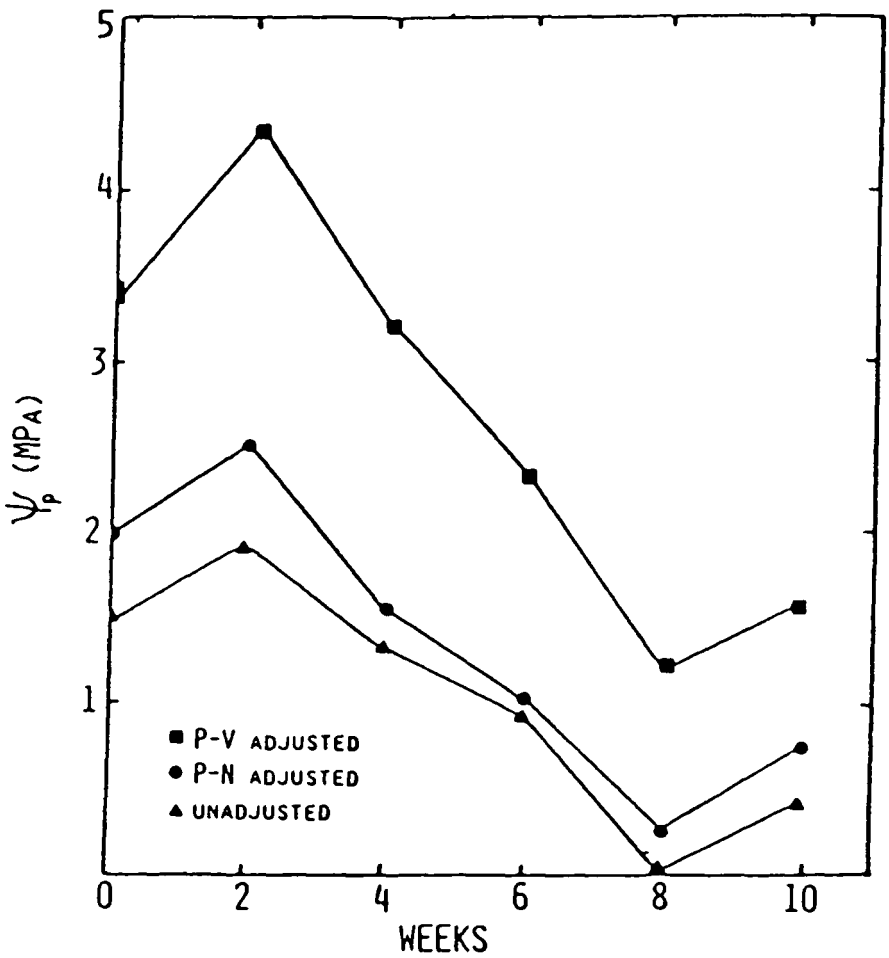

Fig. 1.6. Effect of adjusting turgor pressure using % APO calculated by pressure/volume or pressure/needle methods. Picea pungens 'Hoopsi' scions were grafted to Picea abies rootstocks. Scion and were measured weekly. Each point is the average of 10 scions.

DISCUSSION

The % APO calculated from the P-N curves were found to be both lower and more precise than the % APO calculated from P-V curves of each species. The lower values derived from the P-N curves are the result of the exclusion of stem apoplastic water from the calculations. As shown for pine and spruce, there is a high correlation between twig dry weight and % APO. The increase in dry weight was due mainly to an increase the woody tissue of the twig. Calculation of total water in the tissue (V_T) in P-V curve calculations by eq. 4 causes an overestimation of V_T by the amount of apoplastic water contained in the woody tissue.

$$V_T = FW_{twig} - DW_{twig} \qquad (4)$$

It follows that this overestimation of V_T leads to an overestimation of the % APO as calculated by eq. · 5, where V_S is the extrapolated volume of water expressed (symplastic water).

$$\% \text{ APO} = V_T - V_S \; / \; V_T \qquad (5)$$

Cheung et al. (1975) observed that the apoplastic water volume (Va) in angiosperms determined by the P-V method was greater for shoots than for leaves alone. Roberts and Knoerr (1977) generated P-V curves for several woody angiosperms and developed equations to estimate the % APO of the leaves only. The P-N method however estimates % APO of the conifer needles directly.

