

**CAMBIAL AND PHOTOSYNTHETIC ACTIVITY RELATIONS IN UNTREATED,
WOUNDED, AND GEOTROPICALLY STRESSED WHITE SPRUCE (PICEA GLAUCA
(MOENCH.) VOSS) SEEDLINGS**

by

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ABSTRACT

This thesis reports results of a study of relationships between photosynthetic activity and developmental parameters, and cambial activity (wood formation rate), during and following the period of active wood formation in untreated white spruce seedlings, and in seedlings stressed either by extensive stem incisions, or by tilting.

The approach involved the use of two non-destructive methods for measuring photosynthetic activity: chlorophyll *a* fluorescence using optical instrumentation, and CO₂ uptake using infrared gas exchange techniques. Photosynthetic development was examined by estimating chlorophyll *a* content from a specific fluorescence parameter (O-level), and by the relative occurrence of specific chloroplast stroma and membrane (thylakoid) proteins using electrophoretic and immunoblotting techniques. Cambial activity was determined using digitized image analysis of prepared cross-sections of seedling stems.

Several fluorescence parameters were strongly correlated to cambial activity in untreated seedlings during the period of active wood formation (in mid-summer). However, the correlations were severely diminished or non-existent when cambial activity was arrested (in late-summer and autumn). Correlations between fluorescence and cambial activity in stressed seedlings were not discernible at any time, suggesting that the induced stresses resulted in a substantial alteration in normal source:sink relationships. Carbon dioxide uptake measures, either uncorrected or corrected to estimated chlorophyll *a* content, were not measurably correlated to cambial activity in untreated or stressed seedlings at any time in this system.

Chlorophyll *a* content estimated from O-level fluorescence, was not related to cambial activity in untreated or stressed seedlings. The relative occurrences of two enzymes and proteins associated with photosynthetic carbon fixation, i.e. ribulose 1,5-bisphosphate carboxylase (Rubisco) and Coupling Factor, did not appear to be influenced by applied wounding and geotropic stresses.

In contrast to the strong correlations found between fluorescence parameters and current season stem vigour, pre-season seedling height and cross-sectional stem areas were not related to stem vigour. These results suggest that in unstressed white spruce seedlings, the measure of specific chlorophyll *a* fluorescence parameters, using the methods

delineated in this study, offers an alternative and more strongly predictive means of assessing current stem vigour, than measures of seedling dimensions.

The results of this study provide strong evidence for, and a degree of elucidation on, the anticipated but previously unestablished existence of a source:sink relationship between leaves and vascular cambium in conifer seedlings. This information should provide an initial foundation for the elucidation of non-invasive methodologies by which to assess stem vigour of white spruce seedlings, and to probe source:sink relationships in other conifer species.

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LIST OF ABBREVIATIONS AND SYMBOLS

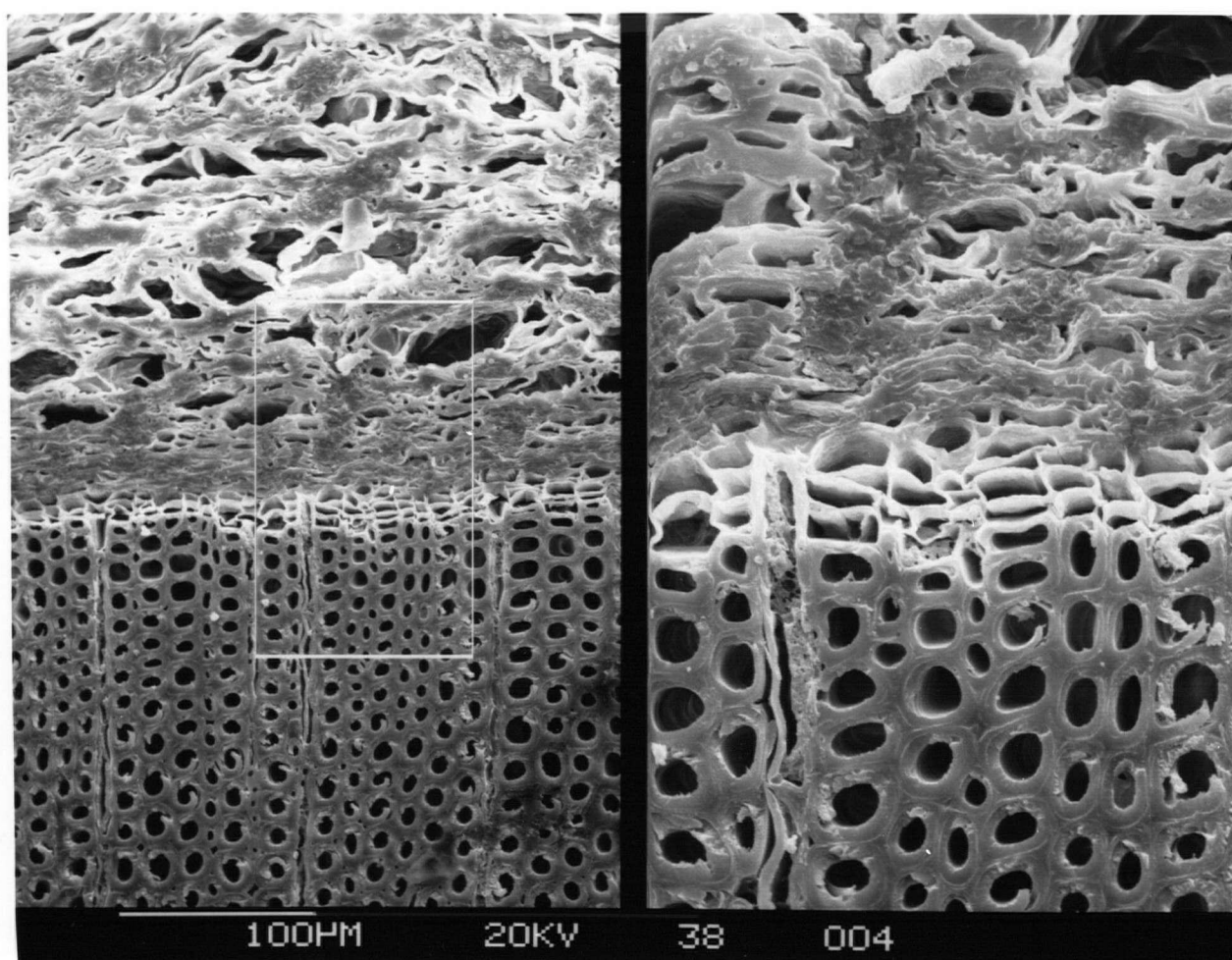
ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
Chl	Chlorophyll <i>a</i>
F _o or O-level	Initial or constant fluorescence
F _{var}	Normalized variable fluorescence
F _v	Non-normalized fluorescence
F _{var} AVG	Mean fluorescence of induction period
F _{var} M	Relative fluorescence maximum
F _{var} T	Terminal fluorescence of induction period
kDa	Kilodalton
LHCI	Light-harvesting complex of PSI
LHCII	Light-harvesting complex of PSII
NADP ⁺	Nicotinamide-adenine dinucleotide phosphate
OEC	Oxygen evolving complex
P680	Reaction centre Chl <i>a</i> , primary electron donor of PSII and associated pigments
P700	Reaction centre Chl <i>a</i> , primary electron donor of PSI and associated pigments
P _i	Inorganic phosphate
PQ	Plastoquinine
PSI	Photosystem I, including reaction centre complex and
PSII	Photosystem II, including reaction centre complex and

Q _A	Primary PQ acceptor of PSII
RC	Reaction centre
Rubisco	ribulose 1,5-bisphosphate carboxylase
RuBP	ribulose 1,5-bisphosphate
TP	Triose phosphate

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Frontispiece. Transverse section of the secondary xylem, cambium, and phloem tissues of a white spruce [*Picea glauca* (Moench.) Voss] seedling. Magnification is approximately 2600 X in the left frame and 7700 X in the right frame. The image was generated on a Cambridge 250 Scanning Electron Microscope at an accelerating voltage of 20 kV, and captured on Polaroid type 55 positive/negative film.

Chapter 1

INTRODUCTION

The vascular cambium of woody plants is dependent upon the photosynthetic structures and processes occurring within leaf chloroplasts for the supply of energy and carbon required to synthesize the most abundant organic compounds on earth, cellulose and lignin.

The energy and structural carbon requirements of the vascular cambium make it a significant sink for photosynthates, particularly during periods of active wood formation (Berlyn and Battey, 1985). Photosynthetic activity has been shown to respond to secondary sink activity in a variety of plant species, (Geiger, 1976; Borchers-Zampini *et al.*, 1980; Shibles *et al.*, 1987; Lauer and Shibles, 1987; Diethelm and Shibles, 1989). The relationship has been suggested to occur in conifers (e.g. Gordon and Larson, 1968; Berlyn and Battey, 1985), but data to support its existence are scarce.

The primary objective of this study was to explore the hypothesis that photosynthetic activity and development in conifer seedlings are correlated to the rate of cambial activity during seasonal periods of active wood formation. This objective was entertained within the practical context of investigating and developing methodologies for non-invasive assessment of seedling vigour, based on source:sink phenomena. Additional objectives were to determine if stresses, imposed to enhance or modify cambial activity (stem wounding and tilting), would result in a measurable alteration of photosynthetic activity, as determined by CO₂ uptake, chlorophyll *a* fluorescence and development, and

chloroplast membrane protein development. A further objective was to compare measures of photosystem efficiency and development to measures of seedling morphology as indicators and predictors of the current year's stem growth vigour.

The species selected for this study was white spruce [*Picea glauca* (Moench.) Voss].

Gas exchange and fluorescence data have been collected for several years for this species (Toivonen, 1985; Toivonen and Vidaver, 1988; Vidaver *et al.*, 1989), and adding to the database for an existing system was considered preferable to beginning work on another conifer species.

Furthermore, and within the context of resource management, white spruce is important to the forest resource industry in British Columbia. With increased efforts to reforest the extensive not sufficiently restocked (NSR) lands of the Central Interior region of British Columbia, methodologies for effective assessment and selection of nursery stock prior to outplanting, should become increasingly valuable.

1.1 HYPOTHESES

The specific hypotheses investigated in this study were:

1. Photosynthetic activity is measurably correlated to cambial activity of white spruce seedlings during the wood formation season.
2. Measures of photosynthetic activity can be used for non-invasive assessment of current stem vigour in conifer seedlings.
3. Correlations between photosynthetic activity and current season's cambial activity are diminished when wood formation is arrested, i.e. when the vascular cambium ceases to be a significant and active sink for photosynthates (in late summer and fall).

4. Cambial activity, modified by wound (incision) and geotropic (tilting) stresses, shows altered photosynthetic and cambial activity relations and photosynthetic development.

1.2 BACKGROUND AND RATIONALE

1.2.1 SOURCE:SINK RELATIONS IN NONWOODY AND WOODY PLANTS

For the purposes of this study, 'source' refers to the chloroplast, i.e. the organellar site of photosynthetic carbon fixation, and 'sink' generally refers to any living non-photosynthetic portions of the plant. Primary sinks refer specifically to the cytoplasm of the photosynthetic cell, and secondary sinks, refer to all other living portions of the plant, including the vascular cambium. Several attempts to define the cambium and associated tissues have been offered (for reviews, see Schmid, 1976, and Catesson, 1980). For the purposes of this study, cambium refers to the layer of dividing and differentiating cells located between the layers of mature xylem and phloem tissues in woody plants. Strictly speaking, cambial activity ought to refer to the rate of production of both phloem and xylem tissues within the cambium. However this study is concerned primarily with wood formation, and unless otherwise stated, cambial activity will refer to the production of xylem tissue.

The existence of a causal relationship between sources and sinks in nonwoody plants is well established and evidence for regulation of photosynthesis by sink activity is substantial. For example, Shibles *et al.* (1987), Lauer and Shibles (1987), and Diethelm and Shibles (1989) have shown recently that reproductive sinks enhance CO₂ exchange rates and ribulose 1,5-bisphosphate carboxylase (Rubisco) activity in soybean (*Glycine max*). Geiger (1976) demonstrated an alteration in CO₂ exchange rates in response to modified sink demand in bean plants (*Phaseolus vulgaris*). Carbon dioxide exchange

rates responded to sink manipulation in cucumber (*Cucumis sativus*), Borchers-Zampini *et al.* (1980). Tschaplinski (pers. comm.) reported increased photosynthetic rate in leaves of hybrid poplars within two days of reducing the shoot:root ratio. Photosynthetic responses may occur within hours or days of sink manipulation and are, at times, pronounced (Geiger, 1976).

Such responses, however, are not universally observed (Herold, 1980). Little information has been published regarding source:sink relations in conifers, beyond suggestions that such relationships are likely to exist (e.g. Gordon and Larson, 1968).

Three major regulators have been identified by which sink requirements might be communicated to the supply from the source: hormones; accumulation of carbohydrate; and orthophosphate. What follows is a brief overview of research into these mechanisms. (For a more thorough discussion, the reader is referred to Herold, 1980).

For hormones to affect photosynthetic activity directly, the chloroplast envelope must be permeable to them. Several plant hormones have been suggested to affect photosynthesis directly: including abscisic acid (McLaren and Smith, 1977); cytokinins (Incoll and Whitelam, 1977); gibberellins (Gale *et al.*, 1974); and indole acetic acid (IAA) (Turner and Bidwell, 1965), although these latter findings have been challenged (Robinson *et al.* 1978). Most research has involved detached leaves and chloroplast suspensions, and attempts to duplicate findings in intact plants have not always been successful (Hall *et al.*, 1978).

Hormones could affect photosynthetic rates indirectly by promoting sucrose transport thereby changing carbohydrate concentrations within leaves (Powell and Krezdorn, 1977; Herold 1980).

Carbohydrate accumulations in leaves have often been associated with decreases in sink activity (Neales and Incoll, 1968; Herold and Walker, 1979). While the chloroplast membrane is not permeable to sucrose, making this sugar an unlikely candidate as a direct messenger, several mechanisms have been offered to explain the apparent sink inhibition by sucrose accumulation at the source. Increased sucrose levels may lead to higher triose phosphate (TP) levels by a "mass action effect" (Herold, 1980), or feedback inhibition of enzyme activity, for example the inhibition of sucrose phosphate synthetase (Salerno and Pontis, 1978) or sucrose phosphate phosphatase (Hawker, 1967). Such a condition should lead to an elevated 3-phosphoglycerate/inorganic orthophosphate (PGA/Pi) ratio in the cytoplasm which will favour starch synthesis (Preiss and Levi, 1979).

Notwithstanding the mechanisms identified above, direct evidence of photosynthetic inhibition by sucrose accumulation in leaves of intact plants, is lacking. In red beet (*Beta vulgaris*), increased sucrose levels did not appear to affect photosynthetic rate (Austin, 1972). In the egg plant (*Solanum melongena*), Clausen and Biller (1977) could not demonstrate a correlation between sucrose (and starch) accumulations and decreased photosynthetic rates. In tobacco (*Nicotiana tabacum*), CO₂ exchange rates were not inhibited until starch content increased to over 20 times the original concentration (Herold and McNeil, 1979). Starch accumulations may affect CO₂ uptake by distorting thylakoid membranes (Herold, 1980), or by interfering with light penetration (Wildman, 1967).

Under normal *in vivo* conditions with relatively high levels of TP, the role of Pi may be much more significant than that of TP in regulating photosynthesis (Herold, 1980). In chloroplast suspensions (Herold and Walker, 1979) and in leaf discs (Herold and Lewis,

1977), P_i appears to play a major role in the regulation of the photosynthetic rate, as it is consumed by the process and, if not replaced, would result in a lower ATP/ADP ratio (Robinson and Walker, 1979). Lowered P_i would also be expected to have a direct effect on Rubisco activity (Heldt, *et al.* 1978).

In summary the modulation of photosynthetic rates in response to sink requirements has been demonstrated repeatedly, although not universally, but it is not yet well enough understood to allow formulation of a comprehensive regulatory mechanism. Several hormones appear to have direct effects on photosynthetic rates, and less direct effects may involve the alteration of chloroplast membrane permeability to metabolites. Carbohydrate accumulations have been linked to changes in sink activity, but causative mechanisms remain ambiguous. In intact plants, P_i , TP, and PGA, each of which is capable of moving across the chloroplast envelope, all have roles in communicating sink requirements to the chloroplast (Herold, 1980).

1.2.2 FACTORS MODULATING CAMBIAL ACTIVITY

A wide variety of factors have direct and indirect influences on cambial activity and vascular development. Berlyn and Battey (1985) have categorized them as physical, mineral, hormonal, stress, genetic, intrabiotic, interbiotic, and anthropogenic. Savidge (1985) classified influencing factors as genetic (those which originate in meiosis and result in permanent changes in gene expression), epigenetic (those which regulate sequential and differential expressions of genes) or physiological (the physical and chemical factors that alter enzyme activity).

Unfortunately, it is not possible to discriminate fully among the influences of these

various factors, because any single environmental component, for example daylength, will affect cambial activity through a relatively complex array of physiological mechanisms and sequences that are spatially and temporally disparate. As a consequence, the following commentary presents research on cambial activity more on the basis of approach, than on a precise classification of the factors involved.

1.2.2.1 SEASONAL AND CLIMATIC INFLUENCES

The least specific approach to studying cambial activity is by correlating it with changing seasons, without a rigorous attempt to discriminate among the influences of individual factors that are associated with seasonal or climatic changes, such as air and soil temperature, air and soil moisture, daylength, radiative flux, etc. These studies, which generally utilize trees in uncontrolled conditions, do not differentiate between indirect responses which arise from hormonal messages sent from crown or roots to the cambium, and direct responses of the cambium to its external environment (e.g. temperature, extracellular pH, O₂ availability, pressure, etc).

Paliwal and Prasad (1970) found correlations of cambial activity with development and season in *Dalbergia sissoo*, an important commercial tree species in India. Peak activity for this species occurred in May, and was suggested to be initiated by long-day, high temperature, low rainfall and low humidity conditions. A more complete account, linking cambial activity to phenological events, was provided for this species, as well as for *Albizia lebbeck* and *Tectona grandis*, by Venugopal and Krishnamurthy (1987) who examined *Calophyllum inophyllum*, *Mangifera indica*, and *Morinda tinctoria*. Several patterns of cambial activity were found among these species, with all but the teak (*Tectona grandis*) demonstrating two periods of cambial activity annually.

Iqbal and Ghouse (1985) demonstrated the more usual single period of cambial activity in *Prosopis spieigera*, in which xylem production was linked to the break of monsoon in July. Deshpande and Rajendrababu (1985) studied secondary phloem development in *Grewia tiliaefolia*, and demonstrated seasonal variations in vascular tissue production and development associated with phenological events and non-specified, crown-mediated climatic cues. Ghouse and Hashmi (1980, 1983) tracked the cambial activity and seasonal production of vascular xylem and phloem tissue in *Mimusops elengi* for three years, and reported a peak in November, without an attempt to correlate activity with climatic cues.

Atkinson and Denne (1988) described a complex, non-simultaneous reactivation of ash (*Fraxinus excelsior*) vessel production in spring, and correlated development with storage and soluble carbohydrate concentrations, as well as phenological events (budbreak and leaf development).

Correlations between cambial activity and specific climatic components such as temperature, humidity, and rainfall, have been demonstrated by Ghouse and Hashmi (1979) for *Polyanthia longifolia*, Ajmal and Iqbal (1987) for *Streblus asper*, and by Liphschitz *et al.* (1981) for *Cupressus sempervirens*.

1.2.2.2 BIOCHEMICAL GROWTH REGULATORS

It is generally understood that many influences of the above climatic factors' are mediated by the crown, which is where several growth regulators are synthesized and released.

The activity of the vascular cambium is clearly responsive to the growth regulators that

are produced in the crown and move basipetally through the vascular tissues toward the root tips (Larson, 1969; Dann *et al.*, 1985; Savidge, 1983; Savidge and Wareing, 1981). Gill, (1971) reported endogenous control of cambial activity in the tropical tree *Avicennia*, and Zamski (1981) demonstrated that cambial activity was unrelated to leaf development in this species. Evidence also exists for endogenous ethylene production by vascular tissues in several species (Yang and Hoffman 1984) after wounding, and reaction wood formation in the upper halves of tilted seedlings of *Eucalyptus gomphocephala* is linked to higher than normal ethylene levels (Nelson and Hillis, 1978). However, crown-derived auxins appear to be the most important regulators, or at least have received the most attention.

Jacobs' (1952) pioneering research into the role of auxins in controlling the production of vascular tissues showed that indole-3-acetic acid (IAA) could induce xylem regeneration in *Coleus*. Since that time, auxin has become recognized as the major growth regulator in vascular tissues of a variety of species (Aloni 1987), including further work on *Coleus* (Bruck and Paolillo 1984). Correlations between auxin and secondary xylem development have been demonstrated in hardwood species such as *Populus deltoides* (DeGroote and Larson, 1984; Meicenheimer and Larson, 1985), and an experiment with ³H-IAA yielded microautoradiographs showing that this hormone occupies a suitable site for the regulation of xylogenesis in *Fagus sylvatica* (Lachaud and Bonnemain 1981).

In coniferous species, Little and Bonga (1974) established that IAA stimulates cambial activity in *Abies balsamea*. However Sundberg *et al.* (1987) assayed IAA levels during an activity-rest-quiescence transition in *Abies balsamea*, and concluded that differentiation of tracheids, but not in their rate of production, was regulated by IAA.

Wodzicki and Wodzicki (1980) identified abscisic acid as an inhibitor of cambial activity

in *Pinus sylvestris*. Savidge and Wareing (1984) examined seasonal cambial activity in *Pinus contorta* but found little relation between IAA and (*S*)-abscisic acid (ABA) levels within the cambium, and its rate of activity.

Most of the research into the roles of cytokinins in regulating vascular development has involved nonwoody plants, often in *in vitro* culture conditions (Dalessandro, 1973; Dalessandro and Roberts, 1971; Fosket and Torrey, 1969; Minocha and Halperin, 1974; Houck and LaMotte, 1977; Saks *et al.* 1984). Cytokinins are produced in root apices, and appear to be more important in controlling the differentiation of vascular tissues than in regulating their rates of production.

Gibberellic acid (GA) plays a role in controlling cambial activity and promoting phloem development (Wareing *et al.*, 1964; DeMaggio 1966). Zakrzewski (1983) showed that gibberellic acid was important in controlling vascular development in *Quercus rubus*, and Lachaud and Bonnemain (1984) found that GA was important, but less so than auxins, or a combination of auxin and GA in *Fagus silvatica*. Digby and Wareing (1966) found that, in *Populus robusta*, high ratios of IAA to GA₃ favoured xylem formation, while the reverse condition favoured phloem formation. Effects on overall cambial activity were not addressed.

Ethylene production by vascular tissues has been correlated to the accelerated cambial activity associated with reaction wood formation in *Eucalyptus gomphocephala* (Nelson and Hillis, 1978) and *Pinus contorta* (Savidge *et al.* 1983). Ethylene enhanced bark development in *Pinus halepensis* (Yamamoto and Kozłowski 1987) and bark and vessel production in *Ulmus americana* (Yamamoto *et al.* 1987).

The ethylene releasing agent, 2-chloroethylphosphonic acid (CEPA), promoted enhanced

cambial activity in pine (Brown and Leopold, 1973) and apple (Robitaille and Leopold, 1974), and vascular development *in vitro* in *Lactuca sativa*, (Miller and Roberts, 1984), while addition of silver, an ethylene inhibitor, reduced xylem lignification.

There are conflicting reports on the effects of sucrose concentration on the development of vascular tissue (Aloni 1987). Most research has been focussed on *in vitro* cultures of plant tissues. Wetmore and Rier, (1963) found that in *Syringa* cultured under constant auxin concentrations, low sucrose concentrations (1.5 - 2.5%) favoured xylem production, while higher concentrations (3-4%) favoured phloem production. Similar results have been reported for fern prothalli (Wetmore *et al.* 1964).

These results are in contrast to those in *Parthenocissus* (Rier and Beslow, 1967), cultured *Helianthus* (Minocha and Halperin, 1974) and internodes of *Coleus* (Beslow and Rier 1969) in which the production of xylem elements was positively correlated with sucrose concentration.

Wright and Northcote (1972) found that in callus cultures of woody plants (e.g. *Platanus*), the sucrose was necessary only as a carbon source, and that many other sugars are equally effective at promoting vascular development.

1.2.2.3 PHYSICAL AND PHYSIOLOGICAL STRESSES

Most research to date indicates that the majority of tree cambial responses to environmental conditions are triggered biochemically by crown-derived growth regulators, and sugars. However, the cambium appears to be responsive to its immediate physical environment independent of the activity of the crown. Temperature, water, gas concentrations, pH, mechanical stress, gravity, and pressure are the most important

physical factors affecting vascular development (Roberts 1976; Roberts *et al.* 1988).

The majority of research into temperature effects on meristematic activity has involved *in vitro* studies of nonwoody plant tissue. In general, xylogenesis is stimulated at elevated temperatures (30 - 35° C.) in tissue cultures of *Helianthus* (Gautheret 1969), and *Pisum* (Shininger 1979). The mechanism of this response has not been identified, although it may be that either a metabolic pathway leading to xylogenesis has a high temperature optimum (Roberts 1976), or the synthesis of a hormone stimulating xylogenesis is stimulated by these relatively high temperatures (Syono and Furuya, 1971).

