

**Environmental and endogenous influences on carbohydrate
assimilation and allocation of apple trees (*Malus domestica*
Borkh.)**

By

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DECLARATION

I, the undersigned, hereby declare that the work contained in this dissertation is my own original work and that I have not previously in its entirety or in part submitted it at any university for a degree.

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SUMMARY

Market preferences for larger fruit have forced producers to adopt cultural practices that will ensure bigger fruit even if this comes at the expense of reduced total yields. In order to obtain acceptable fruit size there must be an adequate supply of photosynthetic carbon products especially during the cell division stage of fruit growth. Competition between fruits and between fruit and vegetative growth, as well as adverse climatic conditions, may limit the carbon supply to the fruits at this critical period and thus limit the final fruit size. Growers are showing renewed interest in the use of girdling or scoring in combination with the usual fruit thinning program to achieve growth control and increase fruit size. A new and milder chemical growth retardant, prohexadione-calcium (ProCa), is now also available for vegetative growth control of apple trees. In this study, the effects of, and potential interaction between scoring, ProCa and fruit thinning were investigated, with respect to shoot and fruit growth, yield and photosynthetic capacity, in 'Royal Gala', 'Fuji' and 'Cripps' Pink' apple trees.

ProCa decreased final extension shoot length in all three cultivars. Extension shoots were generally more sensitive than bourse shoots to scoring and ProCa. ProCa seems to be a more effective way of controlling shoot growth than scoring, with 'Fuji' and 'Cripps' Pink' being more sensitive than 'Royal Gala' to the application of ProCa. Scoring led to increased fruit growth rates during the first 40 days after full bloom (DAFB), and culminated in better fruit size at harvest. Scoring improved the total soluble solids concentration (TSS) of 'Fuji' and 'Cripps' Pink' compared to control and ProCa treatments. ProCa inhibited shoot growth effectively, but no evidence was found for improved carbon allocation to fruits and reproductive buds.

Yield efficiencies of scored trees were significantly improved in all cultivars during the second season, due to better reproductive bud development after the first year of scoring. On 'Royal Gala' and 'Cripps' Pink', the efficiency of scoring to stimulate reproductive bud development on old and new spurs declined after 4 weeks after full bloom (WAFB). In 'Fuji', scoring later than 1 WAFB led to a decreasing positive response on old and new spurs. In contrast, reproductive bud development on long shoots increased with later scoring (6 and 8 WAFB) on all cultivars. It seems that the most beneficial time of scoring is 2-4 WAFB, as

early as possible during the cell division stage of fruit growth, but not before natural drop has occurred. The combination of early-season scoring and application of ProCa seems to hold potential for increasing carbon allocation to the fruit and improving fruit size and quality attributes.

Scoring early in the season reduced photosynthetic capacity, and this reduction in carbon availability led to earlier cessation of shoot growth as well as shorter shoots. Later in the season, reduced fruit numbers led to a decrease in photosynthesis. The optimum temperature range for photosynthesis was found to adjust according to seasonal temperature variations. The harvest-induced reduction in sink strength changed stomatal sensitivity to higher temperature. Due to a reduced demand for carbohydrates by the plant, the maximum rate of photosynthesis (A_{\max}) was reduced post-harvest. Following this reduction in sink strength and A_{\max} , stomata became more sensitive to high leaf temperatures, thus restricting water loss. Pre-harvest there was a strong demand for carbohydrates, therefore stomata were kept open at higher temperatures to ensure a high rate of CO_2 incorporation, but at a cost with regard to water use efficiency. The sharp increase in dark respiration (R_d) in leaves and fruit with an increase in temperature would mean that significant carbohydrate shortages could occur in trees during source limited periods early in the season, especially under warm weather conditions commonly experienced in the Western Cape region.

OPSOMMING

Die effek van omgewings- en interne faktore op koolstofproduksie en -allokasie in appelbome (*Malus domestica* Borkh.).