In 1982 Tyree & Richter reviewed the limitations of isotherm analysis of P-V curves and concluded that the estimate of % APO could not be made with a high degree of accuracy but the value of Ψ_π at 100% RWC could. However, there is good agreement between

the values of Ψ_π calculated from corrected osmometry values using the P-N derived % APO values and those determined from the water potential isotherms.

The values we obtained for % APO by either method fall generally in the range of values reported by others. Boyer (1967) calculated a % APO of 26% for leaves of rhododendron and 11% for yew by the P-V method. Tyree et al. (1973) reported a % APO of 23% for Canadian hemlock by the P-V method. The % APO of Sitka spruce twigs was estimated to be 46% by by the P-V method (Hellkvist et al. 1974). To account for the high determined for Quercus ilex, Kyriakopoulus and Richter (1977) estimated dilution of expressed sap of the leaves to be 17-27%. Estimates of Ψ_π at 100% RWC by P-V and P-N were also similar, and were comparable to those obtained for hemlock (1.65 MPa; Tyree et al. 1973) and for Norway spruce (2.2 MPa; Tyree et al. 1978) by the P-V method.

The influence of correction on P is illustrated by data in Fig. 5 and 6. The use of uncorrected Ψ_π suggested that the Douglas-fir developed P as low as -1.5 MPa. Such values should be associated with severe tissue damage, which was not the case. Correction of Ψ_π with either the P-V or P-N factors resulted in P consistent with the moderate drying that actually took place. In spruce scions, adjustment of Ψ_π with P-V values resulted in estimates of P larger than the Ψ_π at 100% RWC calculated by either method, whereas adjustment of Ψ_π by P-N values produced P less than or equal to Ψ_π at 100% RWC. This large difference between P-V and P-N adjusted Ψ_π is a reflection of the woodier spruce twigs used, as opposed to the less woody Douglas-fir twigs used, resulting in smaller differences

between P-V and P-N calculations of % APO. These points emphasize the importance of estimating % APO of the needles but not the twig and the potential error in calculated P that can result from P-V corrected Ψ_π.

Estimates of % APO of Scots pine and blue spruce were significantly different by the P-V and P-N methods, whereas % APO of grand fir and Douglas-fir by P-V and P-N were not. This was due to the small needle weight:stem weight ratio of the pine and spruce caused by the large stems of spruce and pine (data not shown). The firs, on the other hand, had small stems which did not influence % APO significantly, although the P-V values of % APO were still higher than P-N values of % APO.

In summary, the P-N protocol described here is a method suitable for direct estimation of % APO of conifer needles, contributing to more accurate calculations of needle water relations. The P-N method is particularly appropriate in large-stemmed twigs where the % APO of the stem will substantially alter the estimate of % APO of the needles.

Acknowledgements

We would like to thank Dr. Larry Boersma for stimulating the development of the P-N method. We also acknowledge the advice of Robert Joly and John Jacatta about the P-V procedure. Oregon Agricultural Experiment Station Technical Paper No. 7500.

Literature Cited

Boyer, J. S. 1967. Matrix potentials of leaves. - Plant Physiol. 42: 213-217.

Campbell, E. S., Papendick, R. I., Rabie, E. & Shayo-Ngowi, A. J. 1979. A comparison of osmotic potential, elastic modulus, and apoplastic water in leaves of dryland winter wheat. - Agron. J. 71: 31-36.

Cheung, Y. N. S., Tyree, M. T. & Dainty, J. 1975. Water relations parameters on single leaves obtained in a pressure bomb and some ecological interpretations. - Can. J. Bot. 53: 1342-1346.

Hellkvist, J., Richards, G. P. & Jarvis, P. J. 1974. Vertical gradients of water potential and tissue water relations in Sitka spruce trees measured with the pressure chamber. - J. Appl. Ecol. 11: 637-668.