Cambial development and xylem production in tissue cultures of *Helianthus tuberosus* were reduced under temperatures of 15° C. (Gautheret, 1969). Xylem formation was arrested in suspension cultures of *Picea glauca* grown in temperature conditions of a "late-spring day" (Durzan *et al.*, 1973). Temperature sensitivity appears less of a factor *in vivo*, as the differentiation of tracheary elements continues at 10° C. Such findings place a cautionary note on the interpretation of *in vitro* studies.

Worrall (1980) has demonstrated that lowering temperatures of the cambium independent from those in the crown in *Pseudotsuga menziesii* leads to reduced cambial activity (reported as narrower growth rings) and the production of xylem with a higher specific gravity. He suggested that temperature changes altered the cambial response to hormonal stimuli.

Pressure affects xylem differentiation both *in situ* and *in vitro* (Brown and Sax, 1962; Brown, 1964; Lintilhac and Vesecky, 1981; Makino *et al.*, 1983). The release of soil pressure in *Pinus* roots resulted in enhanced cambial activity (Fayle 1968) but effects on xylem production in tree stems were not quantified.

Mechanical stresses applied through bending and vibration, enhance cambial activity in a variety of nonwoody and woody species, including *Pinus strobus*, *Pyrus malus*, and *Prunus persica* (Brown and Leopold, 1973). Torque stress stimulated cambial activity more than shaking in *Pinus resinosa* (Quirk *et al.*, 1975). Bending stress resulting from winds increased cambial activity in a variety of woody species (Jacobs, 1954; Larson 1964; Bannan and Bindra, 1970; Hunt and Jaffe, 1980), although the effect was not so pronounced in seedlings (Jaeger 1975). Stem discontinuities appeared to enhance cambial activity in *Picea abies* (Steucek and Kellogg, 1972), however the effects of girdling could not be distinguished from effects of mechanical stresses upon tissues adjacent to the treatment zone.

Direct responses of cambium to changed physical conditions are not easily discerned from the chemical stimuli that sometimes mediate responses. This is very much the case with regard to mechanical stress, for example, ethylene is rapidly synthesized in response to mechanical stimuli (Jaffe 1980), and elicits responses from cambium that are identical to those triggered by mechanical agitation (Jaffe and Biro, 1979).

The modifying effects of gravity on xylem differentiation are well documented (e.g. Wardrop, 1965; Kennedy and Farrar, 1964; Low, 1964; Scurfield, 1973; Timell, 1982; Wilson and Archer, 1977; Wilson, 1981; Yoshizawa *et al.*, 1985), but the mechanisms by which enhanced cambial activity is triggered have not been fully elucidated (Aloni 1987).

As a consequence of their weight, tilted trees must undergo mechanical stress, which has been discussed above as a modifier of cambial activity. In addition, a direct response by the cambium to the gravitational field is well supported. The initial perception of a change in normal gravitational orientation may be by the statoliths, which respond to

tilting within minutes (Steward, 1964; Westing 1968).

The enhanced cambial activity related to reaction wood formation has been associated with varying distributions of auxin (Kennedy and Farrar, 1964), and enhanced production of ethylene (Nelson and Hillis, 1978), but gibberellins and cytokinins do not appear to be involved (Pharis *et al.*, 1972; Wilson and Archer, 1977). The literature on biochemical control of the geotropic response is substantial. The reader is referred to papers by Wilson and Archer (1977) and Kubler (1987) for reviews of mechanisms and impacts on wood properties, and by Iwabuchi, *et al.* (1989) for more recent advances in research on geotropism.

The influence of extracellular pH on cell production rates in plants has not been well studied (Roberts 1983), and the few reports are for nonwoody species in tissue cultures. Shiraishi (cited in Roberts, 1983) reported that, as the pH in cultures of lettuce pith rose from 5.1 to 6.8, the numbers of tracheary elements produced decreased, but recovered after the tissue was transferred to a medium buffered to the original pH. However, changes in nutritional factors were not taken into account. In cultured explants of *Plumeria*, initial pH of the medium affected xylem production (Datta *et al.*, 1975).

Light has obvious indirect effects on cambial activity through the crown, which is very sensitive to daylength and light intensity, but there is evidence that the cambium will respond directly to light quality. Cambial activity in *Pinus banksiana* and *P. sylvestris* roots covered in clear plastic, red and blue celluloid, and exposed to light, was increased 2.0 to 6.0 times over controls that were covered in black plastic (Fayle 1968). The relationship between the root cambium responsiveness and that of stem cambium is unclear, and the possibility exists that stem cambium can respond to light, even if it is not a normal influence.

The influences of gases other than ethylene on cambial activity have not been well studied. Oxygen has a regulatory effect on differentiation in suspension cultures of carrot (Kessell and Carr 1972) and, below 5%, there is an inhibition of ethylene synthesis in plant tissues (Beyer 1979). In woody plants, the vascular cambium is separated from the atmosphere by the outer bark and secondary phloem, leading Kimmerer and Stringer (1988) to hypothesize that oxygen consumption in the cambial zone, where gas exchange might be impeded by the layer of bark and secondary phloem, may lead to hypoxia sufficient to induce anaerobic fermentation. Ethanol was found in the vascular cambium of all nine softwood and hardwood species tested, and for *Populus deltoides*, an alcohol dehydrogenase activity of 165 micromoles of NADH oxidized per minute per gram fresh weight in May, was measured. Ethanol was present in xylem sap of several species, but a cambial origin was not established.

Very little work has been reported on the effects of ozone, but it has been shown to inhibit tracheary element production in wounded *Coleus* stems, and *in vitro* cultures of *Parthenocissus* (Rier, 1976).

Carbon dioxide is not considered to have a significant role in modulating cambial activity or differentiation (Martin 1980) *in vitro*, and no information of *in vivo* effects is available. Carbon dioxide concentration may however affect cambial activity indirectly by its influence on ethylene production, as reported from peach mesocarp tissue cultures (Bradley and Dahmen, 1971), and sunflower plants (Bassi and Spencer, 1982).

Little information is available on water relations within the vascular cambium in woody plants. Reduced cambial activity in drought conditions was reported by Kennedy and Farrar (1964), although no attempt was made to monitor cambial conditions. The

production of tracheary elements in cultured explants of *Lactuca sativa* pith was positively correlated to the amount of water adhering to the explant (Johnson and Roberts, 1978).

Silk and Wagner (1980) modelled water potential gradients in growing corn root, and predicted maximal growth in the area with the most negative water potential. *In vitro* studies, primarily addressing the effects of lower osmotic potentials on xylogenesis, provide the majority of information on the effects of water relations on cell production rates.

In *Fraxinus*, lowered osmotic potentials stimulated xylogenesis in the wound callus, but again, these physical effects were not resolved from biochemical ones. For example, reactivity to IAA also changed with water potential, and several studies have reported altered ethylene production under abnormal water conditions (Lieberman, 1979; Adams and Yang, 1981), which would be expected to affect further development.

However, in his review of the biophysics of cell growth, Cosgrove (1986) concludes, that despite a lack of understanding of the mechanisms controlling cell osmotic potential, high internal osmotic pressure is essential for growth.

The modulation of cambial activity within wounded tree stems, is a complex and inadequately studied phenomenon, involving at once a variety of responses to physical and biochemical factors. For example, an abrasion exposing the vascular cambium and removing xylem tissue in a tree, would bring into play the physical factors of gas, light, water, pressure, mechanical stress, and temperature relations, and simultaneously evoke physiological "wounding" responses, if these can be considered disparate. As noted

above, several of these physical factors have been associated with cambial tissue responsiveness to hormone concentrations and to ethylene production by vascular tissues. For this reason, "wounding," within the context of this study, should not be considered a discrete factor involving altered cambial activity, but rather, the cumulative measurable result of a variety of connected and disconnected physical and physiological factors.

The vascular cambium is responsive to direct intrusions, (Wilson, 1967; Rademacher *et al.*, 1984; Smith, 1980; Sharon, 1973; Lowerts, 1986; Makino *et al.*, 1983; Bauch *et al.*, 1980; Phelps *et al.*, 1975; Phelps and McGinnes, 1977; Fisher, 1981; Lo, 1988) as well as indirect intrusions occurring either centrifugally to bark and phloem tissue (Soe, 1959; Mullick 1977), or centripetally to xylem tissue (Shigo and Dudzik 1985). Direct intrusions generally result in an initial reduction in cambial activity (Savidge and Farrar, 1984) followed by enhanced cambial activity.

Shigo and Dudzik (1985) and Tippet and Shigo (1981) demonstrated enhanced cambial activity in angiosperms and conifers, after xylem injury centripetal to uninjured cambium, and the former suggested that the regulation of cambial activity may be imposed by the entire symplast.

Extensive intrusions to bark often result in enhanced cambial activity, apparently as a consequence of the release of growth regulators, e.g. ethylene, from injured cells (Mullick 1977). However, a bark injury, depending on its severity, results in a number of cambial activity modifying factors being brought into play, including possible alterations in water relations, gas concentrations, light intensity, and pressure. As a result, it is not yet possible to describe a single, discrete mechanism for enhanced cambial activity associated with wounding of bark or secondary phloem.

1.2.3 MEASURES OF PHOTOSYNTHETIC ACTIVITY

1.2.3.1 CO₂ ASSIMILATION

The measurement of CO₂ gas exchange by foliage remains a commonly used approach for photosynthetic studies of vascular plants. Individual leaves, groups of leaves, or an entire plant may be enclosed in an airtight chamber, with atmospheric air pumped into the chamber at a metered rate. Carbon dioxide levels of the air are measured by infrared gas analyzers placed at the entrance and exit of the chamber. Subtraction of the latter value from the former is the net uptake associated with the "dark reactions" of photosynthesis. Net uptake is a consequence of the gross assimilation necessary for carbohydrate synthesis in the dark reactions or Calvin cycle (Bassham, 1965), less respiratory losses from the foliage and whatever tissues are enclosed in the chamber. As a consequence, these values represent relative rather than absolute measures of gas uptake.

Carbon dioxide exchange rates have been shown to increase in response to enhanced sink demands in soybean leaves (*Glycine max*) (Lauer and Shibles, 1987; Diethelm and Shibles, 1989).

1.2.3.2 VARIABLE CHLOROPHYLL *a* FLUORESCENCE

Chlorophyll *a* fluorescence occurs as a consequence of the absorption and re-emission of photons by the antenna pigments within the chloroplast's thylakoid membrane. Variable fluorescence (Fvar) is modulated primarily as a consequence of the redox state of Q_A, but is also affected by pH, ionic strength, temperature, and the distribution of chlorophyll-protein complexes (Briantais *et al.*, 1986). The occurrence of photochemical

events also impinge on F_{var} yields and, as a result, the measuring of chlorophyll a fluorescence is becoming increasingly widespread as a non-invasive means to assess photosynthetic activity of higher plants (Renger and Shreiber, 1986), including conifer seedlings (e.g. Toivonen and Vidaver, 1988). In particular, the fluorescence induction kinetics of the dark-light transition, or "Kautsky effect," have proved useful in assessing the *in vivo* photosynthetic performance of plants.

Stokes (1864) made the first significant study of the fluorescence of photosynthetic pigments, demonstrating that the fluorescence spectrum of a pigment is independent of the wavelength of excitation. Using crude filters and the human retina, Muller (1874) studied fluorescence and speculated that emission yields were correlated with photosynthetic rates. Kautsky and Hirsch (1931) described a correlation between CO_2 fixation and chlorophyll a fluorescence, again using the human retina as a detector.

Kautsky was the first to report that, by directing blue light at foliage and placing a blue filter in front of a photodetector directed at the foliage, the emission of red photons could be quantitatively detected independent of incident or reflected light. He offered a model for photosynthetic electron transport with two photoreactions (Kautsky *et al.* 1960). Subsequently, Duysens and Sweers (1963) showed that two pigment systems with differing absorbances are driven by two light reactions, and made the key discovery that variable fluorescence (F_{var}) emission reflects the redox changes of the "quencher" (Q_A) in Photosystem II.

The development of more refined detection apparatus and analytical techniques has facilitated a growing list of applications of fluorescence techniques to physiological research, particularly in assessing photosynthetic activity of plants in natural and experimental conditions (Renger and Shreiber, 1986). Fluorescence is now considered a

"universal probe" of photosynthetic events (Lavorel *et al.*, 1986), being particularly useful in tracking the light reactions.

Before discussing variable fluorescence (F_{var}) theory, I will review briefly the models of pigment excitation and electron transfer upon which interpretations of F_{var} are based. (For full details see Parson and Ke, 1982).

Pigment excitation occurs within 10^{-15} to 10^{-6} seconds of the thylakoid being struck by light within the absorptive range of the pigments. Upon absorbing photons, chlorophyll is projected from the ground state to the excited singlet or triplet state at which time one electron in the outer shell is raised to the higher energy level. Once in the excited state, the electron is available for a photochemical reaction, and an oxidized chlorophyll molecule results. Alternatively, the chlorophyll may return to its ground state by emitting fluorescence, phosphorescence, infrared radiation, or by resonance transfer.

If a photochemical reaction occurs, the results are ATP formation and NADP reduction, under which circumstances energy emission via fluorescence is somewhat quenched. The photochemical reactions occur in reaction centres (RC) which are contained in groups of pigments termed photosystems. Light energy impinging on the surrounding (accessory) pigments is quickly ($\sim 10^{-9}$ seconds) transferred by resonance to the RC long-wavelength trap.

The transport of electrons from lysed water to the terminal acceptor, NADP, has been described as the "Z-scheme" in the most recent reviews (e.g. Codgell (1983). Briefly, in the light reactions, photosystem II (PSII) captures light energy that is used to transfer electrons derived from water-splitting from chlorophyll a to Q, the primary electron acceptor. The electrons then flow through several more acceptors, including

plastoquinone (PQ) and plastocyanin (PC), to Photosystem I (PSI). ATP production (photophosphorylation) is coupled to this non-cyclic flow by a mechanism similar to the chemiosmotic process hypothesized by Mitchell (1966) for mitochondria. In this process, chloroplasts accumulate protons within the thylakoid space, producing a pH gradient and thus a voltage gradient across the thylakoid membrane. The energy potential of the gradient is used to produce the ATP required for carbohydrate biosynthesis in the dark reactions of the Calvin cycle.

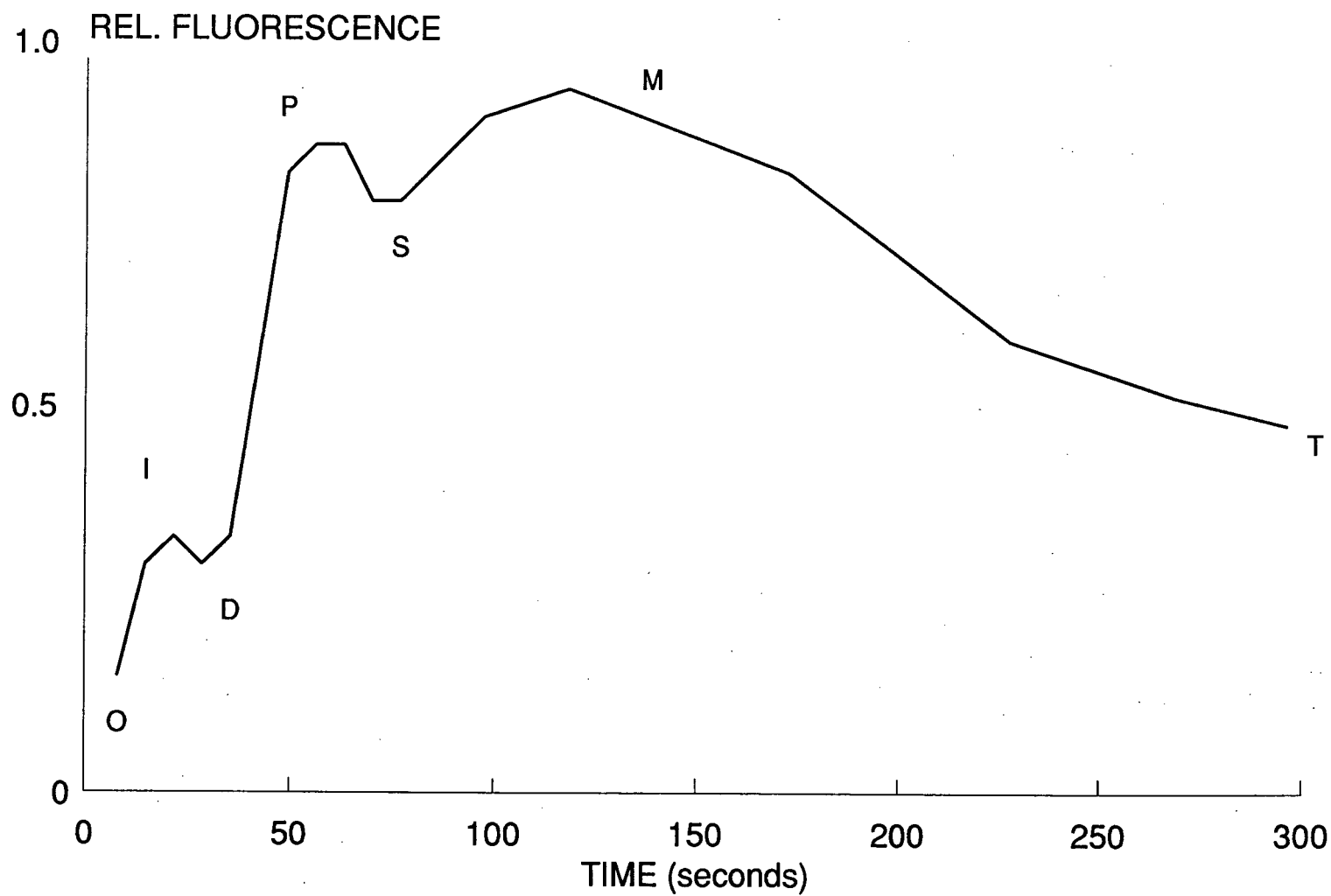
Fluorescence induction curves are useful because they reflect dynamic changes in the photosynthetic apparatus, as it becomes active in response to a transition from dark to light conditions.

Fig. 1.1 represents a typical variable fluorescence induction curve, with O,I,D,P,S,M,T levels and transients identified. Currently accepted interpretations of these transients, adapted from Govindjee and Satoh, (1986), are as follows:

1. O-level. The initial photochemically independent fluorescence emitted from PSII (with all photochemical traps open), indicating the total number of excited chlorophyll *a* (Toivonen and Vidaver, 1988). The O-level is determined within the first several ms. of the shutter opening after a dark preincubation, indicating a fully oxidized Q_A (the first quinone receptor).
2. O-I rise. The rise from the O-level to I (inflection or intermediate peak) reflects partial photoreduction of Q_A by PSII (Munday and Govindjee, 1969).
3. I-D plateau/decline. Reduction of Q_A is counteracted by oxidation by PSI (Satoh and Katoh, 1981). The event is

delayed because of the plastoquinone pool (PQ) situated between PSI and Q.

4. D-P rise. All the electron acceptors in the electron transport chain up to X are reduced. NADPH accumulates and reduced PQ results in reduction of Q due to low rate of carbon assimilation. The rate is dependent upon water splitting in PSII.



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Figure 1.1. Chlorophyll a fluorescence as a function of time of illumination. See text for symbol definitions.

5. P-S decline. The Calvin cycle begins, and re-oxidation of the chain accelerates. The pH potential builds across the thylakoid membranes, as fluorescence declines to S. (This phenomenon is complex, and has been reviewed by Govindjee and Satoh, 1986).

6. S-M-T oscillation and decay. Before reaching a steady state (T), the systems may oscillate due to the reactions "overshooting." In pea and spinach chloroplast culture systems, the time taken to reach the M peak within the induction curve, has been attributed to the time taken to reduce the NADP pool (Horton, 1983). The M-T decline has been linked to ATP production in *Chlorella* (Govindjee and Satoh, 1986). More specifically, ATP has been demonstrated to have a quenching effect on Fvar yields (Black and Horton, 1984), most likely as a consequence of phosphorylation of the LHCP.

1.2.4 MEASURES OF PHOTOSYNTHETIC DEVELOPMENT

1.2.4.1 O-LEVEL AND ESTIMATED CHLOROPHYLL

The O-level fluorescence is measured from PSII before photochemical events begin. Also referred to as "constant" (as opposed to variable) fluorescence, the O-level is measurable only when Q_A is fully oxidized, i.e. following dark preincubation. O-level fluorescence has been used to estimate chlorophyll content (Toivonen, 1985) and therefore photosynthetic development. It may also be used to normalize data to allow comparison of samples of different sizes. (All fluorescence amplitude parameters in this study have been corrected to O-level.)

1.2.4.2 RUBISCO AND THYLAKOID MEMBRANE PROTEIN COMPOSITION

Carbon dioxide fixation involves the reaction of atmospheric CO₂, with ribulose 1,5-bisphosphate (RuBP) to form 3-phosphoglyceric acid (3-PGA). This reaction is catalyzed by ribulose bisphosphate carboxylase (Rubisco), reportedly the most abundant protein on earth (Miziorko and Lorimer, 1983), and commonly making up one-quarter to one-half of the protein content of chloroplasts. The Rubisco content of soybean plants has recently been linked to sink demands (Diethelm and Shibles, 1989).

Less abundant, but equally essential components of the chloroplast protein complement are the coupling factor (CF), and the extrinsic polypeptides.

The photophosphorylation that occurs in chloroplasts requires the movement of protons down an electrochemical gradient from the lumen of the thylakoid to the stroma. This is accommodated by the coupling factor complex. This complex is comprised of two parts: the extrinsic protein CF₁, and the intrinsic protein CF₀. Together these two components span the thylakoid membrane from lumen to stroma, and function to catalyze ATP formation.

If induced stresses or alterations in sink activity do indeed affect photochemistry, then the relative proportion of these critical components, i.e. Rubisco and CF, might determine the ability to accommodate altered activity.

The following chapter describes the stock origin, treatments, and measurement techniques used to investigate photosynthetic and cambial activity relations in the current study.

Chapter 2

MATERIALS AND METHODS

2.1 STOCK ORIGIN AND CULTURE CONDITIONS

Thirty-six white spruce seedlings (seedlot #60526-8981, origin 55°N, 124°W), in their third growing season were obtained from the British Columbia Ministry of Forests Surrey Nursery, 3605-196th Street, Surrey B.C. Each seedling was removed from its styroblock (PSB-313) bedding container in mid summer (June 15, 1988) and planted in a two litre pot containing a 3:1 mixture (by volume) of commercial potting soil and sand. The seedlings were kept in an unheated greenhouse on the south campus of the University of British Columbia (49°N, 121°W) for twenty-eight days before treatment.

The period between potting and treatment was to allow time for recovery from possible transplant shock, and to allow budset and shoot elongation to complete, thereby leaving the vascular cambium as the primary meristematic sink for photosynthates. With respect to transplant shock, white spruce stock of this age and origin, and transplanted under conditions described, normally recovers root and caliper development within one week, and virtually always within two weeks (pers. comm. G. Lister, Biological Science Dept., Simon Fraser University, Burnaby, Canada; and N. Pelton, Pelton Nurseries, Maple Ridge, Canada). The four week period between transplanting and the onset of treatments was considered adequate to exceed the duration in which abnormal cambial and photosynthetic activity due to transplanting, would be a significant factor.

2.2 TREATMENTS

To investigate the effects that biophysical stresses might have on the source:sink relationships, two treatments were chosen. The first treatment was a vertically extensive wound to the cambial tissues of the stem. Such a wound would be expected to impinge on source:sink relations in several ways. Stem injuries may reduce or enhance cambial activity (Savidge, 1985; Steucek and Kellogg, 1972; Rademacher *et al.*, 1984) in woody plants. Reduced cambial activity might be expected to reduce the demands from the source of photosynthetic products, while enhanced activity would be expected to increase demands. Furthermore, wound-related metabolism and the biosynthesis of anti-pathogenic products such as phenolics, might also require higher than normal carbohydrate translocation. Finally, a stem incision would impinge directly on the translocatory system for crown-produced carbohydrates, i.e. the phloem, possibly resulting in "leaks" of photosynthates to the external environment.

The second treatment was that of tilting. Tilting induces a geotropic response in conifer seedlings, resulting in enhanced cambial activity (Kennedy and Farrar, 1964) and an alteration in secondary xylem cell wall biochemistry and architecture, i.e. the formation of reaction wood (Wilson and Archer, 1977; Kubler, 1987). Either or both of these effects might be expected to impinge on photosynthetic activity as a consequence of changing carbon demands relating to altered or enhanced biosynthetic activity.

The treatment procedure was as follows. The seedlings were sorted randomly into treatment groups (1, 2 and 3) of twelve and individually labelled. After 28 days (on July 12, 1988), when shoot elongation and budset were completed, groups 2 and 3 were, respectively, incised and tilted. The incision was made by drawing a razor blade from the acropetal terminus of the second year stem segment, basipetally to 1 cm above the

soil on the first year stem segment. Tilting was accomplished by placing the pots on their sides at ninety degrees to normal. The plants in group 1 were not treated.