Mark voorkeur vir groter vrugte forseer produsente om tegnieke te gebruik wat groter vrugte sal verseker selfs al beteken dit verlaagde totale opbrengste. Om aanvaarbare vruggrootheid te bereik moet daar voldoende voorsiening van fotosinteties geproduseerde koolstofprodukte aan die vrug wees, veral gedurende die selverdelingstadium van vruggroei. Kompetisie tussen vrugte, en tussen vrugte en vegetatiewe groei sal die voorsiening van koolstof gedurende die kritieke stadium aan die vrug beperk en so vruggrootheid benadeel. Daar is dus hernude belangstelling in die gebruik van ringelering in kombinasie met die gewone vrug uitdunprogramme om groei te beheer en vruggrootheid te verbeter. 'n Nuwe, sagter chemiese groeireguleerder, proheksadioon-kalsium (ProCa) is ook nou beskikbaar vir groei-beheer op appelbome. In hierdie studie is die effekte van, en moontlike interaksie tussen ringelering, ProCa en vruguitdunning ondersoek ten opsigte van loot- en vruggroei en opbrengs, sowel as die effekte op gaswisseling op 'Royal Gala', 'Fuji' en 'Cripps' Pink' appelbome.

ProCa veroorsaak korter lote in al drie kultivars. Verlengingslote is meer sensitief as beurslote vir ringelering en ProCa. ProCa is effektiewer as ringelering om lootgroei te beheer, en 'Fuji' en 'Cripps' Pink' is sensitiewer as 'Royal Gala'. Ringelering lei tot verbeterde vruggroei-tempos gedurende die eerste 40 dae na volblom en verbeterde vruggrootheid by oes. Ringelering verbeter die totale oplosbare vastestofkonsentrasie (TOVS) van 'Fuji' en 'Cripps' Pink' in vergelyking met kontrole- en ProCa-behandelings, onderskeidelik. ProCa inhibeer lootgroei effektief, maar geen bewyse van verbeterde koolstofallokasie na vrugte en reprodutiewe knoppe is gevind nie.

Opbrengseffektiwiteit van geringeleerde bome verhoog gedurende die tweede seisoen van ringelering weens beter reprodutiewe knopontwikkeling. Ringelering later as 4 weke na volblom (WNVB) is minder effektief om reprodutiewe knopontwikkeling op ou en nuwe spore in 'Royal Gala' en 'Cripps' Pink' te stimuleer. Die positiewe effek van ringelering op reprodutiewe knoppe op 'Fuji' verminder sodra dit later as 1 WNVB gedoen word. Op langlote het 'n laat ringelering (6 en 8 WNVB) die beste effek op reprodutiewe knopontwikkeling. Die mees voordelige tyd om te ringeleer was 2-4 WNVB, so vroeg as moontlik gedurende die selverdelingstadium van vruggroei, maar nie voor natuurlike vrugval

voltooi is nie. Die kombinasie van vroeë ringelering en die toediening van ProCa het potensiaal om koolstofallokasie na die vrug te verbeter, met die gepaardgaande verbetering in vruggrootte en -kwaliteit.

Ringelering vroeg in die seisoen inhibeer fotosintese en hierdie verlaging in koolstof beskikbaarheid het tot gevolg dat lootgroei vroeër gestaak word. Later in die seisoen veroorsaak verlaagde vruggetalle 'n afname in fotosintese. Die optimum temperatuur vir fotosintese verander na gelang van heersende lugtemperatuur. Die oes-geïnduseerde verlaging in sinksterkte verander stomatale sensitiwiteit vir hoë temperature. Weens 'n verlaagde aanvraag vir koolhidrate deur die plant word fotosintese verlaag na-oes. Weens dié verlaging is stomata sensitiewer vir hoër temperature en beperk dus waterverlies. Voor oes is daar 'n hoë aanvraag na koolhidrate dus word stomata oop gehou selfs by hoë temperature ten koste van watergebruiksdoeltreffendheid. Die skerp styging in donker respirasie in blare en vrugte met 'n verhoging in temperature sal beteken dat koolhidraat-tekorte kan ontstaan in bome gedurende die bron-beperkte periodes vroeg in die seisoen, veral onder warm toestande soos wat algemeen ervaar word in die Wes Kaap.