Kyriakopoulos, E. & Richter, H. 1977. A comparison of methods for the determination of water status in Quercus ilex L.. Z. Pflanzenphysiol. 82: 14-27.

-, & Richter, H. 1981. Pressure-volume curves and drought injury. - Physiol. Plant. 52: 124-128.

Richter, H., Duhme, F., Glatzel, G., Hinckley, T. M. & Karlic, H. 1981. Some limitations and applications of the pressure-volume curve technique In ecophysiological research. - In "Plants and Their Atmospheric Environment". (J. Grace, E. D. Ford & P. G. Jarvis), pp. 263-272. Blackwell Scientific Publisher, Oxford. ISBN 0-470-27125-6.

Roberts, S. W. & Knoerr, K. R. 1977. Components of water potential estimated from xylem pressure measurements in five tree species. - Oecologia (Berl.) 28: 191-202.

Scholander, P. F., Hammel, H. T.,Hemmingsen, E. A., & Bradstreet, E. D. 1964. Hydrostatic pressure and osmotic potentials in leaves of mangrove and some other plants. - Proc. Natl. Acad. Sci. 51: 119-125.

- , Hammel, H. T., Bradstreet, E. D., & Hemmingsen, E. A. 1965. Sap pressure in vascular plants. - Science 148: 339-349.

Talbot, A. J. B., Tyree, M. T. & Dainty, J. 1974. Some notes concerning the measurement of water potentials of leaf tissues with specific reference to Tsuga canadensis and Picea abies. - Can. J. Bot. 53: 784-788.

Tyree, M. T., Hammel, H. T. 1972. The measurement of turgor pressure and the water relations of plants by the pressure-bomb technique. J. Exp. Bot. 23(74): 267-282.

- , Dainty, J. & Benis, M. 1973. The water relations of hemlock (Tsuga canadensis). I: Some equilibrium water relations as measured by the pressure-bomb technique. - Can. J. Bot. 51: 1471-1480.

-, Cheung, Y. N. S., MacGregor, M. E. & Talbot, A. J. B. 1978. The characteristics of seasonal and ontogenetic changes in the tissue - water relations of Acer, Populus, Tsuga, and Picea. - Can. J. Bot. 56: 635-647.

-. & Richter, H. 1982. Altermate methods of analyzing water potential isotherms: some cautions and clarifications. II: Curvilinearity in water potential isotherms. - Can. J. Bot. 60: 911-916.

Appendix 2. Tables of mean separation for the Figures.

Appendix 2.1. Separation of means of total water potential of Greenhouse Water Relations 1983. Means with the same letter are not significantly different at the 5% level as separated by Protected LSDs.

Week	Group 1	Group 2	Group 3	Group 4
0	-0.569 a	-0.574 a	-0.527 a	-0.408 a
1	-1.380 efgh	-1.408 efghij	-1.361 defgh	-1.566 ijklm
2	-1.564 ghijk	-1.565 ghijklm	-1.486 efghijkl	-1.639 ijklm
3	-1.317 defgh	-1.491 efghijkl	-1.332 defgh	-1.397 efghi
4	-1.368 efgh	-1.314 defgh	-1.428 efghijk	-1.513 efghijkl
5	-1.296 def	-1.671 jklmn	-1.553 fghijklm	-1.543 fghijklm
6	-1.234 cde	-1.314 defgh	-1.558 fghijklm	-1.797 mn
7	-1.421 efghij	-1.730 klmn	-1.820 mn	-2.184 p
8	-1.009 b	-1.296 defg	-1.400 efghij	-1.907 no
9	-0.967 b	-1.026 bc	-1.002 b	-1.773 lmn
10	-1.018 bc	-1.047 bcd	-1.363 defgh	-2.213 p
11	-0.928 b	-0.984 b	-0.963 b	-2.111 op
12	-1.007 b	-1.057 bcd	-1.196 bcde	-2.495 q

Appendix 2.2. Separation of means of osmotic potential of Greenhouse Water Relations 1983. Means with the same letter are not significantly different at the 5% level as separated by Protected LSD.