The seedlings were transported to the laboratory eleven days after treatment (July 23, 1988), where CO₂ assimilation and fluorescence amplitudes were measured.

Measurements were repeated in late August and October.

2.3 MEASUREMENT TECHNIQUES

2.3.1 FLUORESCENCE INDUCTION

For measures of variable chlorophyll *a* fluorescence (Fvar), each seedling was allowed a ten minute dark adaptation period, and then transferred to an integrating fluorometer (Toivonen and Vidaver 1984). Variable chlorophyll *a* fluorescence was measured and recorded 1029 times for each seedling over a 300 second time course. The primary fluorescence data (Fv) were normalized to the instantaneous value of Fo which is a measure of the non-photochemical component of fluorescence (Papageorgiou, 1975). The relative value of Fvar was determined by:

$$Fvar = \frac{Fv - Fo}{Fo}$$

where: Fvar is normalized variable fluorescence at time t,

Fv is non-normalized fluorescence at time t, and

Fo is O-level fluorescence

The resulting Fvar data were stored for subsequent induction curve plotting and statistical analyses. Mean average Fvar values were calculated using an algorithm written in the awk language of UNIX (Appendix A).

2.3.2 CARBON DIOXIDE UPTAKE AND CHLOROPHYLL CONTENT

Carbon dioxide fixation measurements were obtained for each seedling using a previously described open gas exchange setup (Toivonen 1985). A 10 cm section of each leader was sealed in a 635 ml transparent gas exchange cuvette, illuminated by light emitted by a CGE projector lamp (Quartz iodide, 650 watts, 125 volts) delivered through a water bath heat filter. The intensity of radiation received by the foliage was maintained at approximately $440 \mu\text{moles quanta m}^{-2} \text{s}^{-1}$ (PAR) by adjusting the distance between the light source and the airtight chamber. Light measurements were made using a LiCor LI-185A light meter and quantum flux detector head (LiCor Inc., Lincoln, Nebraska). Air flow rate through the chamber was maintained at 6.25 ml s^{-1} . Carbon dioxide levels were determined using ADC-225-MK3 LCA2 portable infrared gas analyzers (The Analytical Development Co. Ltd., Hertfordshire, England).

Two CO_2 exchange measurements were determined. The initial measurement was of uptake uncorrected to chlorophyll *a* content, i.e. the uptake by the (10 cm) leader portion that was sealed into the gas exchange chamber. This "uncorrected" uptake measurement represents net exchange on a per unit leader length basis. A second CO_2 uptake measure was determined by taking the uncorrected uptake measure described above, and normalizing it to an estimate of chlorophyll *a* content of the material at the time of the measurement (as chlorophyll *a* development continued through the summer). This "corrected" CO_2 uptake measure represents CO_2 exchange normalized on a per mg chlorophyll *a* basis.

As well as being used for normalizing CO_2 or fluorescence data to sample size, chlorophyll *a* content was used as a measure of photosynthetic development in this study.

Chlorophyll *a* content of each measured leader section was estimated from O-level fluorescence¹ using a method described by Toivonen (1985) for white spruce seedlings using the formula:

$$\text{Total chlorophyll (mg)} = \frac{(\text{O-level}) - 0.48}{36.66}$$

2.3.3 SEEDLING DIMENSIONS AND CAMBIAL ACTIVITY

In early winter the seedlings were removed from their pots and the soil was washed from the root systems with cold tap water. Preseason stem lengths and current season leader lengths were measured for each seedling.

Segments (3 cm long) were removed from each sapling's stem, fixed in 50% ethanol:formalin:glacial acetic acid (90:6:4) (FAA), and the cross-sectioned surfaces prepared using an American Optical Model 860 microtome. Cambial activity was determined from measurements obtained using a KONTRON-SEM Image Processing System (Kontron Bildanalyse GmbH, Breslauer Str 2, D8087 Eching/Munich).

Cross-sectional areas of the 1986 and 1987 stem segments, cambial circumferences, and cambial activities (ratio of the cross-sectional area of the current season's xylem production to the preseason cambial circumference), were determined from cross sections using images generated with a MTI Series 68 video camera mounted to a Zeiss dissecting

1. As O-level represents an output voltage from the detector, and detectors may be calibrated differently, this formula pertains specifically to the instrumentation used in this laboratory, and described by Toivonen and Vidaver (1984).

microscope. These images were then transferred to the Kontron image analyzer for capture and digitization.

A subroutine (Appendix B) developed specifically for calculating incremental areas and cambial circumferences was then initiated. This subroutine required the use of a "mouse" to trace over the latewood/earlywood demarkations and the current cambial circumferences, on the digitized image. The KONTRON microprocessor then calculated cross-sectional areas, and preseason cambial circumferences for the last season's increment. Cambial activities were calculated for the last two years by dividing each season's cross-sectional area by the cambial circumference at the beginning of the growing season.

2.3.4 SDS-POLYACRYLAMIDE GEL ELECTROPHORESIS OF CHLOROPLAST PROTEINS

Immediately following removal of the seedlings from their pots, the needles were stripped from the region of each leader used for CO₂ and fluorescence studies, grouped according to treatment, and then frozen at -70°C. Electrophoretic analyses were performed in the laboratory of Dr. Edith Camm, Botany Dept., University of British Columbia.

Chloroplasts were prepared by the method of White and Green (1987). In this method, conifer leaves are ground in liquid nitrogen and then extracted in buffer containing polyethylene glycol 6000, Polyclar AT and mercaptoethanol to reduce oxidation of proteins by phenolics. Chloroplasts were separated from other cell components by centrifugation on a single step sucrose gradient containing the above reagents and washed in 2mM tris-maleate buffer pH 8.0 before solubilization in a mixture of 0.88% (w/v)

octyl glucoside, 0.22 (w/v) sodium dodecyl sulfate, 10% (v/v) glycerol at a final chlorophyll concentration of 440 $\mu\text{g/ml}$. Aliquots of 25 μg were loaded on a 10% polyacrylamide gel for electrophoresis of chloroplast protein complexes. Chlorophyll was determined in 80% acetone by the method of Lichenthaler and Wellburn (1983).

Electrophoresis of denatured samples was performed after solubilization and denaturation, in the (a) system described in Camm and Green (1989).

The Rubisco large subunit (LSU) and Coupling Factor α and β subunits were identified by immunoblotting methods described by White and Green (1987). Antibodies to the Rubisco LSU and Coupling Factor α and β subunits were prepared in the laboratory of Dr. T. Crawford, University of British Columbia.

2.3.5 STATISTICAL PROCEDURES

The data were tested for normality by a Kolmogorov-Smirnov Lilliefors probability test, as well as by normality plotting. With respect to between-group variances, i.e. the null hypothesis that the gas exchange measures, fluorescence parameters, chlorophyll contents and cambial activities of the three groups had equal means, was tested using a Model I one-way analysis of variance (ANOVA) where data were normal. The Model I ANOVA is considered a relatively robust test, even where a considerable heterogeneity of variance exists, so long as values for n_i are close to being equal (Zar, 1984). Bartlett's test was used to test for homogeneity of variance.

Where data were not normal, a Kruskal-Wallis non-parametric test was used. In this case the data were ranked, and an approximation to a chi-square distribution was substituted for an F -ratio.

The Tukey HSD test was applied where the null hypothesis has been rejected, as a post-hoc multiple comparison test. The Tukey test was chosen because of its effective application in Model I applications (Zar, 1984), and its capacity for handling unequal n 's.

Regression analyses have been used to identify relationships between fluorescence parameters and spruce seedling dimensions (Ronco, 1969) and between fluorescence parameters and CO₂ uptake in spruce seedlings (Toivonen and Vidaver, 1984; Toivonen, 1985; Toivonen and Vidaver, 1988). The occurrence of correlations and functionally dependent relationships between stem vigour and various photosynthetic activity and stem dimension parameters in this study were investigated using parametric correlation and regression analyses as described by Wilkinson (1988). Where data were not normal, a Spearman Rank correlation test was performed, and the resulting coefficient was used to replace R .

Unless otherwise indicated, all analyses were performed using the statistical software routines of The System of Statistics (SYSTAT Inc., Evanston, IL.).

Chapter 3

RESULTS AND DISCUSSION

In sections 3.1 to 3.3, fluorescence, CO₂ uptake, and chlorophyll development parameters are presented with means and ranges, to characterize photosynthetic activities and development in untreated and treated white spruce seedlings during mid-summer, late-summer, and autumn respectively. These data are summarized and contrasted in section 3.4 and, together with the cambial activity and seedling dimension data provided in section 3.5, provide the basis for a series of regression and correlation analyses presented in section 3.6. These analyses are aimed at identifying statistical evidence for functional relationships between stemwood formation rates, and photosynthetic activity and development during and following the wood formation season, as well as testing the reliability of seedling measures as indicators of current year's stem vigour.

Section 3.7 presents the results of SDS-polyacralamide gel electrophoresis of selected chloroplast membrane proteins in treated and untreated seedlings, following destructive sampling in winter¹.

3.1 FLUORESCENCE, CO₂ UPTAKE, AND ESTIMATED CHLOROPHYLL DEVELOPMENT IN MID-SUMMER.

Table 3.1.1 presents fluorescence parameters for the untreated white spruce seedlings in mid-summer. At this period, the M peaks in the untreated group occurred between 18.3

1. Readers who are particularly concerned with the practical results of this study, are directed to section 3.6.1 wherein specific fluorescence parameters are demonstrated to have a strong relationship with cambial activity in untreated plants, but not in stressed ones. Sections 3.6.2 and 3.6.3 reveal a breakdown in photosynthetic and cambial activity relations during periods when wood formation is arrested.

and 57.0 seconds in the induction time course (Time to M), with a mean average of 31.0 ± 12.4 seconds. The M fluorescence (FvM) amplitudes ranged from 0.61 to 1.11, with a mean average of 0.85 ± 0.16 .

Mean average Fvar values (FvAVG) for the untreated group during the 300 second time course ranged from 0.32 to 0.93, with a mean of 0.57 ± 0.18 . Final Fvar values (FvT) ranged from 0.15 to 0.74, with a mean of 0.40 ± 0.18 .

Table 3.1.1: Fluorescence values for the untreated group in mid-summer.

SEEDLING	O-level	Time to M (sec)	FvM	FvT	FvAVG	Fv120
A	6.31	30.9	0.67	0.15	0.37	0.40
B	4.32	52.8	1.11	0.74	0.93	1.02
C	4.36	57.0	0.90	0.57	0.73	0.78
D	5.40	27.6	0.77	0.28	0.44	0.45
E	4.56	24.0	0.81	0.47	0.65	0.71
F	5.86	24.6	0.95	0.60	0.74	0.77
G	4.48	38.1	0.61	0.21	0.36	0.36
H	4.31	18.3	0.64	0.20	0.32	0.31
I	4.43	31.2	1.07	0.53	0.72	0.75
J	5.28	21.0	0.97	0.38	0.60	0.62
K	5.37	23.1	0.90	0.38	0.53	0.51
L	5.86	23.4	0.87	0.28	0.47	0.49
CASES = 12						
Minimum	3.86	18.3	0.61	0.15	0.32	0.31
Maximum	6.31	57.0	1.11	0.74	0.93	1.02
Mean	5.04	31.0	0.85	0.40	0.57	0.60
SD	0.74	12.4	0.16	0.18	0.18	0.21

Figures 3.1.1 and 3.1.2 contrast Fvar induction curves of the two seedlings with the highest cambial activity (see Section 3.5 for data), with those of the lowest. The implications of the apparent differences in Fvar parameters in high and low stem vigour seedlings in mid-summer are addressed in Section 3.6.

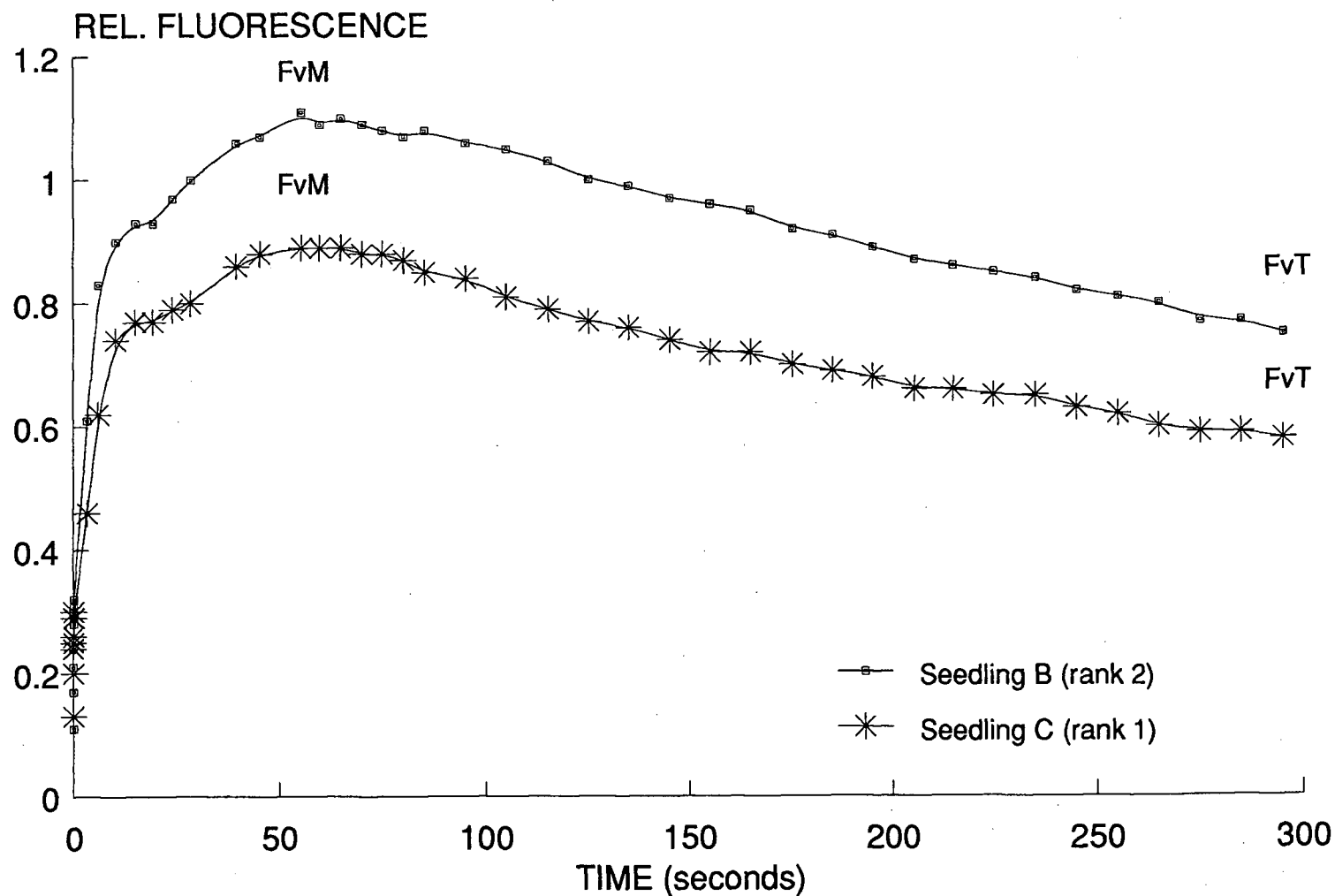
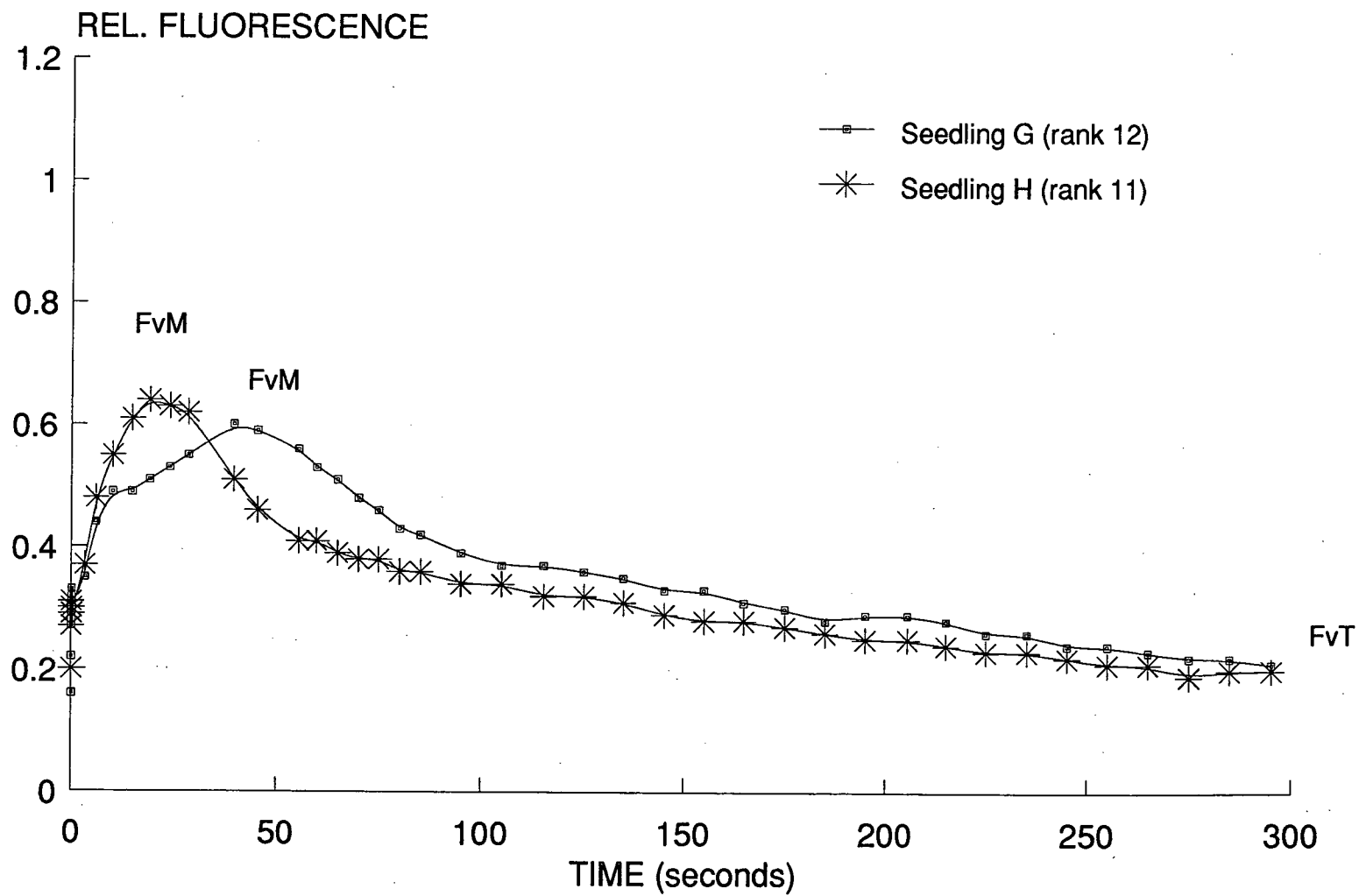


Fig. 3.1.1 Fv plots for the two highest vigour, untreated seedlings, in mid-summer (ranked).

Falls/89



Falls/89

Fig: 3.1.2 Fv plots for the two lowest vigour, untreated seedlings, in mid-summer (ranked).

O-levels were used to estimate total chlorophyll, as a measure of photosynthetic development. Total chlorophyll values were also used to correct CO₂ uptake to the amount of chlorophyll present in the sampled leader section (Table 3.1.2).

Uncorrected CO₂ uptake for the untreated seedlings in mid-summer (Table 3.1.2) ranged from -3.19 to 18.17 µg/min, with a mean average uptake of 8.70±5.92 µg/min. Estimated chlorophyll content ranged from 0.092 to 0.159 mg, with a mean average of 8.70±0.020. Carbon dioxide uptake corrected to estimated chlorophyll content ranged from -30.51 to +129.16 µg/mgChl/min, with a mean average of 72.25±47.78 µg/mgChl/min. The negative uptake of the "H" seedling may have been due to a leaking seal surrounding the stem in the gas measurement chamber. This seedling demonstrated relatively normal uptake rates in the subsequent measurement period (August).

Table 3.1.2: Uncorrected and corrected CO₂ uptake, and estimated chlorophyll development for the untreated group in mid-summer.

SEEDLING	Uncorrected Uptake (µg/min)	Est. Chlorophyll (mg)	Corrected Uptake (µg/mg/min)
A	18.17	0.159	114.09
B	6.13	0.105	58.38
C	12.76	0.106	120.66
D	1.96	0.134	14.63
E	9.45	0.112	84.77
F	14.11	0.147	96.11
G	14.11	0.109	129.16
H	-3.19	0.105	-30.51
I	8.10	0.108	75.14
J	9.57	0.131	73.10
K	3.68	0.134	27.58
L	9.57	0.092	103.70
CASES=12			
Minimum	-3.19	0.092	-30.51
Maximum	18.17	0.159	129.16
Mean	8.70	0.120	72.25
SD	5.92	0.020	47.78

FvAVG for the incised group (Table 3.1.3) ranged from 0.22 to 0.86, with a mean of 0.58 ± 0.19 . FvT ranged from 0.10 to 0.66, with a mean of 0.40 ± 0.18 . The Time to M was between 15.3 and 45.9 seconds, with a mean of 30.1 ± 8.75 seconds. FvM ranged from 0.66 to 1.19, with a mean of 0.85 ± 0.16 .

Table 3.1.3: Fluorescence values for the incised group in mid-summer.

SEEDLING	O-level	Time to M (sec)	FvM	FvT	FvAVG
A	5.60	41.7	0.85	0.30	0.55
B	5.40	41.1	1.06	0.57	0.81
C	5.30	26.4	0.77	0.45	0.58
D	4.70	28.2	0.68	0.26	0.40
E	4.20	28.5	0.81	0.48	0.63
F	4.90	45.9	0.79	0.36	0.54
G	4.40	34.2	0.96	0.64	0.79
H	4.00	15.3	0.94	0.53	0.71
I	5.90	23.1	0.66	0.10	0.22
J	4.80	32.4	0.70	0.14	0.34
K	4.30	30.6	0.74	0.37	0.53
L	4.60	24.0	1.19	0.66	0.86
CASES = 12					
Minimum	4.00	15.3	0.66	0.10	0.22
Maximum	5.90	45.9	1.19	0.66	0.86
Mean	4.84	30.9	0.85	0.40	0.58
SD	0.59	8.7	0.16	0.18	0.19

Uncorrected CO₂ uptake for the incised seedlings (Table 3.1.4) ranged from 2.08 to 26.02 µg/min, with a mean average uptake of 12.49±7.03 µg/min. Estimated chlorophyll content ranged from 0.096 to 0.148 mg, with a mean average of 0.119±0.160 mg. Carbon dioxide uptake corrected to estimated chlorophyll content ranged from 17.71 to 193.93 µg/mgChl/min, with a mean average of 103.64±53.13 µg/mgChl/min.

Table 3.1.4: Uncorrected and corrected CO₂ uptake, and estimated chlorophyll development for the incised group in mid-summer.

SEEDLING	Uncorrected Uptake (µg/min)	Est. Chlorophyll (mg)	Corrected Uptake (µg/mg/min)
A	18.17	0.140	130.09
B	26.02	0.134	193.93
C	20.37	0.131	155.00
D	5.77	0.115	50.12
E	11.17	0.101	110.96
F	13.75	0.121	114.04
G	17.80	0.107	166.47
H	10.06	0.096	104.84
I	9.94	0.148	67.26
J	2.08	0.118	17.71
K	4.42	0.104	42.41
L	10.31	0.112	91.76
CASES = 12			
Minimum	2.08	0.096	17.71
Maximum	26.02	0.148	193.93
Mean	12.49	0.119	103.64
SD	7.03	0.160	53.13

Contrasting corrected CO₂ uptake of incised seedlings with that of controls, the former group exhibited both a higher maximum and mean uptake. However, the mean of the untreated group was depressed by the occurrence of a negative value for one seedling (H).

FvAVG for the tilted group (Table 3.1.5) during the 300 second time course ranged from 0.16 to 0.78, with a mean of 0.58 ± 0.18). FvT ranged from 0.07 to 0.58, with a mean of 0.40 ± 0.15).

Table 3.1.5: Fluorescence values for the tilted group in mid-summer.

SEEDLING	O-level	Time to M (sec)	FvM	FvT	FvAVG
A	5.20	33.6	0.80	0.26	0.53
B ¹					
C	6.50	54.6	0.80	0.51	0.66
D	7.50	29.1	0.36	0.07	0.16
E	4.70	33.3	0.63	0.41	0.52
F	4.90	31.2	0.87	0.54	0.68
G	4.10	63.0	0.71	0.31	0.46
H	3.60	47.7	1.04	0.53	0.77
I	4.43	31.2	1.07	0.53	0.72
J	5.28	21.0	0.97	0.38	0.60
K	5.40	17.7	0.79	0.32	0.47
L	5.20	27.9	1.08	0.50	0.72
CASES = 11					
Minimum	3.60	17.7	0.36	0.07	0.16
Maximum	7.50	63.0	1.08	0.58	0.78
Mean	5.24	35.4	0.82	0.40	0.58
SD	1.07	13.7	0.21	0.15	0.18

1. The leader of seedling B of the tilted group was badly damaged in transport, and as a consequence, Fvar and CO₂ uptake data for this seedling are not reported for any of the measurement periods.