Week	Group 1	Group 2	Group 3	Group 4
0	-2.578 def	-2.574 def	-2.556 def	-2.589 def
1	-3.318 ijkl	-3.592 jkl	-3.269 hijkl	-3.344 ijkl
2	-3.233 hij	-3.234 hijk	-2.959 fghi	-3.155 ghi
3	-3.156 hi	-3.043 fghi	-3.107 fghi	-3.144 ghi
4	-3.122 ghi	-3.137 ghi	-3.190 hij	-3.025 fhgi
5	-2.733 efg	-2.954 fgh	-3.054 fghi	-2.862 fgh
6	-2.391 cd	-2.682 def	-3.044 fghi	-3.210 hij
7	-2.220 bc	-2.770 efg	-3.089 fghi	-3.414 ijkl
8	-1.980 ab	-2.503 cde	-2.968 fghi	-3.682 lm
9	-1.961 ab	-2.359 cd	-2.727 defg	-3.444 ijkl
10	-1.780 a	-1.881 ab	-2.812 efgh	-3.648 klm
11	-1.780 a	-1.760 a	-2.006 abc	-3.675 lm
12	-1.825 a	-1.801 a	-2.040 abc	-4.050 m

Appendix 2.3. Separation of means of turgor pressure of Greenhouse Water Relations 1983. Means with the same letter are not significantly different at the 5% level by Protected LSD.

Week	Group 1	Group 2	Group 3	Group 4
0	2.008 a	2.000 a	2.015 a	2.181 a
1	1.938 ab	2.184 a	1.908 abc	1.778 abcd
2	1.669 bcdef	1.670 bcdefg	1.473 cdefghij	1.516 cdefg
3	1.839 abc	1.553 cdefg	1.775 abcde	1.748 bcde
4	1.754 bcde	1.823 abc	1.763 abcde	1.513 cdefg
5	1.438 defghi	1.283 ghijk	1.502 cdefgh	1.319 fghij
6	1.157 hijk	1.369 efghij	1.487 cdefghi	1.414 defghi
7	0.799 l	1.039 jkl	1.269 ghijk	1.231 ghijk
8	0.971 kl	1.107 hijkl	1.568 bcdefg	1.775 abcd
9	0.994 kl	1.313 fghij	1.725 bcdef	1.671 bcedf
10	0.762 l	0.834 l	1.449 cdefghi	1.435 defghi
11	0.851 l	0.775 l	1.043 ijkl	1.563 cdefg
12	0.817 l	0.744 l	0.844 l	1.555 cdefg

Appendix 2.4. Separation of means of total water potential of Greenhouse Water Relations for 1984. Means with the same letter are not significantly different at the 5% level as separated by Protected LSDs.

Week	Group 1	Group 2	Group 3
0	-0.795 a	-0.949 b	-0.607 a
1	-1.141 cde	-1.214 cdef	-1.079 bcde
2	-1.632 hij	-1.635 hij	-1.662 ij
3	-1.303 def	-1.441 fg	-1.363 efg
4	-1.328 ef	-1.414 fg	-1.441 fg
5	-1.314 ef	-1.540 ghi	-1.464 fgh
6	-1.235 def	-1.256 def	-11.31 cde
7	-1.031 bc	-1.174 cde	-1.258 def
8	-1.034 bc	-1.005 bc	-1.363 fg
9	-1.011 bc	-1.091 cde	-1.222 cdef
10	-0.920 ab	-0.934 ab	-1.783 j

Appendix 2.5. Separation of means of osmotic potential of Greenhouse Water Relations 1984. Means with the same letter are not significantly different at the 5% level as separated by Protected LSDs.

Week	Group 1	Group 2	Group 3
0	-3.302 k	-3.353 k	-3.298 jk
1	-3.135 ij	-3.118 ij	-2.954 h
2	-3.104 ij	-3.071 ij	-3.289 j
3	-2.847 gh	-2.922 h	-2.893 ghi
4	-2.750 gh	-2.749 gh	-3.047 i
5	-2.544 ef	-2.651 g	-2.616 fg
6	-2.202 d	-2.323 d	-2.010 c
7	-1.967 c	-2.148 d	-2.111 cd
8	-1.760 b	-1.770 b	-2.348 de
9	-1.595 a	-1.661 ab	-1.858 bc
10	-1.604 a	-1.600 a	-2.407 ef

Appendix 2.6. Separation of means of turgor pressure of Greenhouse Water Relations 1984. Means with the same letter are not significantly different at the 5% level as separated by Protected LSDs.