Time to M was between 17.7 and 63.0 seconds in the induction time course, with a mean average of 35.5 ± 8.75 seconds. FvM ranged from 0.36 to 1.08, with a mean average of 0.82 ± 0.21 .

Uncorrected CO₂ uptake for the tilted seedlings (Table 3.1.6) ranged from -1.41 to 14.48 µg/min, with a mean average uptake of 7.02±4.46 µg/min. Estimated chlorophyll content ranged from 0.085 to 0.191 mg, with a mean average of 0.130±0.029 mg. Carbon dioxide uptake corrected to estimated chlorophyll content ranged from -16.58 to 106.65 µg/mgChl/min, with a mean average of 52.33±34.05 µg/mgChl/min, in contrast to 72.25±47.78 µg/mgChl/min for the untreated group.

Table 3.1.6: Uncorrected and corrected CO₂ uptake, and estimated chlorophyll development for the tilted group in mid-summer.

SEEDLING	Uncorrected Uptake (µg/min)	Est. Chlorophyll (mg)	Corrected Uptake (µg/mg/min)
A	8.96	0.129	69.60
B			
C	14.48	0.164	88.22
D	5.27	0.191	27.57
E	12.27	0.115	106.65
F	3.06	0.121	25.45
G	4.29	0.099	43.51
H	-1.41	0.085	-16.58
I	8.10	0.121	67.20
J	9.57	0.142	67.25
K	4.54	0.134	33.84
L	8.10	0.129	62.93
CASES = 11			
Minimum	-1.41	0.085	-16.58
Maximum	14.48	0.191	106.65
Mean	7.02	0.130	52.33
SD	4.46	0.029	34.05

In summary, a comparison of mean average fluorescence parameters for each of the three groups during mid-summer, revealed consistent values, with FvAVG for the control, incised and tilted groups of 0.57±0.18, 0.58±0.19, and 0.58±0.18 respectively. Carbon dioxide uptake was much more variable among groups, apparently depressed in the tilted

group, and enhanced in the incised group. The statistical and physiological significances of these findings are addressed in section 3.4.

3.2 CARBON DIOXIDE UPTAKE, FLUORESCENCE, AND O-LEVEL DEVELOPMENT IN LATE-SUMMER.

FvAVG for the untreated group in late-summer (Table 3.2.1) ranged from 0.47 to 0.87, with a mean of 0.67 ± 0.16 . FvT ranged from 0.29 to 0.69, with a mean of 0.47 ± 0.15 .

Table 3.2.1: Fluorescence values for the untreated group in late summer.

SEEDLING	O-level	Time to M (sec)	FvM	FvT	FvAVG
A	13.0	79.5	0.61	0.29	0.48
B	10.0	58.5	0.96	0.65	0.81
C	10.0	97.2	0.99	0.67	0.86
D	12.2	130.2	0.72	0.40	0.61
E	9.5	83.1	0.64	0.34	0.51
F	11.3	103.8	0.98	0.69	0.84
G	9.2	105.3	0.63	0.29	0.47
H	8.7	88.8	0.88	0.43	0.64
I	8.1	44.7	0.77	0.38	0.57
J	10.4	91.8	0.72	0.33	0.56
K	9.8	112.2	1.01	0.65	0.87
L	9.0	99.0	1.04	0.51	0.83
CASES = 12					
Minimum	8.10	44.7	0.61	0.29	0.47
Maximum	13.00	130.2	1.04	0.69	0.87
Mean	10.10	91.2	0.83	0.47	0.67
SD	1.44	23.0	0.16	0.15	0.16

During late-summer, M peaks in the untreated groups occurred substantially later than they did in mid-summer, ranging between 44.7 and 130.2 seconds, with the mean average Time to M being 91.2 ± 23.0 seconds. FvM ranged from 0.61 to 1.04, with a mean average of 0.83 ± 0.16 .

Uncorrected CO₂ uptake for the untreated seedlings (Table 3.2.2) ranged from -2.21 to +25.29 µg/min, with a mean average uptake of 11.14±6.35 µg/min. Estimated chlorophyll content had increased by approximately 118% from mid-summer levels to 0.262±0.039 mg per measured leader section. Carbon dioxide uptake corrected to estimated chlorophyll content ranged from -8.51 to 74.05 µg mgChl⁻¹ min⁻¹, with a mean average of 42.03±20.81 µg/mgChl/min.

Table 3.2.2: Uncorrected and corrected CO₂ uptake, and estimated chlorophyll development for the untreated group in late summer.

SEEDLING	Uncorrected Uptake (µg/min)	Est. Chlorophyll (mg)	Corrected Uptake (µg/mg/min)
A	25.29	0.342	74.05
B	14.85	0.260	57.20
C	-2.21	0.260	-8.51
D	13.75	0.320	43.01
E	9.08	0.246	36.92
F	12.15	0.295	41.18
G	12.39	0.238	52.12
H	13.62	0.224	60.77
I	10.19	0.208	49.02
J	6.87	0.271	25.40
K	7.61	0.254	29.94
L	10.06	0.232	43.31
CASES = 12			
Minimum	-2.21	0.208	-8.51
Maximum	25.29	0.342	74.05
Mean	11.14	0.262	42.03
SD	6.35	0.039	20.81

In summary, fluorescence of untreated seedlings during late-summer was characterized by a substantial delay of the M peak, while fluorescence amplitudes demonstrated a modest overall increase. Estimated chlorophyll content was more than doubled and, while uncorrected CO₂ uptake was higher than in mid-summer, the value when corrected to estimated chlorophyll content was lower.

FvAVG for the incised group in late-summer (Table 3.2.3) ranged from 0.31 to 0.83, with a mean of 0.64 ± 0.18 . FvT ranged from 0.11 to 0.64, with a mean of 0.43 ± 0.18 .

Table 3.2.3: Fluorescence values for the incised group in late summer.

SEEDLING	O-level	Time to M (sec)	FvM	FvT	FvAVG
A	11.9	39.0	0.53	0.22	0.41
B	11.3	96.9	0.83	0.52	0.69
C	12.5	113.4	0.80	0.55	0.73
D	9.6	28.5	0.75	0.29	0.48
E	9.3	47.4	0.98	0.53	0.74
F	10.4	64.5	1.01	0.56	0.82
G	9.1	57.9	1.01	0.64	0.83
H	8.7	48.9	0.90	0.46	0.70
I	10.3	51.9	0.68	0.27	0.49
J	9.6	100.2	0.52	0.11	0.31
K ¹					
L	8.5	37.2	0.95	0.61	0.81
CASES = 11					
Minimum	8.5	28.5	0.52	0.11	0.31
Maximum	12.5	113.4	1.01	0.64	0.83
Mean	10.1	62.3	0.81	0.43	0.64
SD	1.3	28.4	0.18	0.18	0.18

1. Seedling K of the incised group was not measured in late-summer due to a procedural error.

As with the untreated group, M peaks of seedlings in the incised group occurred substantially later than they did in mid-summer, between 28.5 and 113.4 seconds, with the mean average Time to M being 62.3 ± 28.4 seconds. FvM ranged from 0.52 to 1.01, with a mean average of 0.81 ± 0.18 .

Uncorrected CO₂ uptake for the incised seedlings (Table 3.2.4) ranged from 8.60 to 24.79 µg/min, with a mean average uptake of 15.49±4.93 µg/min. Estimated chlorophyll content had increased by approximately 121% from mid-summer levels to 0.263±0.036 mg per measured leader section. Carbon dioxide uptake corrected to estimated chlorophyll content ranged from 38.33 to 76.98 µg/mgChl/min, with a mean average of 58.27±20.81 µg/mgChl/min.

Table 3.2.4: Uncorrected and corrected CO₂ uptake, and estimated chlorophyll development for the incised group in late-summer.

SEEDLING	Uncorrected Uptake (µg/min)	Est. Chlorophyll (mg)	Corrected Uptake (µg/mg/min)
A	13.99	0.312	44.92
B	22.22	0.295	75.28
C	24.79	0.328	76.98
D	19.15	0.249	60.21
E	14.48	0.241	66.24
F	17.92	0.271	66.23
G	12.40	0.235	52.73
H	8.60	0.224	38.32
I	12.89	0.268	48.12
J	12.52	0.249	50.33
K ¹			
L	11.42	0.219	52.29
CASES = 11			
Minimum	8.60	0.219	38.33
Maximum	24.79	0.328	76.98
Mean	15.49	0.263	58.27
SD	4.93	0.036	13.48

¹ Seedling K of the incised group was not measured in late-summer due to a procedural error.

Fluorescence in the incised group was characterized by a substantial delay of the M peak, although this was less than in the untreated group. FvT and FvAVG amplitudes demonstrated a modest overall increase. Estimated chlorophyll content was more than doubled and, while uncorrected CO₂ uptake was higher than in mid-summer, levels corrected to estimated chlorophyll content were lower.

FvAVG for the tilted group in late-summer (Table 3.2.5) ranged from 0.48 to 0.99, with a mean of 0.74 ± 0.14 . FvT ranged from 0.29 to 0.76, with a mean of 0.53 ± 0.13 .

Table 3.2.5: Fluorescence values for the tilted group in late summer.

SEEDLING	O-level	Time to M (sec)	FvM	FvT	FvAVG
A	9.5	119.4	0.71	0.38	0.58
B					
C	13.5	134.7	0.90	0.64	0.82
D	12.6	28.5	0.75	0.29	0.48
E	7.5	48.3	1.26	0.76	0.99
F	8.8	44.7	0.82	0.46	0.64
G	7.9	38.4	1.04	0.49	0.77
H	8.4	40.2	1.11	0.53	0.83
I	10.1	93.0	0.96	0.53	0.79
J	11.9	44.1	1.02	0.57	0.81
K	8.5	141.9	0.85	0.69	0.78
L	10.4	100.5	0.79	0.50	0.69
CASES = 11					
Minimum	7.5	28.5	0.71	0.29	0.48
Maximum	13.5	141.9	1.26	0.76	0.99
Mean	9.9	75.8	0.93	0.53	0.74
SD	1.9	42.7	0.16	0.13	0.14

As with the untreated and incised groups, M peaks from seedlings in the tilted groups occurred later than they did in mid-summer, between 28.5 and 141.9 seconds, with the mean average Time to M being 75.9 ± 42.7 seconds. FvM ranged from 0.71 to 1.26, with a mean average of 0.93 ± 0.16 .

Uncorrected CO₂ uptake for the tilted seedlings (Table 3.2.6) ranged from -12.76 to +24.92 µg/min, with a mean average uptake of 9.32±9.75 µg/min. Estimated chlorophyll content had increased by approximately 97% from mid-summer levels to 0.257±0.054 mg per measured leader section. Carbon dioxide uptake corrected to estimated chlorophyll content ranged from -38.62 to 70.17 µg/mgChl/min, with a mean average of 38.21±33.87 µg/mgChl/min.

Table 3.2.6: Uncorrected and corrected CO₂ uptake, and estimated chlorophyll development for the tilted group in late summer.

SEEDLING	Uncorrected Uptake (µg/min)	Est. Chlorophyll (mg)	Corrected Uptake (µg/mg/min)
A	0.11	0.246	0.45
B			
C	24.92	0.355	70.71
D	-12.70	0.331	-38.62
E	11.90	0.191	62.18
F	13.75	0.227	60.58
G	13.75	0.202	67.93
H	11.04	0.216	51.14
I	15.71	0.262	59.88
J	12.89	0.312	41.38
K	4.05	0.219	18.51
L	7.24	0.271	26.76
CASES = 11			
Minimum	-12.76	0.191	-38.62
Maximum	24.92	0.355	70.17
Mean	9.32	0.257	38.21
SD	9.75	0.054	33.87

A comparison of mean fluorescence parameters for each of the three groups, continued to reveal similar values during late-summer. FvAVG for the control, inclined and tilted groups were 0.67, 0.64, and 0.74 respectively. Late-summer fluorescence was characterized by a delayed M peak (compared to mid-summer), while development was characterized by doubled chlorophyll contents in the measured leader segments.

Uncorrected CO₂ uptake increased marginally, but the higher chlorophyll contents resulted in corrected CO₂ uptake levels that were substantially lower than those of mid-summer. Incised seedlings continued to demonstrate higher CO₂ uptake than either the untreated or tilted seedlings.

3.3 CARBON DIOXIDE UPTAKE, FLUORESCENCE, AND O-LEVEL DEVELOPMENT IN AUTUMN.

FvAVG for the untreated group in autumn (Table 3.3.1) ranged from 0.55 to 1.04, with a mean of 0.79 ± 0.14 . FvT ranged from 0.66 to 1.10, with a mean of 0.89 ± 0.12 .

Table 3.3.1: Fluorescence values for the untreated group in autumn.

SEEDLING	O-level	Time to M (sec)	FvM	FvT	FvAVG
A	11.5	79.8	1.30	0.85	0.98
B	9.2	244.2	1.07	1.04	0.91
C	10.0	240.0	1.15	1.10	1.04
D	10.0	280.5	0.95	0.95	0.79
E	9.3	253.5	0.83	0.81	0.70
F	9.6	279.0	0.90	0.87	0.77
G	8.3	276.9	0.83	0.82	0.68
H	8.3	297.9	0.94	0.94	0.75
I	7.8	279.3	0.85	0.85	0.77
J	9.2	297.3	0.67	0.66	0.55
K	7.9	294.3	0.98	0.97	0.89
L	7.5	294.3	0.78	0.78	0.66
CASES = 12					
Minimum	7.50	79.8	0.67	0.66	0.55
Maximum	11.50	297.9	1.30	1.10	1.04
Mean	9.05	259.8	0.93	0.89	0.79
SD	1.15	60.1	0.17	0.12	0.14

In autumn, M peaks of seedlings in the untreated group occurred substantially later than they did in either mid-summer or late-summer, in a range between 79.8 to 297.9 seconds,

with the mean average Time to M being 259.8 ± 60.1 seconds. FvM ranged from 0.67 to 1.30, with a mean average of 0.93 ± 0.17 .

Uncorrected CO₂ uptake for the untreated seedlings (Table 3.3.2) ranged from 7.24 to 26.67 $\mu\text{g}/\text{min}$, with a mean average uptake of 15.69 ± 6.62 $\mu\text{g}/\text{min}$. Estimated chlorophyll content was slightly reduced from late-summer levels, at 0.234 ± 0.031 mg per measured leader section. Carbon dioxide uptake corrected to estimated chlorophyll content ranged from 29.31 to 126.16 $\mu\text{g}/\text{mgChl}/\text{min}$, with a mean average of 68.33 ± 30.12 $\mu\text{g}/\text{mgChl}/\text{min}$.

Table 3.3.2: Uncorrected and corrected CO₂ uptake, and estimated chlorophyll development for the untreated group in autumn.

SEEDLING	Uncorrected Uptake ($\mu\text{g}/\text{min}$)	Est. Chlorophyll (mg)	Corrected Uptake ($\mu\text{g}/\text{mg}/\text{min}$)
A	26.68	0.301	88.74
B	17.92	0.238	75.35
C	7.61	0.260	29.31
D	15.10	0.260	58.14
E	7.30	0.241	30.36
F	10.71	0.249	43.08
G	15.07	0.213	70.67
H	7.24	0.213	33.95
I	19.27	0.200	96.53
J	19.52	0.238	82.06
K	25.53	0.202	122.16
L	16.38	0.191	85.58
CASES = 12			
Minimum	7.24	0.191	29.31
Maximum	26.67	0.301	126.16
Mean	15.69	0.234	68.33
SD	6.62	0.031	30.12

FvAVG for the incised group in autumn (Table 3.3.3) ranged from 0.48 to 1.04, with a mean of 0.80 ± 0.18 . FvT ranged from 0.31 to 1.10, with a mean of 0.87 ± 0.12 .

Table 3.3.3: Fluorescence values for the incised group in autumn.

SEEDLING	O-level	Time to M (sec)	FvM	FvT	FvAVG
A	11.2	65.7	0.83	0.65	0.70
B	11.1	180.0	1.17	1.07	1.08
C	11.2	285.6	0.87	0.85	0.75
D	9.3	95.1	0.68	0.31	0.48
E	8.7	247.8	0.85	0.83	0.77
F	10.0	251.7	1.09	1.05	0.96
G	9.6	284.7	1.05	1.05	0.90
H	8.2	235.2	1.03	1.00	0.94
I	9.5	252.3	1.11	1.10	1.00
J	8.1	285.3	0.81	0.80	0.59
K	9.2	298.8	0.91	0.90	0.78
L	9.0	299.4	0.77	0.77	0.59
CASES = 12					
Minimum	8.10	65.7	0.68	0.31	0.48
Maximum	11.20	299.4	1.17	1.10	1.08
Mean	9.59	231.8	0.93	0.87	0.80
SD	1.09	78.4	0.15	0.22	0.18

The M peaks of seedlings in the incised group occurred substantially later than they did in late-summer, in a range between 65.7 to 299.4 seconds, with the mean average Time to M being 231.8 ± 78.4 seconds. FvM ranged from 0.68 to 1.17, with a mean average of 0.93 ± 0.15 .

Uncorrected CO₂ uptake for the incised seedlings (Table 3.3.4) ranged from 10.71 to 28.56 µg/min, with a mean average uptake of 16.44±5.47 µg/min. Estimated chlorophyll content was slightly reduced from late-summer levels to 0.249±0.031 mg per measured leader section. Carbon dioxide uptake corrected to chlorophyll content ranged from 38.33 to 76.98 µg/mgChl/min, with a mean average of 58.27±30.12 µg/mgChl/min.

Table 3.3.4: Uncorrected and corrected CO₂ uptake, and estimated chlorophyll development for the incised group in autumn.

SEEDLING	Uncorrected Uptake (µg/min)	Est. Chlorophyll (mg)	Corrected Uptake (µg/mg/min)
A	10.71	0.292	36.65
B	11.98	0.290	41.30
C	18.69	0.292	63.94
D	22.25	0.241	92.53
E	14.30	0.224	63.78
F	20.13	0.260	77.53
G	13.43	0.249	53.99
H	10.96	0.211	52.06
I	28.56	0.246	116.10
J	19.09	0.208	91.84
K	15.92	0.238	66.94
L	11.27	0.232	48.49
CASES = 12			
Minimum	10.71	0.208	36.65
Maximum	28.56	0.292	116.10
Mean	16.44	0.249	67.10
SD	5.47	0.030	23.59

FvAVG for the tilted group in autumn (Table 3.3.5) ranged from 0.58 to 0.97, with a mean of 0.81 ± 0.18 . FvT ranged from 0.49 to 1.07, with a mean of 0.91 ± 0.15 .

Table 3.3.5: Fluorescence values for the tilted group in autumn.

SEEDLING	O-level	Time to M (sec)	FvM	FvT	FvAVG
A	9.6	142.5	0.74	0.49	0.58
B					
C	12.1	245.1	0.93	0.91	0.84
D	11.0	246.6	0.97	0.96	0.83
E	8.0	223.8	0.90	0.84	0.78
F	8.6	299.0	1.02	1.02	0.90
G	8.1	263.4	0.85	0.83	0.71
H	8.4	243.3	1.06	1.04	0.97
I	9.0	297.3	1.08	1.07	0.89
J	11.0	262.8	0.98	0.97	0.87
K	9.0	296.7	0.94	0.93	0.75
L	7.5	294.0	0.97	0.97	0.80
CASES = 11					
Minimum	7.50	142.5	0.74	0.49	0.58
Maximum	12.10	299.0	1.08	1.07	0.97
Mean	9.30	255.9	0.95	0.91	0.81
SD	1.46	45.9	0.09	0.15	0.10

As with the untreated and incised groups, M peaks from seedlings in the tilted group occurred later than they did in summer, in a range between 142.5 to 299.0 seconds, with the mean average Time to M being 255.9 ± 45.9 seconds. FvM ranged from 0.74 to 1.08, with a mean average of 0.95 ± 0.09).

Uncorrected CO₂ uptake for the tilted seedlings (Table 3.3.6) ranged from 6.55 to 24.57 µg/min, with a mean average uptake of 16.79±4.94 µg/min. Estimated chlorophyll content was 0.241±0.040 mg per measured leader section. Carbon dioxide uptake corrected to estimated chlorophyll content ranged from 31.54 to 96.16 µg/mgChl/min, with a mean average of 70.56±30.12 µg/mgChl/min.

Table 3.3.6: Uncorrected and corrected CO₂ uptake, and estimated chlorophyll development for the tilted group in autumn.

SEEDLING	Uncorrected Uptake (µg/min)	Est. Chlorophyll (mg)	Corrected Uptake (µg/mg/min)
A	11.78	0.249	47.37
B			
C	20.95	0.317	63.71
D	14.04	0.287	48.94
E	14.53	0.205	70.86
F	18.36	0.221	82.91
G	6.55	0.208	31.54
H	20.72	0.216	95.92
I	19.66	0.232	84.62
J	24.57	0.287	85.64
K	15.91	0.232	68.46
L	18.41	0.191	96.16
CASES = 11			
Minimum	6.55	0.191	31.54
Maximum	24.57	0.317	96.16
Mean	16.79	0.241	70.56
SD	4.94	0.040	21.10

In summary, a comparison of mean fluorescence parameters for the three groups, few significant differences were apparent during autumn. FvAVG for the control, incised and tilted groups were 0.79, 0.80, and 0.81 respectively, while the mean average M fluorescence values for the three groups were 0.93, 0.93, and 0.95. For all groups, autumn fluorescence was characterized by a further delayed M peak (compared to mid and late-summer), while development was characterized by a slight drop in estimated chlorophyll content of the measured leader segments.

Uncorrected CO₂ uptake increased marginally, but the higher chlorophyll contents resulted in corrected CO₂ uptake levels that were substantially lower than that of mid-summer. Mean average corrected CO₂ uptake was also similar among groups, at 68.33, 67.10, and 70.56 µg/mgChl/min for the untreated, incised, and tilted groups respectively.

3.4 ANALYSES OF VARIANCE IN FLUORESCENCE, CO₂ UPTAKE, AND ESTIMATED CHLOROPHYLL CONTENT IN UNTREATED AND TREATED SEEDLINGS.

The intent of this section is to summarize the results of statistical analyses of data presented in the previous three sections. The statistical procedure for normal data, was that of performing Bartlett's test for homogeneity of variance, and a Model I one-way analysis of variance (ANOVA) to test the null hypothesis that the measured parameters of the three groups have equal means. Where, on the basis of a Model I ANOVA, the null hypothesis has been rejected at the 0.05 level, the Tukey HSD test has been applied.

Data which were determined by normality plotting and Kolmogorov-Smirnov Lilliefors probability testing to be non-parametric, were tested using the Kruskal-Wallis one-way analysis of variance.

Table 3.4.1 summarizes mean fluorescence parameters for the untreated and treated seedlings during the mid-summer, late-summer, and autumn monitoring periods.

Table 3.4.1 Summary of averaged fluorescence parameters for the untreated (1), incised (2), and tilted (3) groups in mid-summer, late-summer, and autumn.

SEASON	GROUP	O-level	Time to M (sec)	FvM	FvT	FvAVG
Mid-Summer:	1	5.0	31.0	0.85	0.40	0.57
	2	4.8	30.1	0.85	0.40	0.58
	3	5.2	35.4	0.82	0.40	0.58
Late-Summer:	1	10.1	91.2	0.83	0.47	0.67
	2	10.1	62.3	0.81	0.43	0.64
	3	9.9	75.7	0.93	0.53	0.74
Autumn:	1	9.0	259.8	0.93	0.89	0.79
	2	9.6	231.8	0.93	0.87	0.80
	3	9.3	255.9	0.95	0.91	0.81

Seasonal changes in photosynthetic activity and development in the untreated group were characterized most dramatically by O-level and M fluorescence parameters. O-level was doubled from mid to late-summer. (The implications of these changes in O-level will be discussed subsequently in terms of estimated chlorophyll content.) The mean average time to the M peak was increased from 31.0 seconds in mid-summer to 91.2 seconds in late-summer, and 259.8 seconds in the autumn. These changes are consistent with reported observations and likely reflect seasonal shifts in demands and allocations of photosynthetic products (Vidaver *et al.*, 1989, in press).

FvM was relatively consistent in mid and late-summer, and showed a marginal rise in autumn. Due to the substantial delay of the M peak, both final and mean average fluorescence values appeared higher in late-summer and autumn than in mid-summer.

Table 3.4.2. summarizes the results of ANOVAs testing for the effect of treatments on Fvar parameters of incised and tilted seedlings during each of the three monitoring periods.

Table 3.4.2: ANOVA and Bartlett's tests of homogeneity of variance for the effects of treatment on fluorescence values during mid-summer, late-summer and autumn. (Degrees of freedom = 2 for all parameters.)