Week	Turgor Pressure
0	2.469 a
1	1.935 b
2	1.464 c
3	1.509 c
4	1.398 c
5	1.160 d
6	0.998 e
7	0.948 e
8	0.747 f
9	0.581 g
10	0.676 fg

Appendix 2.7. Separation of means of total water potential of Lath Water Relations 1984. Means with the same letter are not significantly different at the 5% level as separated by Protected LSDs.

Weeks	Total Water Potential
0	-1.091 bc
1.5	-1.201 d
3	-1.268 de
4.5	-1.417 f
6	-1.410 f
7.5	-1.365 ef
8.5	-1.273 de
9.5	-1.239 d
10.5	-1.073 b
11.5	-1.034 ab
12.5	-0.983 ab
13.5	-0.957 a
14.5	-1.032 ab
15.5	-1.054 ab
16.5	-1.191 cd

Group	Total Water Potential
1	a
2	a
3	b

Appendix 2.8. Separation of means of osmotic potential of Lath Water Relations 1984. Means with the same letter are not significantly different at the 5% level as separated by Protected LSDs.

Week	Group 1	Group 2	Group 3
0	-3.091 b	-3.121 b	-3.314 a
1.5	-2.932 c	-2.973 bc	
3	-2.627 de	-2.675 de	-2.620 de
4.5	-2.683 d	-2.740 d	-2.786 cd
6	-2.649 de	-2.747 d	
7.5	-2.698 d	-2.791 cd	
8.5	-2.665 de	-2.697 de	-2.684 de
9.5	-2.663 de	-2.735 d	
10.5	-2.569 e	-2.617 de	-2.594 de
11.5	-2.477 e	-2.603 de	
12.5	-2.304 f	-2.408 ef	-2.171 g
13.5	-2.087 g	-2.423 ef	
14.5	-2.000 g	-2.210 fg	-2.224 fg
15.5	-1.742 i	-1.945 gh	
16.5	-1.676 i	-1.749 hi	-2.362 ef

Appendix 2.9. Separation of means of turgor pressure of Lath Water Relations 1984. Means with the same letter are not significantly different at the 5% level as separated by Protected LSDs.

Week	Turgor Pressure	Group	Turgor Pressure
0	2.015 a	1	a
1.5	1.743 b	2	a
3	1.362 def	3	b
4.5	1.286 fg		
6	1.267 fg		
7.5	1.360 def		
8.5	1.398 cde		
9.5	1.445 cd		
10.5	1.506 c		
11.5	1.478 c		
12.5	1.331 efg		
13.5	1.224 g		
14.5	1.022 h		
15.5	0.745 i		
16.5	0.542 i		

Appendix 2.10. Separation of means of total and osmotic potentials and turgor pressures of Temperature and Transpiration - 21.1° C. Means with the same letter are not significantly different at the 5% level as separated by Protected LSDs.

	Total Water Potential		Osmotic Potential	
Week	Successful	Unsuccessful	Successful	Unsuccessful
0	-1.087 ab	-1.048 a	-2.913 c	-3.011 cd
1	-1.619 de	-1.706 de	-2.720 bc	-2.971 cd
2	-1.786 ef	-1.982 fg	-2.741 bc	-3.289 d
3	-1.535 cd	-2.058 gh	-2.686 bc	-3.357 de
4	-1.328 bc	-2.065 gh	-2.370 b	-3.470 ef
5	-1.275 ab	-2.239 h	-1.980 a	-3.704 f

Turgor Pressure

Week	Successful	Unsuccessful
0	1.826 e	1.962 e
1	1.101 bc	1.305 bcd
2	0.955 ab	1.265 bcd
3	1.151 bc	1.299 bcd
4	1.042 abc	1.404 cd
5	0.709 a	1.465 d

Appendix 2.11. Separation of means of 0815 hr, 1200 hr and 1600 hr transpiration rates of Temperature and Transpiration - 21.1° C. Means with the same letter are not significantly different at the 5% level as separated by Protected LSDs.

	0815 hr Measurement		1200 hr Measurement	
Week	Successful	Unsuccessful	Successful	Unsuccessful
0	0.0267 b	0.0226 bc	0.0313 bc	0.0290 cd
1	0.0172 bcd	0.0166 cd	0.0132 ef	0.0115 ef
2	0.0058 e	0.0101 de	0.0119 ef	0.0096 f
3	0.0198 bcd	0.0218 bc	0.0206 de	0.0202 de
4	0.0214 bc	0.0245 bc	0.0525 a	0.0461 b
5	0.0414 a	0.0215 bc	0.0519 a	0.0332 bc

1600 hr Measurement

Week	Successful	Unsuccessful
0	0.0284 abcd	0.0223 cdef
1	0.0199 def	0.0148 efg
2	0.0097 g	0.0125 fg
3	0.0232 cde	0.0248 bcde
4	0.0364 a	0.0320 abc
5	0.0342 ab	0.0223 cdef

Appendix 2.12. Separation of means of total and osmotic potentials and turgor pressure of Temperature and Transpiration - 26.7° C. Means with the same letter are not significantly different at the 5% level as separated by Protected LSDs.

	Total Water Potential		Osmotic Potential	
Week	Successful	Unsuccessful	Successful	Unsuccessful
0	-1.325 ab	-1.271 a	-3.045 d	-3.093 d
1	-1.734 cd	-1.960 e	-2.847 c	-3.014 cd
2	-1.692 c	-1.950 de	-2.936 cd	-3.461 e
3	-1.538 bc	-2.143 e	-2.558 b	-3.455 e
4	-1.414 ab	-2.530 f	-2.200 a	-3.624 e
5	-1.341 ab	-2.609 f	-2.054 a	-3.977 f

Turgor Pressure

Week	Turgor Pressure
0	1.761 a
1	10.83 bc
2	1.366 b
3	1.153 bc
4	0.927 c
5	1.013 c

Appendix 2.13. Separation of means of 0815 hr, 1200 hr and 1600 hr transpiration rates of Temperature and Transpiration - 26.7° C. Means with the same letter are not significantly different at the 5% level as separated by Protected LSDs.

	0815 hr Measurement		1200 hr Measurement	
Week	Successful	Unsuccessful	Successful	Unsuccessful
0	0.1345 cd	0.1717 cd	0.3100 bc	0.3366 bc
1	0.1215 d	0.1622 cd	0.1375 e	0.1624 e
2	0.1744 cd	0.2188 bc	0.1623 e	0.1897 e
3	0.2643 b	0.2100 bcd	0.4754 a	0.3997 ab
4	0.3558 a	0.2699 b	0.2901 cd	0.2005 de

1600 hr Measurement

Week	Successful	Unsuccessful
0	0.2390 bc	0.2640 bc
1	0.1443 d	0.1812 cd
2	0.1511 d	0.2037 cd
3	0.3998 a	0.3942 a
4	0.4142 a	0.2990 b

Appendix 2.14. Separation of means of relative water content, total and osmotic potentials and turgor pressures of Scion Water Loss. Means with the same letter are not significantly different at the 5% level as separated by Protected LSDs.

Relative Water Content

Week	Twig	Failure	Successful
0	0.984 ab	0.997 a	0.990 a
1	0.837 abcde	0.833 abcd	0.859 abcd
2	0.721 defg	0.821 abcdef	0.872 abcd
3	0.655 efg	0.825 abcdef	0.837 abcd
4	0.604 ghi	0.800 cdef	0.831 abcdef
5	0.560 ghi	0.808 bcdef	0.874 abcd
6	0.473 hi	0.726 defg	0.895 abcd
7	0.450 i	0.650 fgh	0.964 abc