PARAMETER	SS treatment	SS error	F	P	BARTLETT'S TEST Chi-square 2 prob	
Mid-Summer:						
O-level	0.562	21.356	0.42	0.66	3.89	0.14
Time to M ¹	N.A.	N.A.	1.36	0.51	N.A.	N.A.
FvM	0.004	1.042	0.06	0.93	1.13	0.56
FvT	0.000	0.963	0.01	0.99	0.59	0.74
FvAVG	0.001	1.109	0.01	0.99	0.14	0.93
Late-Summer:						
O-level	0.258	79.905	0.05	0.95	1.97	0.37
Time to M ¹	N.A.	N.A.	3.76	0.15	N.A.	N.A.
FvM	0.085	0.898	1.46	0.24	0.07	0.96
FvT	0.054	0.769	1.09	0.34	0.77	0.08
FvAVG	0.065	0.802	1.26	0.30	0.70	0.70
Autumn:						
O-level	1.763	49.378	0.57	0.57	1.03	0.60
Time to M ¹	N.A.	N.A.	0.57	0.75	N.A.	N.A.
FvM	0.002	0.668	0.05	0.95	3.11	0.21
FvT	0.013	0.965	0.21	0.81	4.07	0.13
FvAVG	0.003	0.710	0.05	0.94	3.01	0.22

1. Data are non-parametric and have been tested with a Kruskal-Wallis analysis of variance. In this case the F-ratio has been replaced by a test statistic, and the probability is based on a chi-square distribution with two degrees of freedom. N.A. signifies that the parametric statistical terms are not applicable.

These results demonstrate that inter-group differences in means of the five measured Fvar parameters were not statistically significant during any of the three monitoring periods following treatment, and thus indicate that, at least in terms of Fvar amplitudes, the two

induced stresses did not have a measurable effect. The means of the Time to M parameter, however, appeared shorter (than for the untreated) in both treated groups in late-summer. A Kruskal-Wallis ANOVA performed with these data yielded a test statistic of 3.76, at a probability level of 0.15.

Group mean average CO₂ uptakes and chlorophyll development in untreated and treated groups during mid-summer, late-summer, and autumn are summarized in Table 3.4.3.

Table 3.4.3 Summary of averaged CO₂ uptake parameters and estimated chlorophyll development for the untreated (1), incised (2), and tilted (3) groups in mid-summer, late-summer, and autumn.

SEASON	GROUP	Uncorrected CO ₂ (µg/min)	Estimated Chlorophyll (mg)	Corrected CO ₂ (µg/mg/min)
Mid-Summer:	1	8.70	0.120	72.25
	2	12.49	0.119	103.64
	3	7.02	0.130	52.33
Late-Summer:	1	11.14	0.262	42.03
	2	15.49	0.263	58.27
	3	9.32	0.257	38.21
Autumn:	1	15.69	0.234	68.33
	2	16.44	0.249	67.10
	3	16.79	0.241	70.56

The estimated chlorophyll content of the leader segments used to measure fluorescence and CO₂ uptake indicate similar development in all three groups. Each group approximately doubled chlorophyll content during the five weeks of the mid-summer to late-summer interval, and each showed a slight decline during the final measurement period in October. Treatments did not appear significantly to affect chlorophyll development.

Corrected CO₂ uptake mean averages were somewhat variable among the treatment groups in mid-summer, but this was at least in part due to the occurrence of two negative uptake values. Analysis of variance (Table 3.4.4) indicates that differences in mean corrected CO₂ uptakes during in mid-summer are significant at the 0.04 level of probability.

Table 3.4.4: ANOVA and Bartlett's tests of homogeneity of variance for the effects of treatment on uncorrected and corrected CO₂ uptake and estimated chlorophyll during mid-summer, late-summer and autumn. (Degrees of freedom = 2 for all parameters.)

PARAMETER	SS treatment	SS error	F	P	BARTLETT'S TEST Chi-square 2-prob	
Mid-Summer:						
Uncorrected CO ₂	176.463	1150.951	2.45	0.10	2.19	0.33
Est. Chlorophyll	0.001	0.016	0.84	0.44	3.63	0.16
Corrected CO ₂	15490.096	67506.076	3.67	0.04	1.92	0.37
Late-Summer:						
Uncorrected CO ₂	220.573	1630.400	2.10	0.14	4.63	0.10
Est. Chlorophyll	0.001	0.062	0.04	0.95	1.88	0.39
Corrected CO ₂ ¹	N.A.	N.A.	3.80	0.15	N.A.	N.A.
Autumn:						
Uncorrected CO ₂	7.972	1060.015	0.12	0.88	0.84	0.65
Est. Chlorophyll ¹	N.A.	N.A.	2.06	0.35	N.A.	N.A.
Corrected CO ₂	68.199	20139.608	0.05	0.95	1.20	0.55

1. Data are non-parametric and have been tested with a Kruskal-Wallis analysis of variance. In this case the F-ratio has been replaced by a test statistic, and the probability is based on a chi-square distribution with two degrees of freedom.

The Tukey multiple comparison matrix (Table 3.4.5) indicates that corrected CO₂ uptake means of the two treatments in mid-summer were unequal at better than a 0.05 probability level, but neither is significantly different from the untreated group.

Table 3.4.5. Tukey HSD matrix of pairwise comparison probabilities for corrected CO₂ uptakes of untreated and treated seedlings in mid-summer.

	UNTREATED	INCISED	TILTED
UNTREATED	1.00		
INCISED	0.25	1.00	
TILTED	0.50	0.03	1.00

If the lower CO₂ uptake values in the tilted group do reflect real effects of tilting, such effects might be a result of a temporary loss of apical dominance in the leaders. In their tilted positions, the leaders were positioned well below the tips of some lateral branches. During the experimental period, compression wood formation in the main stems did return the leaders to a semi-upright position. Uptake data during late-summer and autumn indicate a recovery of uptake in tilted seedlings to mean average levels measured for the untreated seedlings.

Corrected CO₂ uptake thus may have been retarded as a consequence of tilting, but the effect, if it is real, is somewhat masked by relatively large variances in uptake level in mid-summer and late-summer. Elevated uptakes by incised seedlings, if substantive, may have been due to carbon demands for wound respiration occurring in the treated portions of the stems. The fact that final mean average uptake measurements taken in autumn were very similar among the three groups suggests that the unequal means may have been temporary and treatment-related.

In the absence of statistical significance, further speculation on possible causes of reduced and elevated CO₂ uptakes will not be pursued.

3.5 SEEDLING DIMENSIONS AND CAMBIAL ACTIVITY

Individual and mean averaged measures of current season cambial activity, preseason xylem production (area) of 1987 and 1986 stem segments, preseason length, and current season leader length for the untreated, incised, and tilted seedlings, are presented in Tables 3.5.1, 3.5.2, and 3.5.3 respectively. Statistical data are summarized in Table 3.5.4.

Table 3.5.1: Cambial activities, cross-sectional areas of 1987 and 1986 stems, preseason lengths, and leader lengths of untreated group.

SEEDLING	Cambial Activity (mm ² /mm)	Area 1987 Stem (mm ²)	Area 1986 Stem (mm ²)	Seedling Height (cm)	Leader Length (cm)
A	0.68	4.50	5.35	23.1	11.8
B	1.23	5.41	7.09	25.1	15.3
C	1.25	5.45	6.50	24.0	13.0
D	0.46	10.33	10.53	28.7	9.2
E	1.02	3.59	4.49	19.8	16.2
F	0.92	5.07	5.65	21.0	9.5
G	0.40	2.96	3.23	21.2	10.5
H	0.44	4.30	5.49	18.7	9.8
I	0.90	4.04	6.96	19.5	10.1
J	1.00	3.76	4.50	17.1	13.3
K	0.56	3.00	4.02	19.0	12.5
L	0.75	2.54	3.37	18.1	12.0
CASES = 12					
Minimum	0.40	2.54	3.23	17.1	9.2
Maximum	1.25	5.45	10.33	28.7	16.2
Mean	0.80	4.02	5.60	21.7	11.9
SD	0.30	0.96	2.02	3.4	2.3

Table 3.5.2: Cambial activities, cross-sectional areas of 1987 and 1986 stems, preseason lengths, and leader lengths of incised group.

SEEDLING	Cambial Activity (mm ² /mm)	Area 1987 Stem (mm ²)	Area 1986 Stem (mm ²)	Seedling Height (cm)	Leader Length (cm)
A	0.72	6.69	8.79	22.5	13.2
B	1.15	4.08	5.30	24.0	10.2
C	1.21	6.91	9.41	25.0	12.5
D	1.07	10.00	9.99	30.0	10.8
E	0.66	6.10	6.17	25.3	16.5
F	0.94	5.10	7.10	23.0	13.2
G	0.84	6.35	7.76	21.5	12.5
H	1.10	2.81	3.72	18.2	15.5
I	0.32	5.39	5.12	29.0	10.5
J	0.59	4.81	6.91	22.0	9.0
K	0.82	5.07	7.89	18.2	8.3
L	0.75	4.08	5.58	20.8	9.5
CASES = 12					
Minimum	0.32	2.81	3.72	18.2	8.3
Maximum	1.21	10.03	9.99	30.0	16.5
Mean	0.85	5.61	6.98	23.3	11.8
SD	0.26	1.83	1.88	3.7	2.5

Table 3.5.3: Cambial activities, cross-sectional areas of 1987 and 1986 stems, preseason lengths, and leader lengths of tilted group.

SEEDLING	Cambial Activity (mm ² /mm)	Area 1987 Stem (mm ²)	Area 1986 Stem (mm ²)	Seedling Height (cm)	Leader Length (cm)
A	0.76	7.73	10.23	25.5	10.0
B					
C	1.10	5.86	6.74	23.8	14.5
D	1.25	6.02	7.36	25.0	10.5
E	0.94	5.80	8.66	22.5	12.5
F	1.09	3.79	5.98	22.2	8.6
G	1.27	2.63	4.76	18.8	11.6
H	1.33	4.99	6.10	26.4	10.5
I	0.66	5.41	5.28	21.2	11.2
J	1.20	2.52	4.61	20.0	11.5
K	0.75	4.00	3.80	19.0	11.0
L	0.74	4.17	7.64	20.0	12.5
CASES = 11					
Minimum	0.66	2.52	3.81	18.8	8.6
Maximum	1.33	7.73	10.23	26.4	14.5
Mean	1.00	4.81	6.47	22.2	11.3
SD	0.25	1.57	1.91	2.6	1.5

Table 3.5.4: Summary of cambial activities, cross-sectional areas of 1987 and 1986 stems, preseason lengths, and leader lengths for all groups.

GROUP	Cambial Activity (mm²/mm)	Area 1987 Stem (mm²)	Area 1986 Stem (mm²)	Seedling Height (cm)	Leader Length (cm)
UNTREATED:					
Minimum	0.40	2.54	3.23	17.1	9.2
Maximum	1.25	10.33	10.53	28.7	16.2
Mean	0.80	4.57	5.60	21.7	11.9
SD	0.30	2.03	2.02	3.4	2.3
INCISED:					
Minimum	0.32	2.81	3.72	18.2	8.3
Maximum	1.21	10.03	9.99	30.0	16.5
Mean	0.85	5.61	6.98	23.3	11.8
SD	0.26	1.83	1.88	3.67	2.5
TILTED:					
Minimum	0.66	2.52	3.81	18.8	8.6
Maximum	1.33	7.73	10.23	26.4	14.5
Mean	1.00	4.81	6.47	22.2	11.3
SD	0.25	1.57	1.91	2.7	1.5

Cambial activities were relatively variable in all three groups, ranging from 0.40 to 1.25 mm²/mm for the untreated group, 0.32 to 1.21 mm²/mm in the incised group, and 0.66 to 1.33 mm²/mm in the tilted group. Mean averages for the three groups were 0.80±0.30, 0.85±0.26, and 1.00±0.25 mm²/mm, respectively.

Table 3.5.5: Analyses of variance and Bartlett's tests of homogeneity of variance for cambial activity and seedling dimensions. (Degrees of freedom = 2 for all parameters.)

PARAMETER	SS	SS	<i>F</i>	P	BARTLETT'S TEST	
	treatment	error			Chi-square	2 prob
Cambial Activity	0.268	2.239	1.83	0.17	0.39	0.82
Area 1987 Stem	6.809	107.603	1.01	0.37	0.66	0.72
Area 1986 Stem	11.986	119.883	1.60	0.22	0.05	0.97
Seedling Height	24.434	345.428	1.13	0.33	1.01	0.60
Leader Length	2.474	151.165	0.26	0.77	2.48	0.29

The most significant effect apparent from the treatments is that of a 26% higher cambial activity in tilted seedlings than in the untreated group, but the analysis of variance indicates this difference among groups to be significant only at a 0.17 level of probability. If the effect is attributed to the treatment, it is a consequence of enhanced cambial activity and compression wood formation on the seedlings' lower sides.

Preseason seedling heights, cross-sectional stem areas, and current season leader lengths were obviously unaffected by treatment (the development of each being complete at the initiation of the experiment), but are included to demonstrate that the treated and untreated groups were morphologically similar. Mean average preseason seedling heights were 21.7 ± 3.37 , 23.3 ± 3.67 , and 22.2 ± 2.67 cm for the untreated, incised, and tilted seedlings respectively. Mean averaged season leader lengths were 11.9 ± 2.26 , 11.8 ± 2.54 , and 11.3 ± 1.54 cm for the untreated, incised, and tilted seedlings respectively.

Mean averaged cross-sectional areas of the 1987 stem segment were 4.57 ± 2.03 ,

5.61±1.83, and 4.81±1.57 mm² for the untreated, incised and tilted seedlings respectively. Mean averaged cross-sectional areas of the 1986 stem segment were 5.60±2.02, 6.98±1.88, and 6.47±1.91 mm² for the untreated, incised and tilted seedlings respectively.

3.6 CORRELATION AND REGRESSION ANALYSES

In this section, the occurrence of correlations and functionally dependent relationships between stem vigour, and various photosynthetic activity and stem dimension parameters is examined. The terminology adopted for these "simple linear regression" analyses is based on Zar (1984).

3.6.1 Fluorescence parameters.

Fvar parameter - cambial activity regressions in mid-summer.

Table 3.6.1.1 summarizes the results of regression analyses between various fluorescence parameters and cambial activity of the untreated group in mid-summer.

Table 3.6.1.1. Regression analyses of cambial activity and fluorescence parameters during mid-summer in the untreated group.

Statistic	O-level	Time to M ¹	FvM	FvT	FvAVG	Fv120
R	0.182	0.322	0.736	0.827	0.879	0.900
R²	0.033	N.A.	0.542	0.684	0.773	0.809
SE	0.768	N.A.	0.115	0.109	0.094	0.097
α	5.247	N.A.	0.536	-0.010	0.128	0.084
β	-0.455	N.A.	0.399	0.511	0.544	0.641
T	-0.586	N.A.	3.443	4.648	5.830	6.515
P	0.571	N.A.	0.006	0.001	0.000	0.000
ANOVA <i>F</i>	0.344	N.A.	11.852	21.604	33.993	42.411

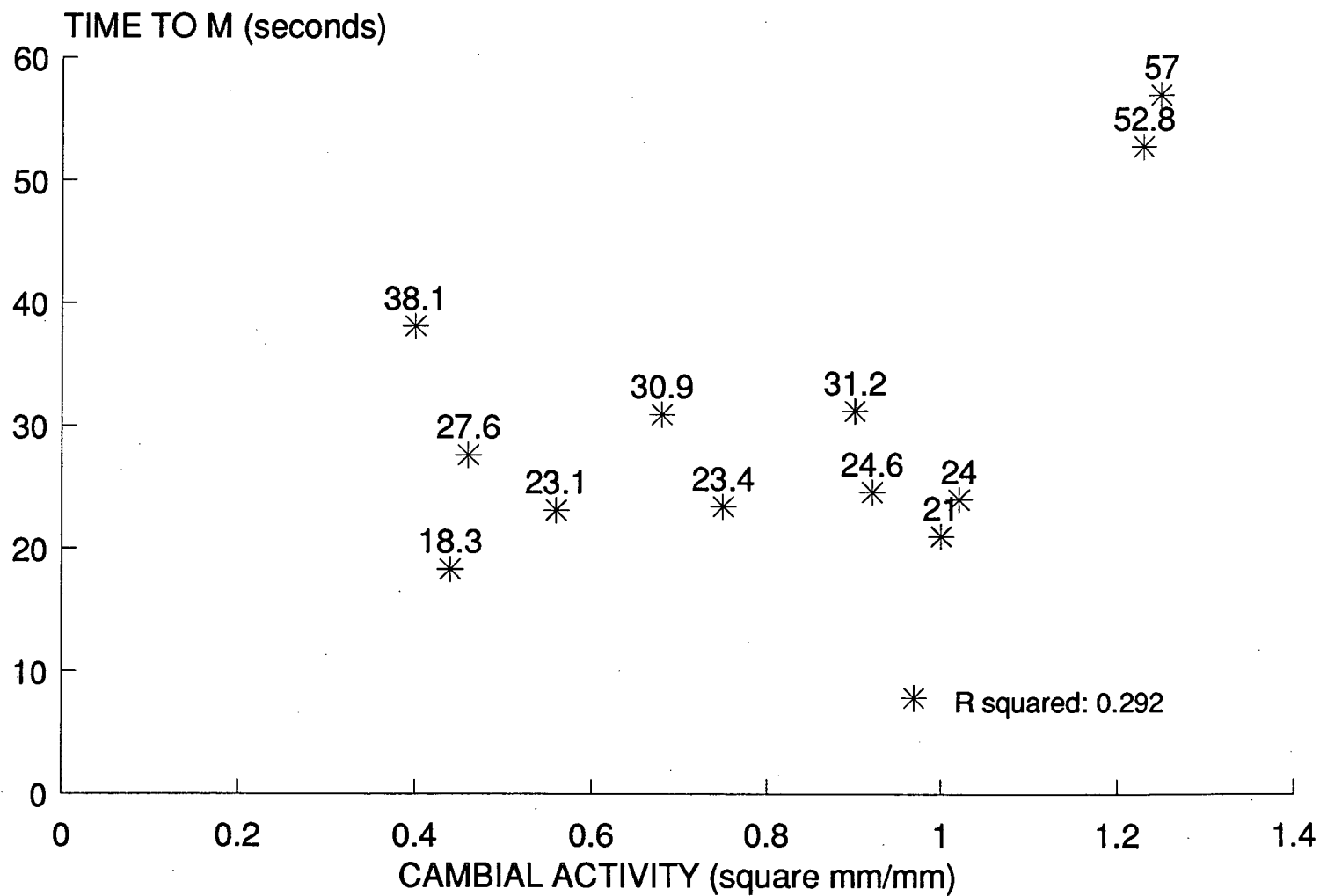
1. These data are non-parametric. A Spearman Rank coefficient replaces R. The notation N.A. indicates the parametric statistical terms are not applicable.

For the untreated group, all fluorescence amplitude parameters exhibited a functional relationship to cambial activities during mid-summer. R^2 values ranged from 0.542 ($p = 0.006$) and 0.684 ($p = 0.001$), for FvM and FvT respectively, to a stronger relationship for FvAVG, with an R^2 value of 0.773, ($p < 0.001$). Time to M peak was weakly correlated ($R^2 = 0.292$), and the O-level parameter was not measurably correlated. Scatter plots demonstrating regressions for Time to M, Fvar at M, Fvar at T, and mean average Fvar, are provided in Figures 3.6.1.1, 3.6.1.2, 3.6.1.3, and 3.6.1.4 respectively.

Figures 3.1.1 and 3.1.2 show Fvar induction curves for the seedlings with the highest and lowest stem vigour (as measured by cambial activity) respectively. Two apparent characteristics of seedlings of low stem vigour are visible. The overall height of the induction curve, corresponding to corrected Fvar amplitude, appears lower in the low

vigour seedlings, and the post-M decay appears more abrupt in seedlings of low stem vigour. FvAVG values, (which were determined by calculating the areas under the curves, and dividing by the induction period), correspond to this observation, as do the regression analyses.

If a lower mean average Fvar, and a somewhat faster decay after M, does indeed characterize low vigour seedlings, one would expect that by measuring Fvar at a single point following the decay after M, a stronger R^2 value might be attained. Regression analyses between cambial activity and Fvar at 120 seconds yielded an R^2 value of 0.809 ($p < 0.001$). This relatively strong relationship is demonstrated in Figure 3.6.1.5.



Falls/89

Figure 3.6.1.1. Scatter plot of Time to M and cambial activity of untreated seedlings in mid-summer.

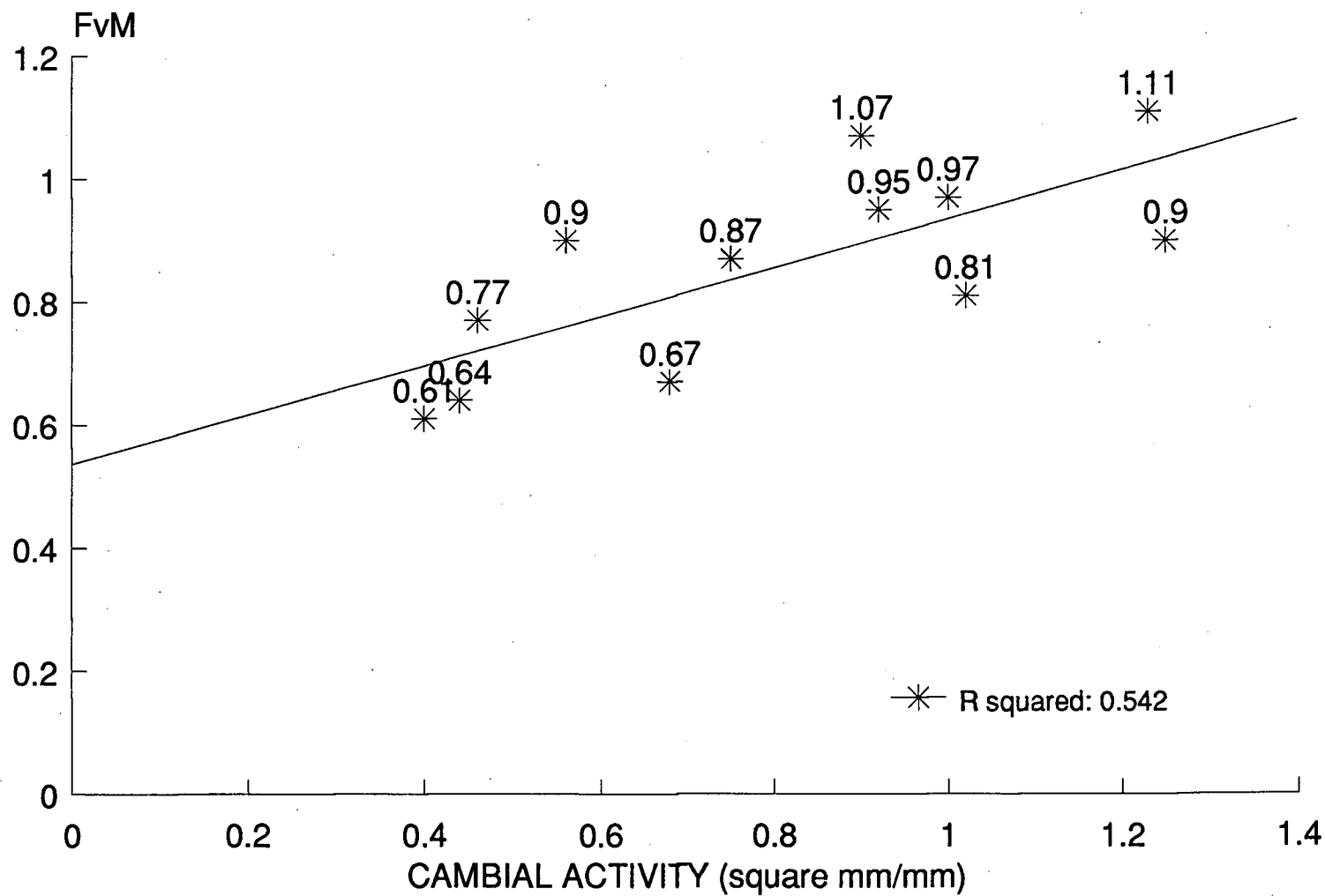
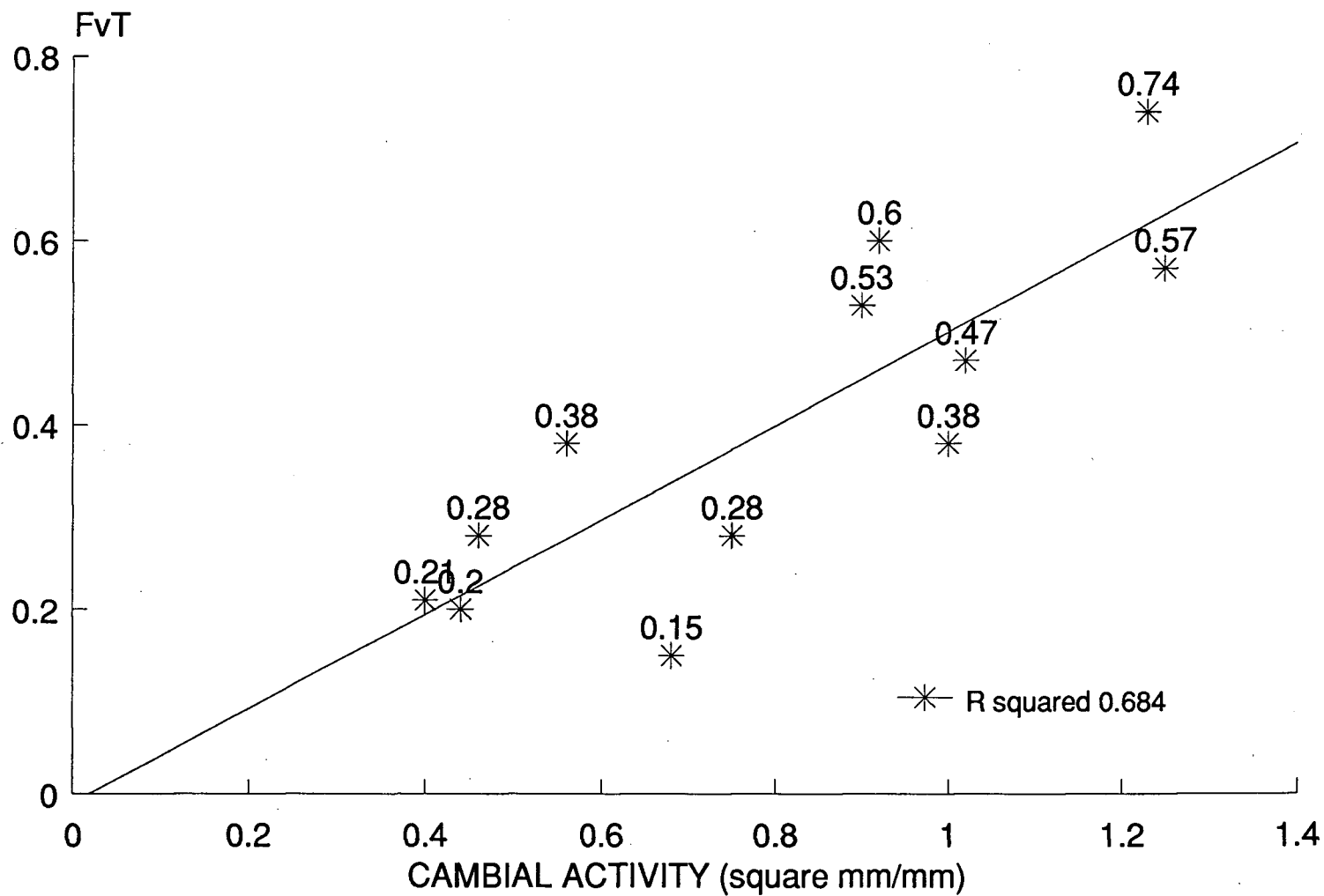


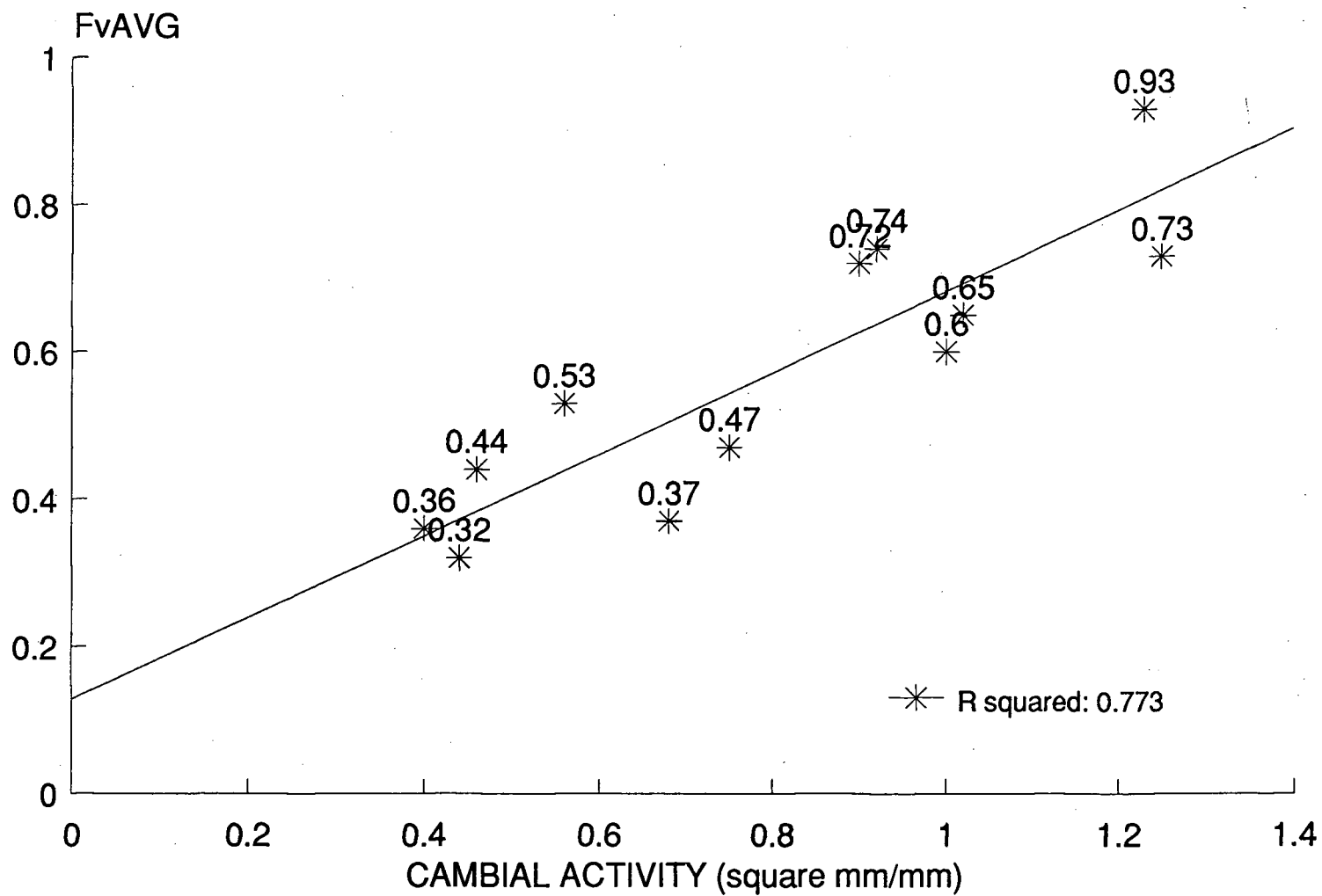
Figure 3.6.1.2. Scatter plot of FvM and cambial activity of untreated seedlings in mid-summer.

Falls/89



Falls/89

Figure 3.6.1.3. Scatter plot of FvT and cambial activity of untreated seedlings in mid-summer.



Falls/89

Figure 3.6.1.4. Scatter plot of FvAVG and cambial activity of untreated seedlings in mid-summer.

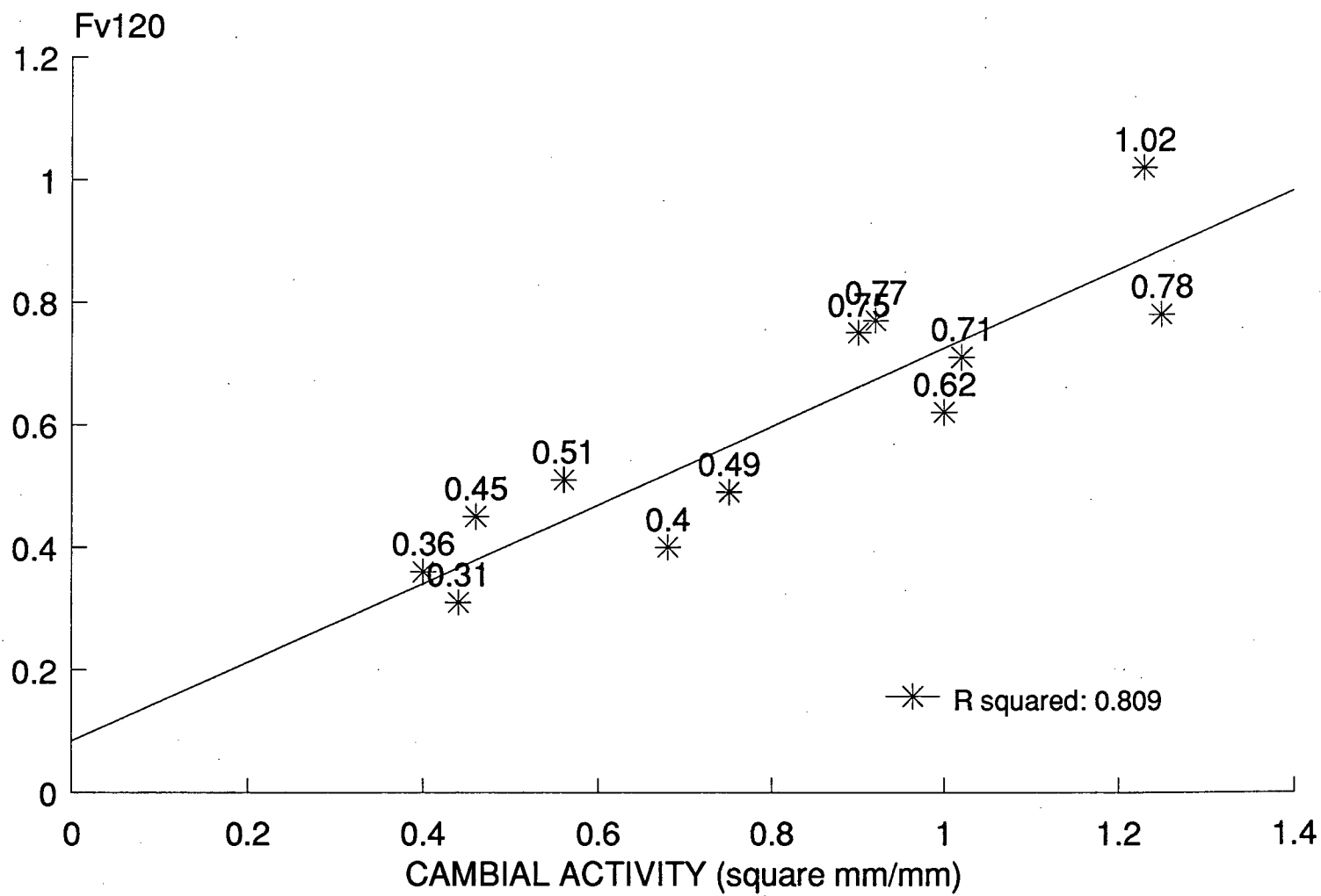


Figure 3.6.1.5. Scatter plot of Fv120 and cambial activity of untreated seedlings in mid-summer.

Falls/89

The reported findings are consistent with the stated hypothesis (1) that during the season of rapid wood formation, when the vascular cambium represents a major sink for photosynthates, photosynthetic activity as reflected by relative fluorescence amplitudes, is strongly correlated to cambial activity in seedlings.

In as much as chlorophyll fluorescence is generally considered to be a dissipative emission of radiative energy from excited chlorophyll pigments that has not been effectively transferred to a reaction center for use in photosynthetic processes, one might expect an inverse correlation between F_{var} and photosynthetic production. And low photosynthetic production is not a condition expected in plants with relatively active photosynthate sinks, such as highly active cambia.

However, the relationships between F_{var} yield and photosynthetic/photochemical yield are not nearly as simplistic as the expectations derived from the above model imply. Positive correlations between F_{var} and photochemical yields have been reported for intact angiosperm leaves (Weis and Berry, 1987) and temperate zone conifer seedlings (Vidaver *et al.*, 1988). There exist several possible mechanisms to account for a positive correlation between the F_{var} yields and the photosynthetic production. These mechanisms involve fluorescence quenching associated with low *in situ* CO_2 levels, PSII state changes associated with the phosphorylation of thylakoid proteins, interruption of electron flow from the oxygen evolving complex (OEC), and the level of inorganic phosphate (Pi) available to the Calvin cycle.

Chlorophyll fluorescence emissions have been closely correlated to CO_2 assimilation and concentrations in white spruce and other species (Toivonen and Vidaver, 1988; Walker *et al.*, 1983; Ireland 1984). Artificially lowered atmospheric CO_2 levels have resulted in

increased fluorescence emissions in pea leaves (Bradbury *et al.*, 1985), reportedly as a consequence of non-photochemical quenching. Similarly, spinach leaves subjected to a CO₂ depleted atmosphere increased fluorescence emissions (Sivak *et al.*, 1984).

The enhanced CO₂ uptake that would be associated with high photosynthetic activity might be expected to produce lower than atmospheric ambient CO₂ levels in associated photosynthetic tissues, and thus enhance Fvar in plants with high demands for carbon based photosynthetic products, such as the faster growing seedlings reported in this study, under conditions similar to those described by Sivak *et al.* (1984).

In contrast, Weis and Berry (1987) have proposed a transition of PSII from a state in which both fluorescence and photochemistry are high to a state where both are low, in response to lowered CO₂ levels, in intact sunflowers leaves.

Larsson *et al.*, (1986) and Deng and Melis, (1986), have suggested that phosphorylation of proteins of a high-fluorescent form of PSII, results in a state change to a low-fluorescence PSII form. Each of these proposals involves a positive correlation between fluorescence and photochemical efficiency. Such phosphorylation of proteins might be associated with a build-up of ATP in the thylakoids if a major sink, such as the cambium of a low-vigour seedling, was relatively inactive.

Alternatively, Keck and Boyer (1974), and Toivonen (1985) have reported that in water-stressed seedlings, a reduction in M fluorescence is associated with lower ATP production, when water-splitting declines to the point where there is an insufficient flow of electrons to maintain the trans-thylakoid electrochemical membrane potential.

The level of inorganic phosphate also impinges on Fvar emissions. P_i , used in light reaction ATP synthesis, is an important component of phosphorylation potential ($ATP/ADP \cdot P_i$), and its relative concentration may be reflected by the fluorescence induction curves (Horton, 1983). If low inorganic P_i levels directly impinge on photosynthetic production, it is conceivable that low fluorescence seedlings were being limited in photosynthate production by relatively low (P_i) levels at the thylakoids, due to lower levels of available phosphate in the soil bed.

A final mechanism by which low photochemical production may be linked to low Fvar amplitudes, is the interruption or blockage of electron flow from the oxygen evolving complex, to P680. If excited electrons do not reach P680, Fvar will not occur. Vidaver (pers. comm.) has suggested that such a blocking mechanism indeed occurs in white spruce, and is responsible for the apparent positive correlation between photochemistry and Fvar in this species.

Having established a correlation between cambial activity and Fvar amplitudes in the present study, and having described several mechanisms to account for a positive correlation between Fvar and photochemical production, it appears clear that source (vascular cambium) and sink (thylakoid photochemistry) are linked in this system. The regression analyses strongly imply that there exists a relationship between Fvar and cambial activity, but it is important to note that these types of analyses cannot establish a biological cause and effect phenomenon (Zar 1984).

The next considerations relate to the nature of the relationship, and plausible sources of the variabilities. For the reasons stated above, the present study cannot provide data to answer these questions, but speculations will be offered.

The introductory chapter identified a variety of environmental factors, such as temperature, humidity, light intensity, gas concentrations, daylength, and humidity that impinge on cambial activity either directly, or as a consequence of crown and root mediated growth regulator (eg IAA, sucrose, GA, and cytokinins) synthesis and/or release. In as much as the seedlings were grown under relatively similar conditions in a glasshouse, it seems unlikely that the environmental cues (eg temperature, photoperiod, humidity, etc) experienced by each seedling were significantly different, and so variations in environmental conditions are not considered to be likely sources for the variations in cambial and photosynthetic activity.

The seedlings were of the same seedlot but not genetically identical, thus genetically derived variability in cambial and/or photosynthetic activities may be a source of variation. A regression analysis using xylem production in the previous year as the independent variable, and xylem production in the current year as the dependent variable, yields an R^2 value of 0.609, indicating that variability in xylem production in the previous year may predict 60% of the variability in xylem production in the current year. However, this issue is not clear for, as will be shown in section 3.6.3, the R^2 values for the regression between the current year cambial activity and the total cross-sectional (xylem) areas of the 1987 and 1986 stems were 0.376 ($p = 0.034$) and 0.007 ($p = 0.796$), indicating a weak relationship.

An alternative explanation is based on individual variations in the relatively high sensitivity of white spruce to temperature and water stresses. Toivonen and Vidaver (1988), have demonstrated that white spruce will curtail photosynthetic activity (and F_{var}) in response to water stress. During a period of particularly hot weather, white spruce seedlings might spend considerable periods in a state of photosynthetic inactivity, and this might be expected to impinge on the quantity of photosynthate available for

xylem synthesis in the stem. In this context, variability in the responsiveness of seedlings (in terms of shutting down their photochemistry) to changes in, for example, humidity, would lead to variations in stem development simply on the basis of carbohydrate supplies.

Alternatively, the cambia themselves might become dormant under stress, thus impinging on photosynthetic activity by reducing the demand for photosynthates.

A primary intent of this study was to investigate the possible existence of a relationships between cambial and photosynthetic activities, particularly during the period of rapid wood formation. At this point, it appears that a relatively strong and demonstrable correlation exists between the two activities during the period of rapid wood formation. This information strongly supports the original hypothesis, and may have implications in forest tree production and management.

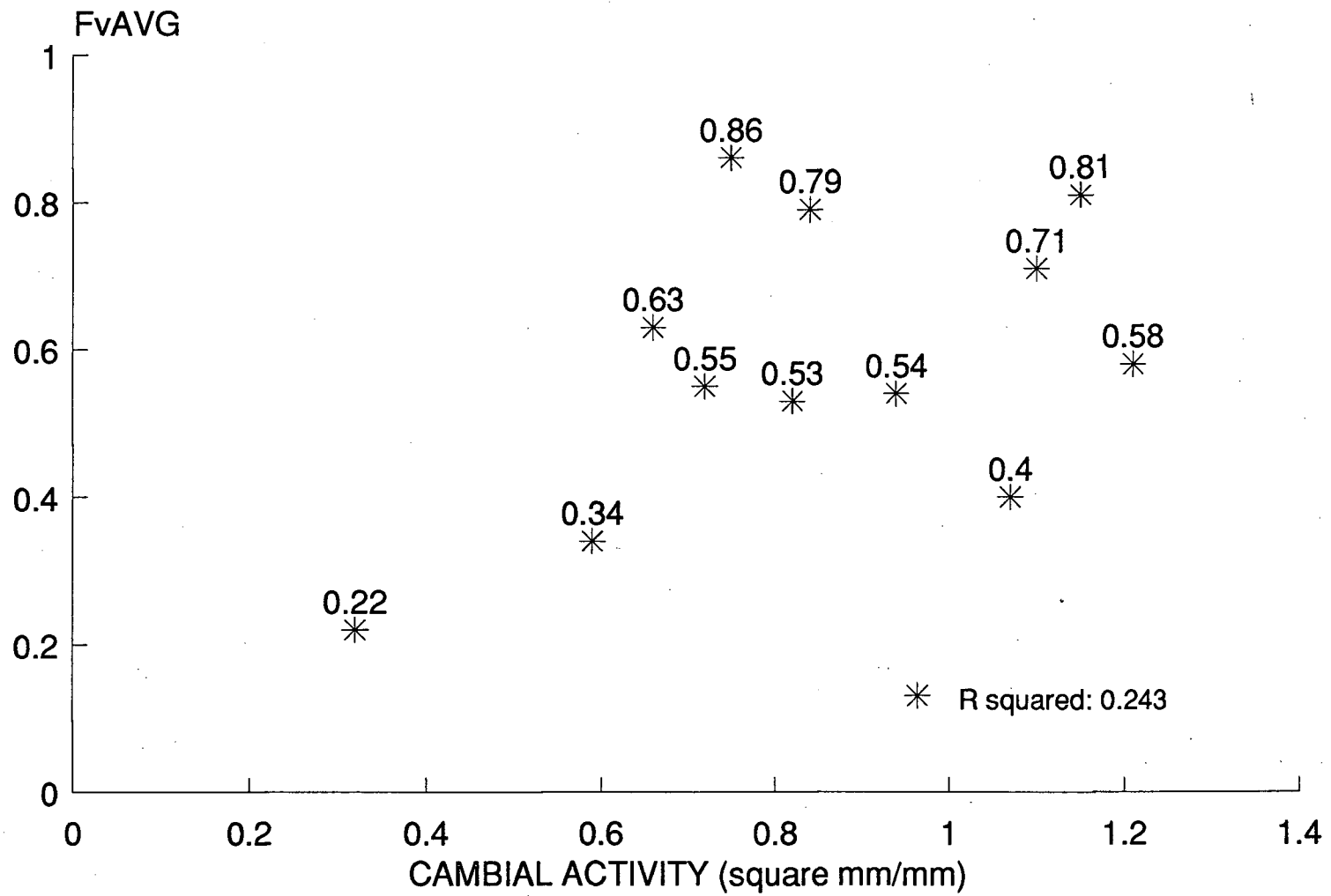
Table 3.6.1.2 presents analyses for regressions between cambial activity and Fvar parameters in mid-summer for the incised group. R^2 values indicate that the relatively strong relationship between cambial activity and Fvar demonstrated in the untreated group, are not apparent in the incised group.

Table 3.6.1.2. Regression analyses of cambial activity and fluorescence parameters during mid-summer in the incised group.

Statistic	O-level	Time to M ¹	FvM	FvT	FvAVG	Fv120
R	0.226	0.014	0.276	0.500	0.493	0.515
R ²	0.051	N.A.	0.076	0.250	0.243	0.265
SE	0.609	N.A.	0.164	0.166	0.178	0.191
α	5.279	N.A.	0.700	0.107	0.267	0.252
β	-0.515	N.A.	0.172	0.351	0.369	0.420
T	-0.732	N.A.	0.907	1.827	1.792	-0.732
P	0.481	N.A.	0.386	0.098	0.103	0.481
ANOVA <i>F</i>	0.536	N.A.	0.823	3.338	3.212	0.536

1. These data are non-parametric. A Spearman Rank coefficient replaces R. The notation N.A. indicates the parametric statistical terms are not applicable.

The weakness in the relationship between any of the Fvar values and cambial activity ($R^2 = 0.250, 0.243, 0.265$ for FvT, FvAVG, and Fv120 respectively) is likely a consequence of a complex of factors. The scatter plot presented Figure 3.6.1.6 shows that the relatively strong relationship between FvAVG and cambial activity demonstrated in the untreated seedlings, is not apparent in the incised seedlings. Extensive incisions were expected to increase the potential for dessication, interrupt normal photosynthate translocation patterns, and induce a wound response. Cambial activity is often arrested following wounding (Savidge and Farrar, 1984), and this response, along with the above mentioned impingements on the normal physiological state, has likely reduced the relative significance of the cambium as a sink for photosynthates.



Falls/89

Figure 3.6.1.6. Scatter plot of FvAVG and cambial activity of incised seedlings in mid-summer.

Table 3.6.1.3 presents regressions for the tilted seedlings in mid-summer, and once again, the treatment has appeared to interrupt normal relationships as demonstrated in the untreated group. Tilting appears to have an even greater impact on cambial activity - fluorescence parameter relations than incising, resulting in negligible R^2 values for all Fvar parameters.

Table 3.6.1.3. Regression analyses of cambial activity and fluorescence parameters during mid-summer in the tilted group.

Statistic	O-level	Time to M ¹	FvM	FvT	FvAVG	Fv120
R	0.011	0.618	0.223	0.052	0.038	0.017
R^2	0.000	N.A.	0.054	0.003	0.001	0.000
SE	1.128	N.A.	0.215	0.161	0.189	0.214
α	5.197	N.A.	1.025	0.371	0.605	0.630
β	0.048	N.A.	-0.199	0.033	-0.028	-0.014
T	0.033	N.A.	-0.720	0.157	-0.115	-0.052
P	0.974	N.A.	0.490	0.878	0.911	0.960
ANOVA <i>F</i>	0.001	N.A.	0.519	0.025	0.013	0.003

1. These data are non-parametric. A Spearman Rank coefficient replaces R. The notation N.A. indicates the parametric statistical terms are not applicable.

Figure 3.6.1.7 demonstrates the lack of correlation between cambial activity and FvAVG in the leaders of the tilted group.