Total Water Potential

Week	Twig	Failure	Successful
0	-0.30 a	-0.30 a	-0.30 a
1	-1.83 def	-1.47 bcd	-1.43 bcd
2	-2.36 efg	-1.48 bcd	-1.33 bcd
3	-2.47 fgh	-1.67 cde	-1.50 bcd
4	-3.19 hi	-1.55 bcd	-1.30 bcd
5	-4.20 j	-1.61 cde	-0.91 abd
6	-5.40 k	-2.79 ghi	-0.79 ab
7	-5.89 k	-3.55 ij	-0.83 ab

Osmotic Potential

Week	Twig	Failure	Successful
0	-3.03 e	-3.00 e	-2.98 e
1	-2.94 de	-2.81 cd	-2.89 de
2	-3.27 f	-27.5 bcd	-2.47 ab
3	-3.88 g	-2.66 abcd	-2.77 bcd
4	-4.25 h	-2.92 de	-2.54 abc
5	-4.73 i	-2.88 de	-2.62 abcd
6	-5.05 i	-3.18 ef	-2.49 bc
7	-5.57 j	-3.28 f	-2.41 a

Turgor Pressure

Week	Twig	Failure	Successful
0	2.73 a	2.70 a	2.68 a
1	1.11 bcde	1.35 bcd	1.46 bcd
2	0.92 def	1.27 bcd	1.13 bcd
3	1.42 bcd	0.99 cde	1.27 bcd
4	1.06 cde	1.38 bcd	1.23 bcd
5	0.53 ef	1.27 bcd	1.71 b
6	-0.35 g	0.39 f	1.69 b
7	-3.3 g	-0.28 g	1.59 bc

Appendix 2.15. Separation of means of starch content of Greenhouse Water Relations 1983. Means with the same letter are not significant at the 5% level as separated by Protected LSDs.

Week	Starch content	Group	Starch Content
0	3.49 g	1	46.17 b
1	7.22 d	2	49.97 a
2	7.78 cd	3	49.12 a
3	8.48 c	4	38.32 c
4	10.30 b		
5	9.69 b		
6	12.58 a		
7	7.70 cd		
8	6.32 e		
9	4.97 f		
10	3.23 gh		
11	2.58 h		
12	2.68 gh		

Appendix 2.16. Separation of means of starch content of Greenhouse Water Relations 1984. Means with the same letter are not significantly differently at the 5% level as separated by Protected LSDs.

Week	Starch Content
0	5.40 de
1	7.47 cd
2	10.93 b
3	9.32 bc
4	13.79 a
5	14.13 a
6	9.01 bc
7	4.46 e
8	2.12 f
9	2.33 f
10	1.64 f

Appendix 2.17. Separation of means of starch content of Lath Water Relations 1984. Means with the same letter are not significantly different at the 5% level as separated by Protected LSDs.

Week	Starch Content	Group	Starch Content
0	6.09 ef	1	a
1.5	5.98 ef	2	a
3	6.94 e	3	b
4.5	4.96 ef		
6	8.30 e		
7.5	13.12 d		
8.5	16.10 cd		
9.5	17.58 bc		
10.5	24.97 a		
11.5	24.96 a		
12.5	27.15 a		
13.5	20.07 b		
14.5	16.31 cd		
15.5	6.66 e		
16.5	3.02 f		

Appendix 2.18. Separation of bark sugars, needle sugars, and needle starch means of Total Carbohydrate Reserves. Mean with the same letter are not significantly different at the 5% level as separated by Protected LSDs.

			Starch	
Week	Bark Sugar	Needle Sugar	Successful	Unsuccessful
0	1.389 g	126.04 a	0.537 a	0.381 a
1	3.721 ab	89.80 c	4.71 b	5.63 b
2	2.864 d	84.40 cd	6.42 b	4.93 b
3	3.433 bc	105.67 b	10.61 de	10.93 de
4	3.299 c	75.59 de	9.38 cd	6.36 b
5	3.879 a	75.92 de	14.42 f	10.42 cde
6	2.323 e	68.64 ef	5.84 b	5.31 b
7	1.933 f	58.80 f	12.78 ef	6.13 b
8	1.403 g	58.80 f	16.92 g	7.23 bc
			Needle Sugar	
			85.2 a	82.8 b

Appendix 2.19. Separation of bark and needle sugar means of Total Carbon Reserves - Covered. Means with the same letter are not significantly different at the 5% level as separated by Protected LSDs.