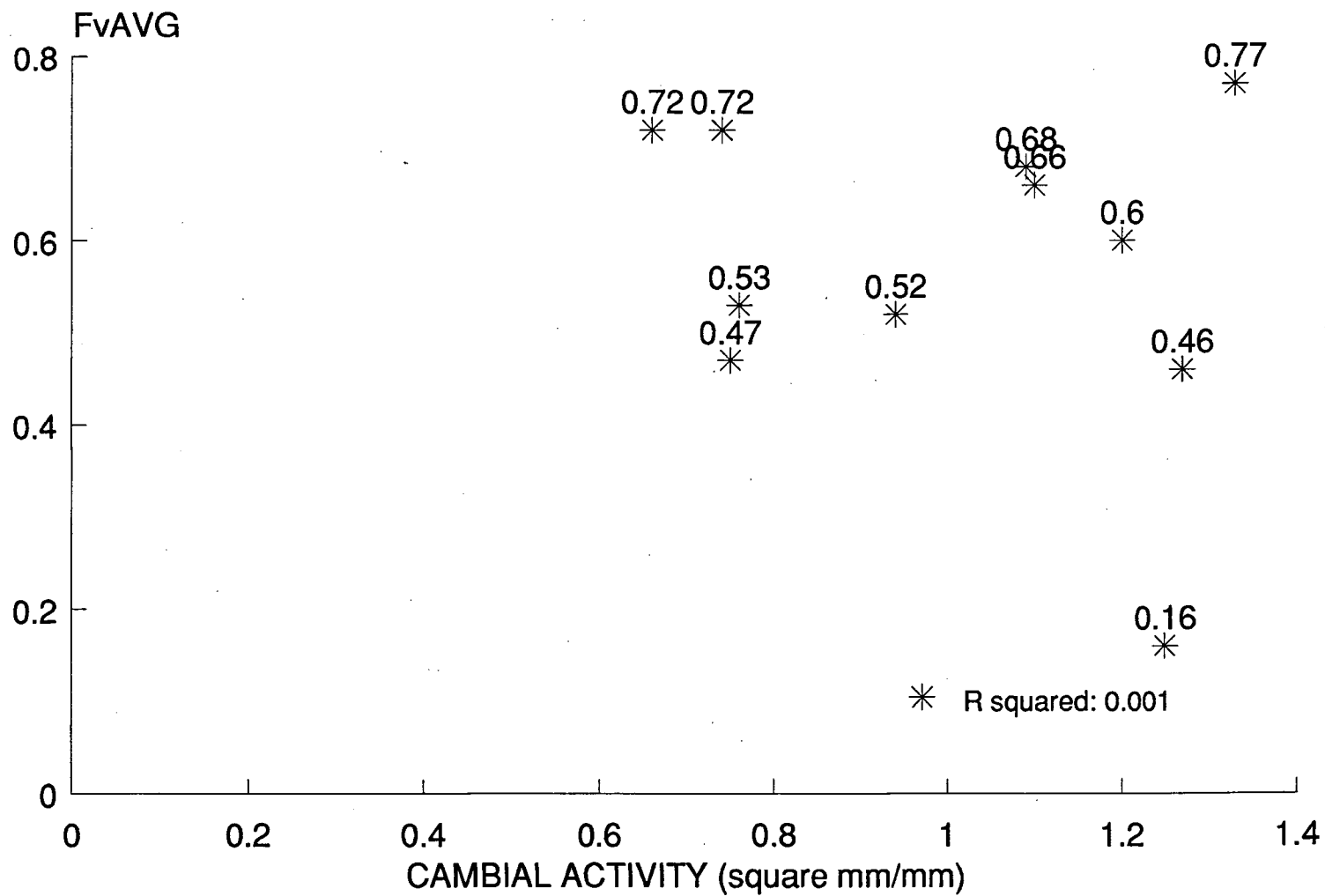


Figure 3.6.1.7. Scatter plot of FvAVG and cambial activity of tilted seedlings in mid-summer.

Falls/89

A more likely reason for this is a loss of apical dominance of the leader, which was the portion of the stem used for Fvar and CO₂ assimilation measurements. Initially, before compression wood was formed to "right" the stem, the leader was well below what were the lower whorl laterals before tilting. Furthermore, the lowered leaders were more apt to be shaded when the sun was low on the horizon, further affecting their relative roles as photosynthetic elements. Measures of leader weights of tilted seedlings were approximately 17.4% lower than those of the untreated group, and while not statistically significant in this sample, this does suggest the possibility of reduced meristematic as well as photosynthetic activity.

Fvar parameter - cambial activity regressions in late-summer.

Fvar induction curves for the untreated group in late-summer (Table 3.6.1.4) were characterized by a delayed M peak that made the Fv120 value an inappropriate parameter. The balance of Fvar parameters, e.g. FvAVG (Figure 3.6.1.8) demonstrated a very weak or non-existent relationship between cambial activity and photosynthetic activity in late-summer. It is expected that in late-summer, wood formation is complete for seedlings of this (northern) origin, and that photosynthetic activity is declining significantly (Vidaver *et al.* 1989). Thus with the cambium being a much diminished "sink" at this time, relationships with photosynthetic activity were not expected.

The treated groups (Tables 3.6.1.5 and 3.6.1.6), similarly and predictably, demonstrated no significant relationship between cambial activity and fluorescence parameters during this season.

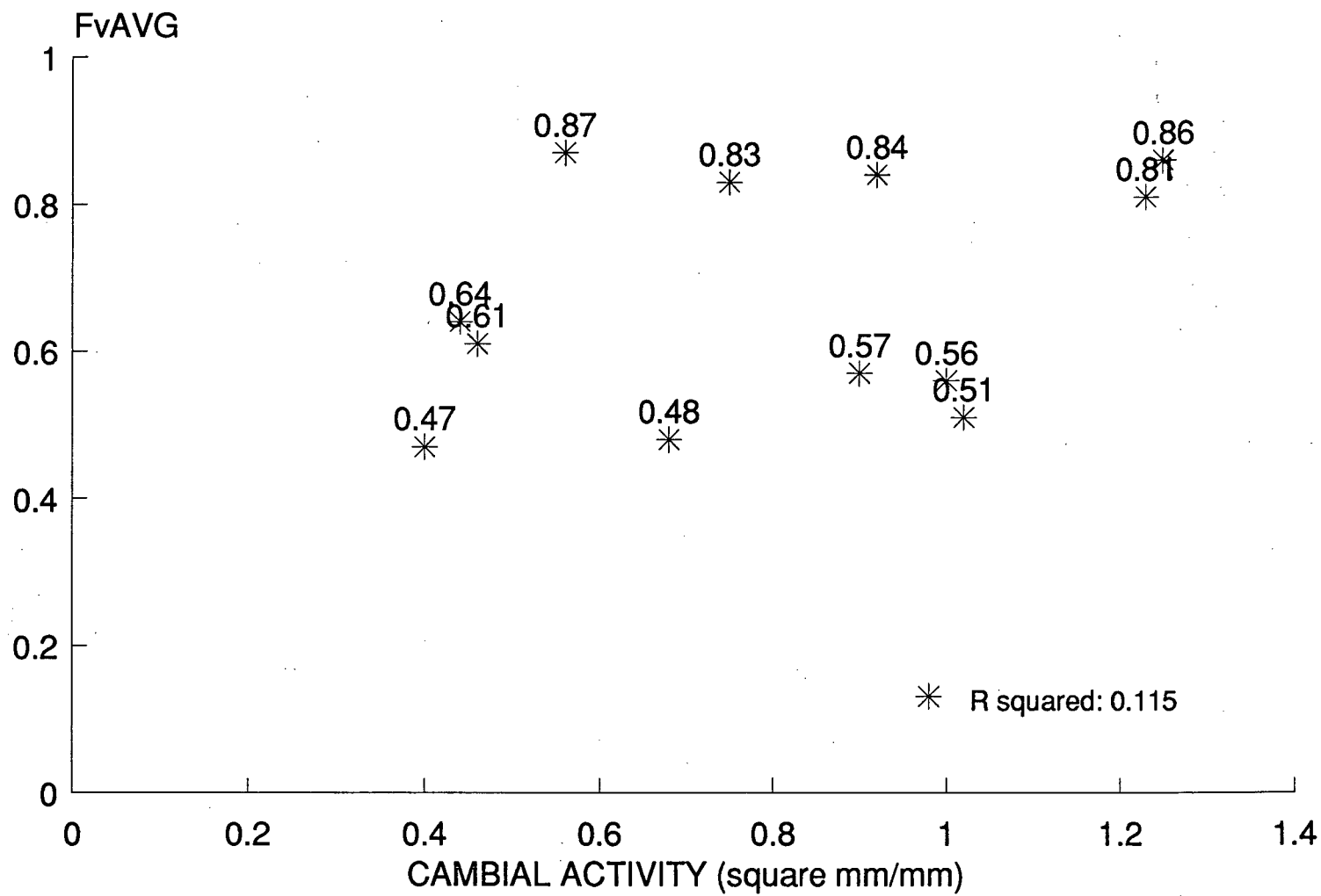


Figure 3.6.1.8. Scatter plot of FvAVG and cambial activity of untreated seedlings in late-summer.

Falls/89

Table 3.6.1.4. Regression analyses of cambial activity and fluorescence parameters during late-summer in the untreated group.

Statistic	O-level	Time to M ¹	FvM	FvT	FvAVG
R	0.060	-0.455	0.275	0.417	0.339
R²	0.004	N.A.	0.076	0.174	0.115
SE	1.507	N.A.	0.165	0.150	0.157
α	10.344	N.A.	0.708	0.293	0.526
β	0.292	N.A.	0.151	0.219	0.181
T	-0.192	N.A.	0.906	1.453	1.139
P	0.852	N.A.	0.386	0.177	0.281
ANOVA <i>F</i>	0.037	N.A.	0.820	2.111	1.298

Table 3.6.1.5. Regression analyses of cambial activity and fluorescence parameters during late-summer in the incised group.

Statistic	O-level	Time to M ¹	FvM	FvT	FvAVG
R	0.251	0.236	0.329	0.437	0.400
R²	0.063	N.A.	0.108	0.191	0.160
SE	1.339	N.A.	0.178	0.169	0.176
α	9.085	N.A.	0.632	0.190	0.411
β	1.204	N.A.	0.215	0.285	0.267
T	0.778	N.A.	1.045	1.459	1.311
P	0.457	N.A.	0.323	0.178	0.222
ANOVA <i>F</i>	0.605	N.A.	1.092	2.130	1.719

1. These data are non-parametric. A Spearman Rank coefficient replaces R. The notation N.A. indicates the parametric statistical terms are not applicable.

Table 3.6.1.6. Regression analyses of cambial activity and fluorescence parameters during late-summer in the tilted group.

Statistic	O-level	Time to M ¹	FvM	FvT	FvAVG
R	0.140	0.727	0.314	0.203	0.011
R ²	0.020	N.A.	0.099	0.041	0.000
SE	2.084	N.A.	0.168	0.138	0.146
α	0.781	N.A.	0.711	0.643	0.750
β	39.096	N.A.	0.215	-0.111	-0.006
T	0.424	N.A.	0.992	0.622	-0.034
P	0.682	N.A.	0.347	0.549	0.974
ANOVA F	0.180	N.A.	0.985	0.387	0.001

1. These data are non-parametric. A Spearman Rank coefficient replaces R. The notation N.A. indicates the parametric statistical terms are not applicable.

Fvar parameter - cambial activity regressions in autumn.

As predicted, regression analyses between cambial activity and Fvar parameters in autumn (with cambial activity arrested) yielded no significant relationships in any of the three treatment groups (Tables 3.6.1.7, 3.6.1.8, and 3.6.1.9). Figure 3.6.1.9 demonstrates the lack of correlation between cambial activity and FvAVG in the untreated group at this time.

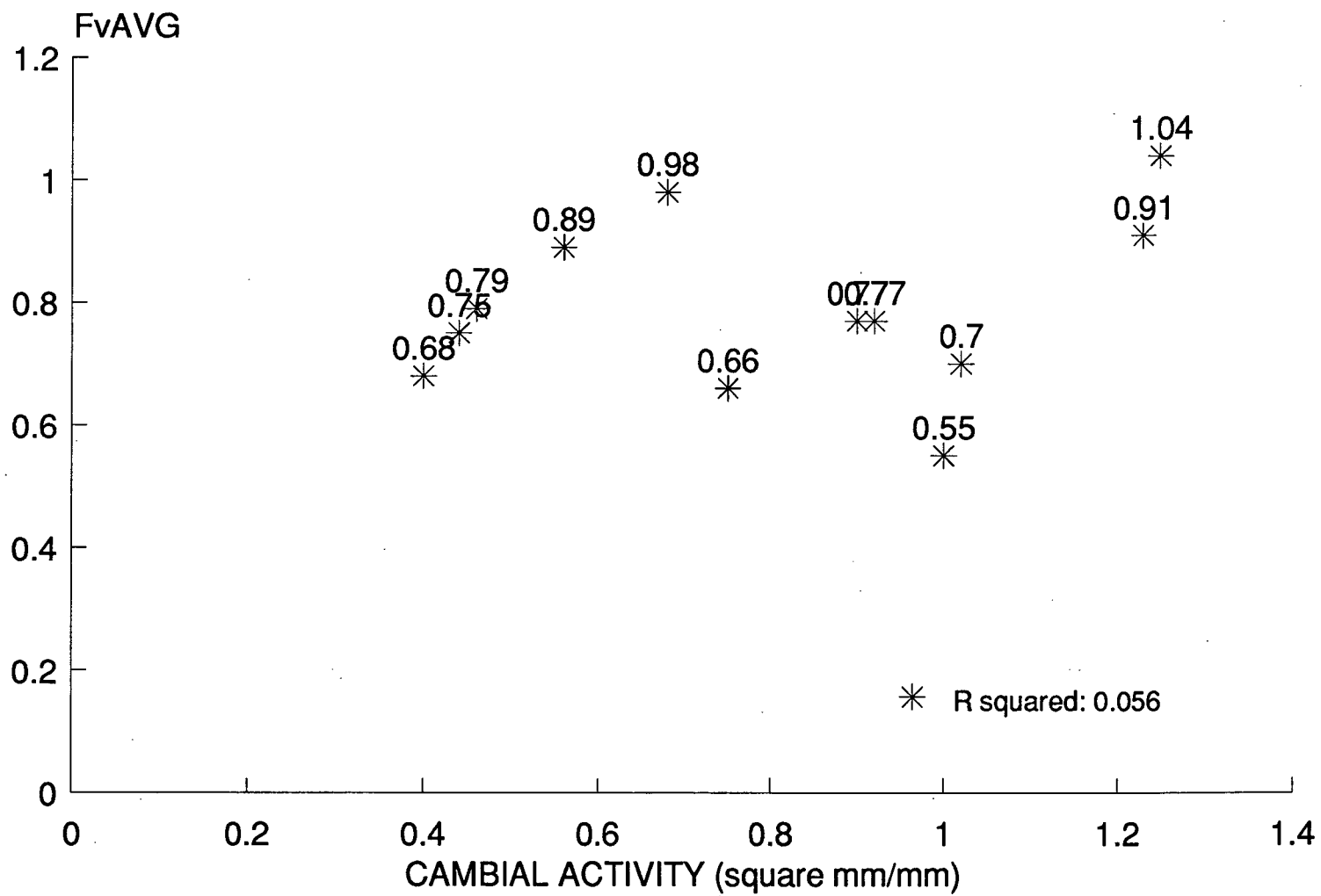


Figure 3.6.1.9. Scatter plot of FvAVG and cambial activity of untreated seedlings in autumn.

Falls/89

Table 3.6.1.7. Regression analyses of cambial activity and fluorescence parameters during autumn in the untreated group.

Statistic	O-level	Time to M ¹	FvM	FvT	FvAVG
R	0.218	-0.427	0.096	0.177	0.237
R ²	0.047	N.A.	0.009	0.031	0.056
SE	1.180	N.A.	0.179	0.124	0.144
α	8.376	N.A.	0.893	0.830	0.701
β	0.842	N.A.	0.055	0.071	0.112
T	0.706	N.A.	0.305	0.568	0.771
P	0.496	N.A.	0.766	0.583	0.459
ANOVA <i>F</i>	0.498	N.A.	0.093	0.322	0.594

Table 3.6.1.8. Regression analyses of cambial activity and fluorescence parameters during autumn in the incised group.

Statistic	O-level	Time to M ¹	FvM	FvT	FvAVG
R	0.356	-0.154	0.031	0.128	0.660
R ²	0.127	N.A.	0.001	0.016	0.004
SE	1.072	N.A.	0.162	0.233	0.194
α	8.326	N.A.	0.915	0.958	0.759
β	1.492	N.A.	0.018	-0.110	0.043
T	1.204	N.A.	0.098	-0.407	0.190
P	0.256	N.A.	0.924	0.692	0.853
ANOVA <i>F</i>	1.450	N.A.	0.010	0.166	0.036

1. These data are non-parametric. A Spearman Rank coefficient replaces R. The notation N.A. indicates the parametric statistical terms are not applicable.

Table 3.6.1.9. Regression analyses of cambial activity and fluorescence parameters during autumn in the tilted group.

Statistic	O-level	Time to M ¹	FvM	FvT	FvAVG
R	0.278	-0.355	0.134	0.221	0.389
R ²	0.077	N.A.	0.012	0.049	0.152
SE	1.487	N.A.	0.101	0.163	0.103
α	7.620	N.A.	0.896	0.767	0.641
β	1.663	N.A.	0.053	0.143	0.168
T	0.869	N.A.	0.405	0.681	1.268
P	0.408	N.A.	0.695	0.513	0.237
ANOVA <i>F</i>	0.755	N.A.	0.164	0.464	1.607

1. These data are non-parametric. A Spearman Rank coefficient replaces R. The notation N.A. indicates the parametric statistical terms are not applicable.

3.6.2 Corrected and uncorrected CO₂ uptake, and estimated chlorophyll.

No substantive relationship was detected between CO₂ uptake and cambial activity, at any of the three measurement periods (Tables 3.6.2.1 - 3.6.2.9). Excepting relatively trivial amounts of carbon available from carbohydrates in the soil, atmospheric CO₂ is the sole source of carbon for the biosynthesis of xylem tissue. It might be expected that relatively high rates of xylem formation would require relatively high rates of CO₂ uptake, and that this gas exchange activity could be exploited to identify vigorously developing plants. However, in this system, such a relationship between cambial activity and CO₂ exchange was not revealed. This result may not be so anomalous however, as it is understood that CO₂ exchange involves a large portion of carbon traffic that is unrelated to cambial activity, for example the assimilation of carbon for the production of

storage compounds, and the release of carbon from respiratory processes of living tissues that would be enclosed in the gas exchange chamber (i.e. the leaves, phloem, vascular cambium, and xylem of the leader). It is quite possible that even a substantial enhancement in assimilation that might occur as a consequence of the higher photosynthate requirements of relatively active cambia would be undetectable within the context of large volume of carbon traffic associated with a variety of metabolic processes which produce and consume CO_2 .

Further difficulty in determining accurate CO_2 uptake rates may have arisen from variabilities in stomatal conductance, which would affect apparent CO_2 transfer into the foliage. Stomatal conductance was not measured or corrected for in this study.

Beyond the apparent inability to detect a significant relationship between CO_2 uptake and cambial activity, the gas exchange procedures require extensive handling of the seedlings which can induce changes in translocation patterns and xylem development (Kellogg and Steucek, 1977). Furthermore, they are time consuming, and produce few data when compared to the procedures associated with fluorescence measurements. In any case, CO_2 uptake (either with or without correction to chlorophyll *a* content) using the system as described, is not recommended as a means of elucidating source:sink relations in conifer seedlings, or for assessing stem vigour non-invasively.

Estimates of chlorophyll content of the sampled foliage, derived from O-level fluorescence indicate a doubling of chlorophyll from the mid-summer to late-summer period in all three groups. No evidence was provided by the regression analyses for a relationship between chlorophyll *a* development and cambial activity (Tables 3.6.2.1 to 3.6.2.9) in white spruce.

Table 3.6.2.1. Regression analyses of cambial activity and CO₂ uptake and estimated chlorophyll during mid-summer in the untreated group.

Statistic	Uncorrected CO ₂ Uptake	Chlorophyll Content	Corrected CO ₂ Uptake
R	0.313	0.001	0.382
R ²	0.098	0.033	0.146
SE	5.899	0.021	46.318
α	3.727	0.130	23.260
β	6.217	-0.012	61.174
T	1.043	-0.586	1.308
P	0.321	0.571	0.220
ANOVA F	1.089	0.344	1.710

Table 3.6.2.2. Regression analyses of cambial activity and CO₂ uptake and estimated chlorophyll during mid-summer in the incised group.

Statistic	Uncorrected CO ₂ Uptake	Chlorophyll Content	Corrected CO ₂ Uptake
R	0.439	0.226	0.486
R ²	0.192	0.051	0.236
SE	6.633	0.017	48.713
α	2.460	0.131	19.822
β	11.832	-0.014	98.869
T	1.544	-0.732	1.757
P	0.154	0.481	0.110
ANOVA F	2.383	0.536	3.085

Table 3.6.2.3. Regression analyses of cambial activity and CO₂ uptake and estimated chlorophyll during mid-summer in the tilted group.

Statistic	Uncorrected CO ₂ Uptake	Chlorophyll Content	Corrected CO ₂ Uptake
R	0.350	0.011	0.448
R ²	0.122	0.000	0.201
SE	4.409	0.031	32.095
α	13.448	0.129	115.077
β	-6.360	0.001	-62.135
T	-1.120	0.033	-1.503
P	0.292	0.974	0.167
ANOVA <i>F</i>	1.254	0.001	2.259

Table 3.6.2.4. Regression analyses of cambial activity and CO₂ uptake and estimated chlorophyll during late-summer in the untreated group.

Statistic	Uncorrected CO ₂ Uptake	Chlorophyll Content	Corrected CO ₂ Uptake ¹
R	0.420	0.060	0.476
R ²	0.176	0.004	N.A.
SE	6.052	0.041	N.A.
α	18.309	0.269	N.A.
β	-8.949	-0.008	N.A.
T	-1.464	-0.192	N.A.
P	0.174	0.852	N.A.
ANOVA <i>F</i>	2.143	0.037	N.A.

1. These data are non-parametric. A Spearman Rank coefficient replaces R. The notation N.A. indicates the parametric statistical terms are not applicable.

Table 3.6.2.5. Regression analyses of cambial activity and CO₂ uptake and estimated chlorophyll during late-summer in the incised group.

Statistic	Uncorrected CO ₂ Uptake	Chlorophyll Content	Corrected CO ₂ Uptake ¹
R	0.565	0.251	0.527
R ²	0.319	0.063	N.A.
SE	4.294	0.037	N.A.
α	6.817	0.235	N.A.
β	10.201	0.033	N.A.
T	2.055	0.778	N.A.
P	0.070	0.457	N.A.
ANOVA <i>F</i>	4.220	0.605	N.A.

Table 3.6.2.6. Regression analyses of cambial activity and CO₂ uptake and estimated chlorophyll during late-summer in the tilted group.

Statistic	Uncorrected CO ₂ Uptake	Chlorophyll Content	Corrected CO ₂ Uptake ¹
R	0.028	0.140	0.191
R ²	0.001	0.020	N.A.
SE	10.274	0.057	N.A.
α	8.194	0.226	N.A.
β	1.124	0.031	N.A.
T	0.085	0.424	N.A.
P	0.934	0.682	N.A.
ANOVA <i>F</i>	0.007	0.180	N.A.

1. These data are non-parametric. A Spearman Rank coefficient replaces R. The notation N.A. indicates the parametric statistical terms are not applicable.

Table 3.6.2.7. Regression analyses of cambial activity and CO₂ uptake and estimated chlorophyll during autumn in the untreated group.

Statistic	Uncorrected CO ₂ Uptake	Chlorophyll Content ¹	Corrected CO ₂ Uptake
R	0.194	0.267	0.229
R ²	0.038	N.A.	0.053
SE	6.818	N.A.	30.758
α	19.151	N.A.	86.860
β	-4.312	N.A.	-23.134
T	-0.626	N.A.	-0.745
P	0.545	N.A.	0.474
ANOVA <i>F</i>	0.392	N.A.	0.554

Table 3.6.2.8. Regression analyses of cambial activity and CO₂ uptake and estimated chlorophyll during autumn in the incised group.

Statistic	Uncorrected CO ₂ Uptake	Chlorophyll Content ¹	Corrected CO ₂ Uptake
R	0.361	0.399	0.470
R ²	0.130	N.A.	0.221
SE	5.357	N.A.	21.846
α	22.869	N.A.	103.114
β	-7.577	N.A.	-42.475
T	-1.224	N.A.	-1.683
P	.249	N.A.	0.123
ANOVA <i>F</i>	1.499	N.A.	2.831

1. These data are non-parametric. A Spearman Rank coefficient replaces R. The notation N.A. indicates the parametric statistical terms are not applicable.

Table 3.6.2.9. Regression analyses of cambial activity and CO₂ uptake and estimated chlorophyll during autumn in the tilted group.

Statistic	Uncorrected CO ₂ Uptake	Chlorophyll Content ¹	Corrected CO ₂ Uptake
R	0.006	0.151	0.179
R ²	0.000	N.A.	0.032
SE	5.210	N.A.	21.890
α	16.676	N.A.	86.134
β	0.120	N.A.	-15.421
T	0.018	N.A.	-0.547
P	0.986	N.A.	5.980
ANOVA <i>F</i>	0.000	N.A.	0.299

1. These data are non-parametric. A Spearman Rank coefficient replaces R. The notation N.A. indicates the parametric statistical terms are not applicable.

3.6.3 Cambial activity and Seedling Dimensions Correlations.

Tables 3.6.3.1 - 3.6.3.3 provide the results of correlation analyses between the current season's cambial activity and four seedling dimensions. The objective of these analyses was to determine if there is a basis in specific stem dimensions for culling or grading seedlings for current years' stem vigour. This was examined by calculating the strength of correlations between the current year's cambial activity, (as a measure of stem vigour) and: (a) the cross-sectional area of the first year (1986) stem segment; (b) the cross-sectional area of the second year (1987) stem segment; (c) the overall preseason stem length; and (d) the current year's leader length.

Table formatting and the reported statistics are consistent with the previous section in order to facilitate comparisons. Even though a primary intent was to determine a correlation coefficient (R), by squaring R, the strength of the straight-line relationship was also tested.