	Bark Sugars		Needle Sugars	
Week	Successful	Unsuccessful	Successful	Unsuccessful
0	1.245 i	1.589 i	126.27 a	125.80 a
1	3.776 bcd	4.094 abc	103.78 bc	109.53 b
2	3.376 def	4.315 ab	77.93 fgh	65.64 ijkl
3	4.368 a	3.462 de	71.47 hij	74.86 hi
4	3.626 cd	3.330 def	93.22 de	98.12 cd
5	2.908 f	2.991 ef	84.75 fg	85.50 ef
6	2.285 g	2.154 gh	57.80 lm	63.21 jklm
7	1.972 gh	1.844 ghi	66.40 ijk	56.52 mn
8	1.263 i	1.230 i	61.58 klm	48.61 n

Appendix 2.20. Separation of needle starch means of Total Carbon Reserves - Covered. Means with the same letter are not significantly different at the 5% level as separated by Protected LSDs.

Week	Starch Content
0	0.46 f
1	4.84 a
2	3.41 bc
3	3.87 b
4	3.01 cd
5	2.58 de
6	2.07 e
7	0.80 f
8	0.92 f

Appendix 2.21. Separation of needle sugar means between Total Carbohydrate Reserves and Total Carbon Reserves - Covered. Means with the same letter are not significantly different at the 5% level as separated by Protected LSDs.

Week	TCR	TCRC
0	126.04 a	126.04 a
1	89.80 c	105.85 b
2	84.41 cde	76.18 def
3	105.67 b	77.82 f
4	75.59 ef	94.20 c
5	75.92 ef	85.02 cd
6	68.64 fg	59.53 h
7	58.77 h	62.45 gh
8	58.80 h	55.72 h

Appendix 2.22. Means and standard deviations of the peaks of Total Carbon Reserves - Covered. Means with the same letter are not significantly different at the 5% level as separated by Protected LSDs.

Means

Week	Lamba	Delta-C	COH	Tri	Di	Alpha	Beta	FFA	Mono	Rho	Delta-P	Omega
0	5699	7151	13997	5950	2776	5773	16921	671	1524	25098	21220	20359
1	7145	10933	12248	7710	3167	4636	9554	382	982	14537	21008	19967
2	9389	6002	9826	3046	1445	2818	5457	233	436	20256	15529	15220
3	5959	5293	9346	3491	1426	3754	6251	215	576	20526	13201	12661
4	5733	7390	10716	3294	1485	3923	10155	182	795	17270	13527	12889
5	6316	5782	13558	4390	1531	4949	7548	249	1393	21289	14881	11274
6	7224	5097	6880	4070	1572	4010	5326	193	739	14091	9572	12396
7	6441	7749	7766	6574	3306	3140	8378	162	462	14837	12042	10514
8	4688	5217	13485	5808	2950	2167	7547	179	861	12862	18790	18790

Standard Deviations

Week	Lamba	Delta-C	COH	Tri	Di	Alpha	Beta	FFA	Mono	Rho	Delta-P	Omega
0	2991	5316	8049	2883	1497	2973	17053	323	1071	21343	14041	12874
1	4227	13909	5240	4221	1179	2508	4316	309	507	9296	11823	11604
2	5476	3093	5135	1722	914	1884	2954	145	302	15995	11201	10250
3	3952	3508	9346	1650	376	1954	5645	125	461	16554	11427	5757
4	5507	5094	5023	1877	691	1982	8659	151	473	18672	12430	12458
5	2972	2758	7717	1700	453	5502	7413	144	1080	14715	18665	7444
6	4040	3504	2986	1539	585	2491	3159	131	535	9168	4586	9233
7	3634	5516	4029	3482	863	1562	6821	65	236	9220	10289	5095
8	4413	3502	3519	2640	752	814	5095	76	550	6360	9830	9830