Table 3.6.3.1. Regression analyses of cambial activity and seedling dimensions in the untreated group.

Statistic	Area 1987 Stem	Area 1986 Stem	Seedling Height	Leader Length
R	0.613	0.084	0.038	0.648
R ²	0.376	0.007	0.001	0.420
SE	0.247	0.312	0.313	0.239
α	0.034	0.731	0.730	-0.219
β	0.191	0.012	0.000	0.086
T	2.452	0.266	0.120	2.688
P	0.034	0.796	0.907	0.023
ANOVA <i>F</i>	6.013	0.071	0.014	7.228

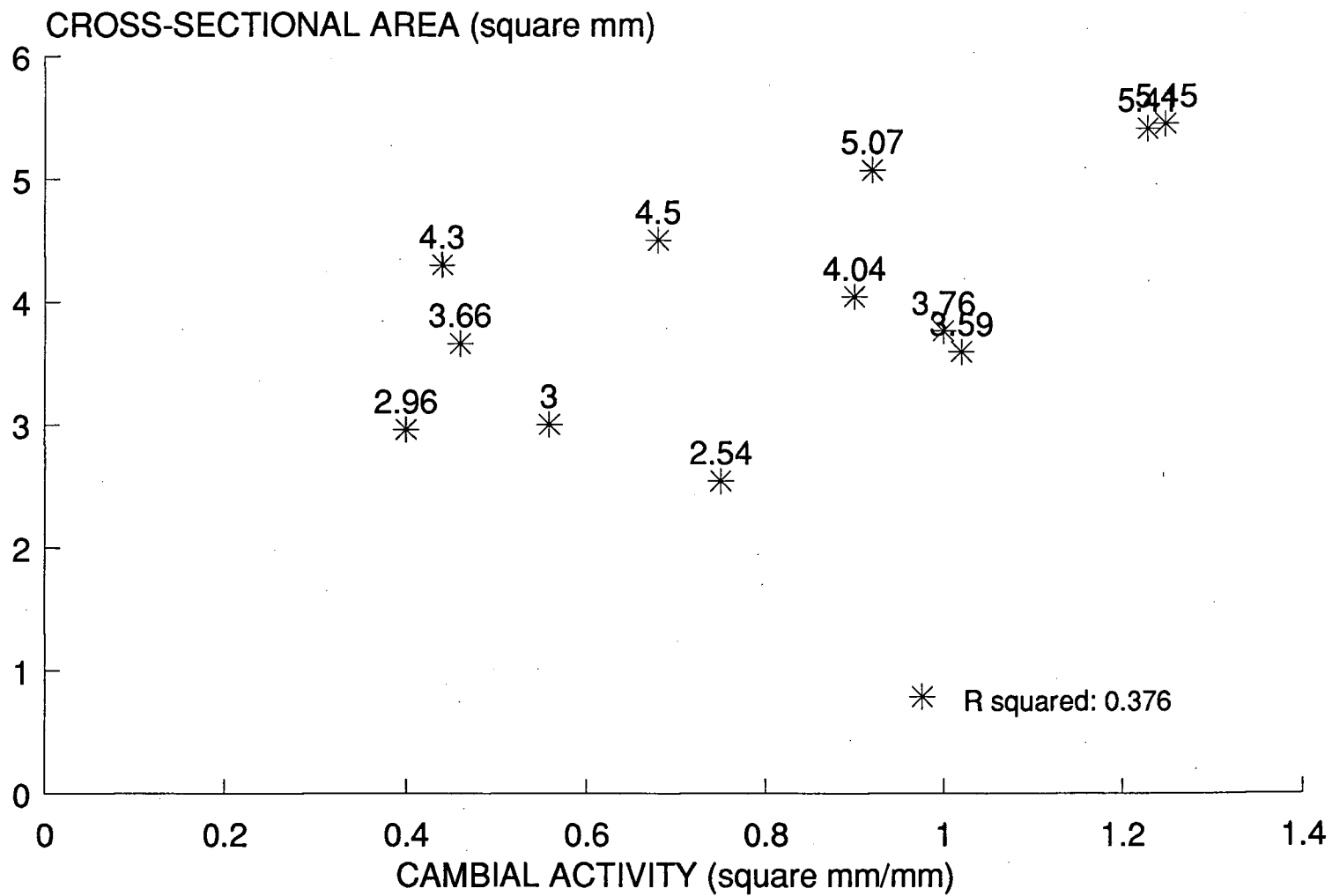
Table 3.6.3.2. Regression analyses of cambial activity and seedling dimensions in the incised group.

Statistic	Area 1987 Stem	Area 1986 Stem	Seedling Height	Leader Length
R	0.092	0.232	0.147	0.173
R ²	0.009	0.054	0.022	0.030
SE	0.273	0.266	0.271	0.270
α	0.774	0.624	1.091	0.639
β	0.013	0.032	-0.010	0.018
T	0.293	0.753	-0.469	0.554
P	0.775	0.469	0.649	0.591
ANOVA <i>F</i>	0.086	0.567	0.220	0.307

Table 3.6.3.3. Regression analyses of cambial activity and seedling dimensions in the tilted group.

Statistic	Area 1987 Stem	Area 1986 Stem	Seedling Height	Leader Length
R	0.331	0.218	0.268	0.059
R ²	0.109	0.048	0.072	0.003
SE	0.244	0.253	0.249	0.258
α	1.259	1.192	0.463	1.116
β	-0.052	-0.028	0.025	-0.009
T	-1.051	-0.672	0.835	-0.176
P	0.321	0.519	0.425	0.864
ANOVA <i>F</i>	1.105	0.451	0.697	0.031

These results indicate that the preseason seedling dimensions used in this analysis, i.e. measures of the 1987 (Figure 3.6.3.1) and 1986 stem segment cross sectional areas ($R^2 = 0.376$ and 0.007 respectively), and the seedling height before the current year's growing season ($R^2 = 0.001$), have little value in predicting the current year's stem vigour. This finding places in question any culling and selection practices based on diameter and height dimensions, with the possible exception of current season's leader length (Figure 3.6.3.2), which yielded a weak ($R^2 = 0.420$) relationship to cambial activity.



Falls/89

Figure 3.6.3.1. Scatter plot of 1987 stem area and cambial activity of untreated seedlings.

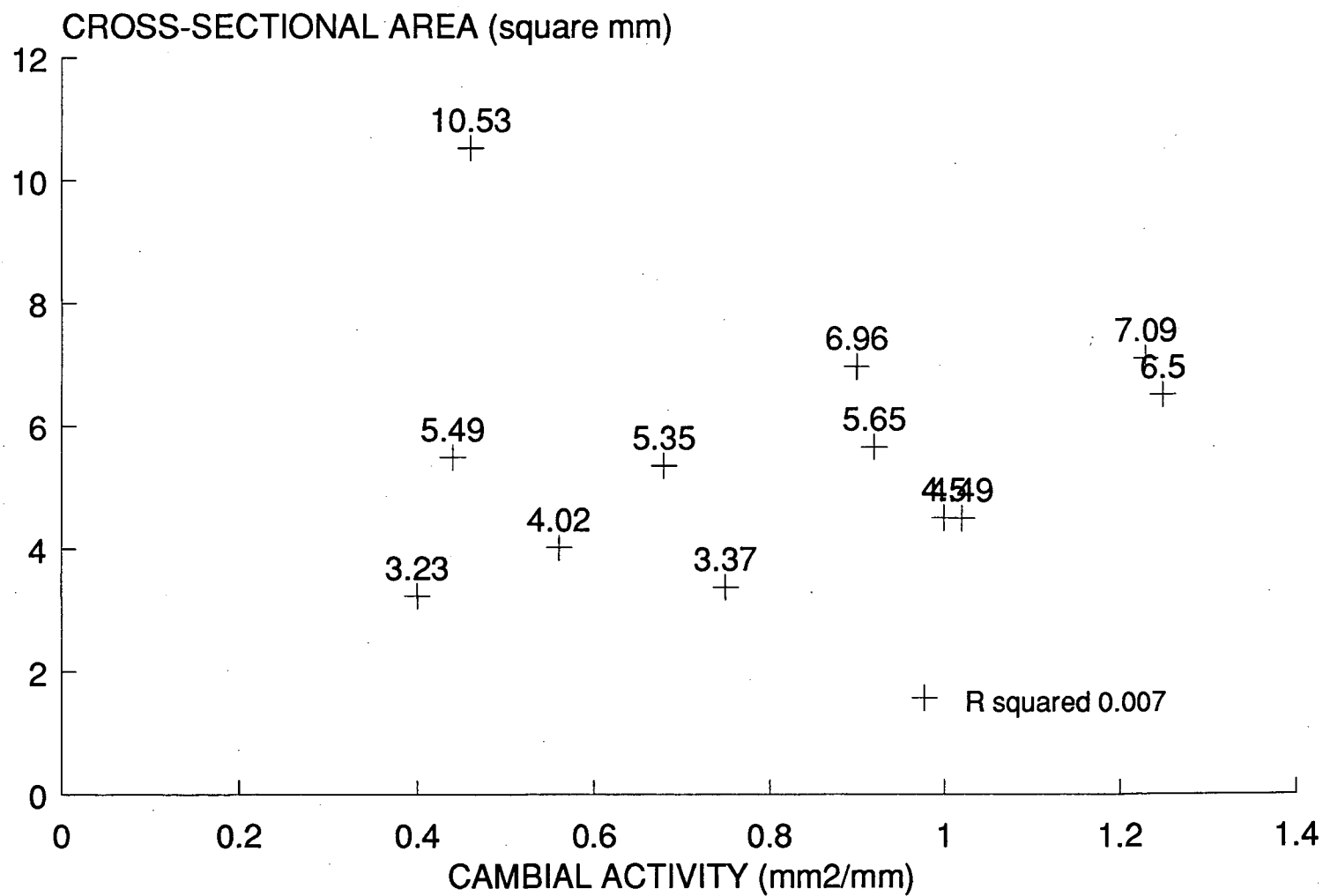


Figure 3.6.3.2. Scatter plot of 1986 stem area and cambial activity of untreated seedlings.

Falls/89

3.6.4 Summary of Results of Regression Analyses

The results of the regression analyses of section 3.6.1 and 3.6.2 demonstrate that during the period of active wood formation, specific measures of photosynthetic activity, i.e. specified fluorescence parameters,¹ are weakly to strongly correlated to cambial activity. This relationship is severely diminished or eliminated following the wood formation period, and under circumstances of wounding or geotropic stressing. The results strongly support the original hypotheses that:

1. Photosynthetic activity, as measured by chlorophyll *a* fluorescence, is correlated to cambial activity in the wood formation season in white spruce seedlings; and
2. Measures of chlorophyll *a* fluorescence (but not CO₂ uptake as described) provide an opportunity for non-invasive assessment of the current stem vigour (as measured by cambial activity) in white spruce seedlings; and
3. Correlations between chlorophyll *a* fluorescence and current season's cambial activity are diminished when wood formation is arrested, i.e. when the vascular cambium ceases to be a significant and active sink for photosynthates (in late summer and fall); and
4. The "normal" relationship between cambial activity and fluorescence parameters occurring in untreated seedlings is severely diminished by stressing as a result of either substantial stem wounding (incision) or abnormal gravitational orientation (tilting), although the abnormal relations exhibited under these circumstances may have been indirectly related to altered cambial activity.

1. Carbon dioxide gas exchange was not found to be measurably correlated to the cambial activity of any of the treatment groups, at any time, in this experimental system.

3.7 ELECTROPHORETIC ANALYSIS AND IMMUNOBLOTTING OF CHLOROPLAST MEMBRANE PROTEINS

The initial intent of the protein analysis was to investigate the relationships between altered cambial activity and the relative occurrence of Rubisco and Coupling Factor, which play key roles in the dark and light reactions and processes of photosynthesis. In as much as the treatments did not significantly alter cambial activity, the protein analyses could neither confirm nor deny the existence such relationships. Furthermore, as the treatments did not appear to affect mean Fvar values within the treated groups, these analyses could not be correlated to photosynthetic activity as measured by fluorescence parameters.

However, if the biophysical stresses had any significant effect on the photosynthetic protein composition of the treated seedlings, these should have been apparent. Plates 3.7.1 and 3.7.2 show the results of electrophoretic analysis and immunoblotting of chloroplast preparations. The banding of the Rubisco LSU and the Coupling Factor α and β subunits of the untreated, incised, and tilted seedlings did not differ remarkably, indicating that the ratios were not dramatically altered by treatments.

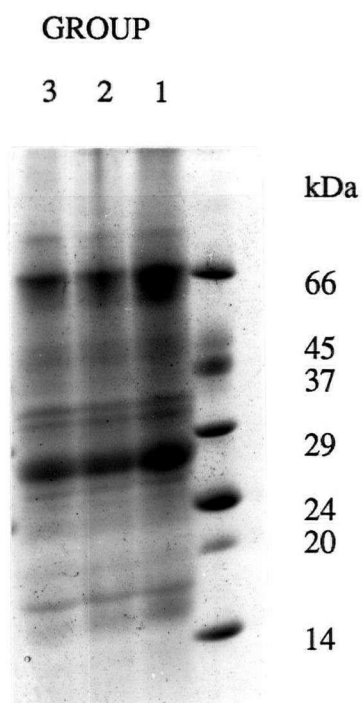


Plate 3.7.1. SDS Polyacrylamide gel electrophoresis of chloroplast proteins of group 1 (untreated), group 2 (incised), and group 3 (tilted).

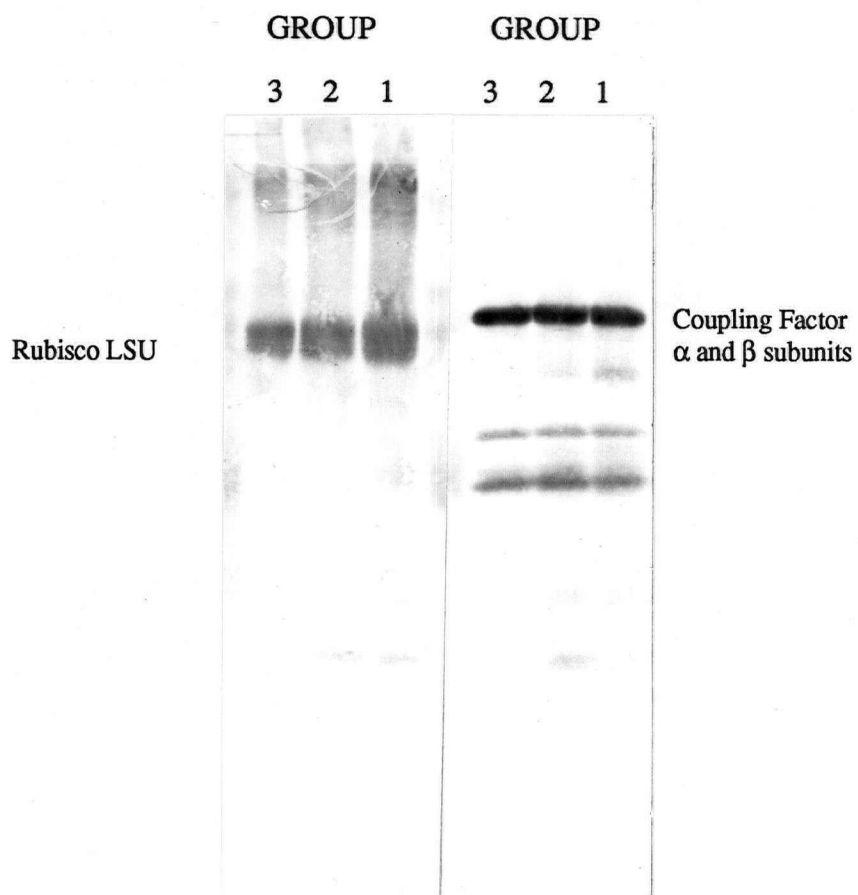


Plate 3.7.2. Immunoblotting of the Rubisco LSU and Coupling Factor α and β subunits of group 1 (untreated), group 2 (incised), and group 3 (tilted).

There has been little information published on stress or sink activity effects on the occurrence of Rubisco or Coupling Factor in conifers. However, for soybean, there exists evidence that the relative activity of secondary sinks can impinge on photosynthetic activity and development (Shibles *et al.*, 1987, Lauer and Shibles, 1987).

In recent work on soybeans (Diethelm and Shibles, 1989), reproductive development enhanced by plant stand thinning resulted in an increase in the total amount of soluble protein in soybean leaves of the remaining plants. As the percentage of soluble protein that was Rubisco did not change, the amount of Rubisco per unit leave tissue appears to have significantly increased. However, the thinning may have altered the quality and quantity of light impinging on the remaining plants, and it is not clear that the altered Rubisco (and total soluble protein) content was not a consequence of external environmental factors (i.e. light) impinging directly upon photosynthetic development, as opposed to development resulting from internal sink activity. In this case in particular, the possibility that externally modulated photosynthetic (source) activity was influencing reproductive (sink) development, persists.

With respect to the current study, the apparent ability to identify vigorously growing seedlings using fluorescence measures, may present an opportunity to investigate the relationship of chloroplast development and activity, to secondary sink (i.e. cambial) activity in conifer seedlings.

Chapter 4

CONCLUSIONS AND RECOMMENDATIONS

The comparative study of photosynthetic activity and development, and cambial activity in untreated white spruce seedlings, attracts the following conclusions:

1. During mid-summer, specific fluorescence parameters were strongly correlated to current season cambial activity. Mean average fluorescence over the 300 second time course (FvAVG), Fvar at the M peak (FvM), Fvar at 120 seconds (Fv120), and Fvar at 300 seconds (FvT), provided R^2 values, respectively, of 0.773., 0.542, 0.809 and 0.684 ($P < 0.05$ for all identified parameters). These results strongly suggest that specified fluorescence measurements may be used to assess current season stem vigour non-invasively, when taken during the period of active wood formation.
2. During late-summer and autumn, correlations between measured Fvar values and current season cambial activity were not significant.
3. Carbon dioxide uptake in the leader was not measurably correlated to cambial activity during mid-summer, late-summer, or autumn. In light of these results, and the apparent effectiveness of the fluorescence methods, CO_2 uptake measurements using the techniques described, are not recommended for the study of source:sink relations in white spruce seedlings.
4. Estimated chlorophyll *a* development was not significantly correlated to cambial activity at any time.

The following conclusions were drawn from the comparative study of stem dimensions and cambial activity in untreated seedlings:

1. The preseason cross-sectional area of the first year (1986) stem segment was not correlated to cambial activity ($R^2 = 0.007$). The pre-season cross-sectional area of the second year (1987) stem segment was weakly correlated to the current season cambial activity ($R^2 = 0.376$).
2. Preseason seedling height was not correlated to current season cambial activity ($R^2 = 0.001$). Current season leader length was weakly correlated to cambial activity ($R^2 = 0.420$).

These results place into question the effectiveness of grading or culling procedures based upon stem dimensions in white spruce seedlings, and illuminate the need for alternative methods.

The following conclusions were drawn from the study of the effects of biophysical stresses on photosynthetic activity and development, and cambial activity:

1. Cambial activity of "wounded" seedlings was not significantly different from that of the untreated group.
2. Extensive stem wounding severely diminished or eliminated the correlation between fluorescence parameters and cambial activities. This may be a consequence of abnormal source:sink relations resulting from altered translocation patterns and wound-associated respiration and biosynthetic activity.
3. Cambial activity in tilted seedlings was 27% greater than that of untreated seedlings, but the increase was not statistically significant ($p > 0.05$).

4. Correlations between cambial activity and fluorescence measures were not demonstrable in tilted seedlings. The reasons for this are not clear, but lower CO₂ uptakes by tilted seedlings suggest that the leaders may have lost apical dominance due to positioning, resulting in a localized reduction in photosynthetic activity.
5. The relative post-treatment occurrence of the Rubisco LSU and Coupling Factor α and β subunits in chloroplasts was not markedly affected by either wounding or tilting stresses.

Arguably the most useful results of this study are the description of methodologies that provide a foundation for non-invasive approaches to assess stem vigour, based on chlorophyll *a* fluorescence, and the identification of photophysiological and developmental relationships that provide evidence for, and some elucidation of, the occurrence of source:sink relationships in conifer seedlings. While this study investigated but one coniferous species, the hypotheses tested herein were based upon fundamental physiological theories, and the methods could prove effective for investigating source:sink relationships in a variety of (woody plant) species and age classes, and could have practical applications in resource management. For example, if correlations between FvT and cambial activity are strongly manifested in outplanted stock and in older age classes, there may exist an opportunity for remote sensing and monitoring of stand development. However, owing to variations in fluorescence characteristics among differing age classes, and different species, and under varying environmental conditions, it is likely that a significant effort aimed at establishing a broad database will be prerequisite to the full development of fluorescence-based management techniques.

Also of importance are the analyses demonstrating weak (at best) correlations between pre-season seedling dimensions and the current season stem vigour. This information should be helpful in developing methodologies for assessing seedling vigour before outplanting.

Extension of this work should explore the occurrence of photophysiological and developmental relationships in other conifer species, and focus on seasonal variabilities in the strength of the demonstrated relationships, particularly in the earlier part of the wood formation season, to determine more precisely when the steepest regression slope and highest R^2 values occur.

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APPENDIX A

Algorithm for calculating average fluorescence values, written in the 'awk' language of UNIX.

```
#put in the average
$0 ~ /[A-Z]/ {next}

prev[1]=" " {for(i=1;i<=NF;i++) prev[i]=$i
              sum=0; for(i=2;i<=NF;i++) sum+=$i
              pavg=sum/(NF-1)
              next}

{
sum=0; for(i=2;i<=NF;i++) sum+=$i
avg=sum/(NF-1)
for(i=2;i<=NF;i++) area[i]+=($i+prev[i])*(1-prev[1])
areavg += (avg+pavg)*(1-prev[1])
pavg=avg
for(i=1;i<=NF;i++) prev[i]=$i
num=NF
}

END{
print "The areas: (last one is average)"
for(i=2;i<=num;i++)printf("%.2f %.2f\n",area[i]/2,area[i]/600)
printf("%.2f %.2f\n",areavg/2,areavg/600)
}
```

APPENDIX B

Subroutine for calculating incremental areas and cambial perimeters from a digitized image using the KONTRON-SEM Image Processing System (Kontron Bildanalyze GmbH, Breslauer Str2, D8087 Eching/Munich).

IBAS PROGRAM LIST PRGM # 733

CAMBIAL AREAS FALLS

FUNCTION	INTEGER	VARIABLES:		REAL
		LOGICAL		
1 IMSIZE	FILE 450			
2 SCALE	SCNO 17	NEW		
3 RESET		TREAT		
		SINGLE CLASS		
		PARAM		
4 PERIM	NCLS 10 MODX 1 MODY 1 NCLT 1 MDLX 10 MDLY 1	SINGLE	LOW 0.000 HIGH 1000.0 LOLT 1.0000 HILT 0.000	
5 AREA	NCLS 10 MODX 1 MODY 1	SINGLE	LOW 0.000 HIGH 1000.0	
6 TVON		ONLINE		
7 PAUSE				
8 TVINP	INP 2			
9 NORMGR	INP 2 OUT 1 THRS 3			

10 SCALIM

INP 1
 OUT 2
 INLO 0
 INHI 255
 OTLO 12
 OTHI 255
 PHLO 0
 PHHI 1
 SCMD 2

11 LUTAB

SEL 794

12 GRSCAL

INP 2
 LINE 10

13 EDIT

INP 2
 OUT 1
 AUX 7

14 DISC2L

INP 1
 OUT 2
 LEV1 0
 LEV2 3

BINARY

15 DISC2L

INP 1
 OUT 3
 LEV1 4
 LEV2 7

BINARY

16 DISC2L

INP 1
 OUT 4
 LEV1 8
 LEV2 11

BINARY

17 CLOSE

INP 2
 OUT 2
 PHAS 255
 CNT 2
 MODE 7

18 CLOSE

INP 3
 OUT 3
 PHAS 255
 CNT 2
 MODE 7

19 CLOSE

INP	4
OUT	4
PHAS	255
CNT	2
MODE	7

20 BTHINN

INP	2
OUT	5
TSTP	0
MARG	0

21 COPYIM

INP	5
OUT	2

22 BTHINN

INP	3
OUT	5
TSTP	0
MARG	0

23 COPYIM

INP	5
OUT	3

24 BTHINN

INP	4
OUT	5
TSTP	0
MARG	0

25 COPYIM

INP	5
OUT	4

26 AFILL

INP	2
OUT	2

27 AFILL

INP	3
OUT	3

28 AFILL

INP	4
OUT	4

29 IDENT

INP	2
OUT	2
MARG	0

B-CONN

30 IDENT

INP 3
OUT 3
MARG 0

8-CONN

31 IDENT

INP 4
OUT 4
MARG 0

8-CONN

32 MEASUR

INP 2
GYRF 1
CHAN 1
SPAC 10

OBJ

33 MEASUR

INP 3
GYRF 1
CHAN 1
SPAC 10

OBJ

34 MEASUR

INP 4
GYRF 1
CHAN 1
SPAC 10

OBJ

35 DISPLY

INP 1

36 LUTAB

SEL 794

37 OUTSGL

HALT

38 PAUSE