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1. Literature review: Girdling and thinning as techniques to improve apple fruit size

1.1 Introduction

In modern fruit culture and particularly within the present western marketing system, total yield is less important than the amount of saleable first class fruit. In South Africa more than 50% of the apples produced are destined for the export market. Poor prices are obtained for smaller fruit in most overseas markets and this situation worsens if above-average crops are harvested in Europe. This has forced South African producers to alter their cultural practices to increase fruit size.

Fruit thinning is the most important technique in apple growing for improving fruit size and quality (Looney, 1993). Since thinning can be performed mechanically or chemically, thinning intensity may vary not only with the method used but also with the physiological condition of the trees and the cultural practices employed (Link, 2000). For the fresh market, fruit size, appearance, flavour, firmness and storability are of main interest. The grower will therefore have to focus his orchard practices to satisfy these market demands in order to produce high quality fruit consistently at maximum yields (Wünsche and Ferguson, 2005).

In deciduous fruit trees, carbohydrates must not only be produced but also allocated efficiently to fruit and new flower buds (Forshey and Elfving, 1989). Source-sink relationships and the regulation of carbon allocation therefore determine growth of individual organs. This may be restricted by assimilate availability (source limitation) or by the organ's ability to utilise assimilates (sink limitation) (Patrick, 1988). In the hierarchy of sinks fruits and shoots are equal (Wardlaw, 1990), but since shoots develop prior to fruits they compete more efficiently for carbohydrates during the early part of the season (Bangerth and Ho, 1984). Excessive vegetative growth is thus a major concern to the producer because it competes with fruit growth (Forshey and Elfving, 1989).

In the Western Cape region of South Africa dwarfing rootstocks offer no solution to excessive tree vigour due to inadequate winter chilling, causing poor bud break and canopy development, and generally poor soil conditions restricting the growth of dwarfing rootstocks severely (Webster and Wertheim, 2003). Currently, semi-vigorous rootstocks such as MM 106

or MM 793 are used in high-density orchards (1000-1666 trees ha⁻¹). Their lack of precocity is a serious limitation and this makes management of mature high density orchards difficult. These orchards must achieve high early yields, high sustained yield and excellent fruit quality in order for the producer to survive financially (Robinson, 2003). In addition, growth on these rootstocks is often too vigorous and an effective restriction of vegetative growth is required. There is an international trend away from harsh chemical treatments and growth retardants and towards a more environmentally acceptable approach. This has led to renewed interest in girdling as a method to control tree vigour and increase fruit size.

A girdled tree is one in which the phloem is completely severed by the removal from the trunk of a more or less wide cylinder of bark without damage to the underlying tissue (Noel, 1970), whilst scoring is a form of girdling in which a narrow cut is made around the circumference of the trunk. Both techniques block phloem transport and allowing for a better availability of metabolites for developing organs above the girdle (Goren et al., 2004). Girdling has been shown to be an effective technique to reduce vegetative growth, promote flowering, improve fruit set, increase fruit size and advance maturity in citrus (Cohen, 1981), grapevines (Winkler et al., 1974), olives (Lavee et al., 1983), apples and pears (Dennis, 1968), peaches and nectarines (Dann et al., 1985) and several other species (Grierson et al., 1982).

The mechanism through which girdling acts is not yet clearly understood. Changes in translocation and accumulation of carbohydrates as a result of girdling have been reported (Wallerstein et al., 1973), but there is also evidence of changes in plant growth regulator content following girdling (Wallerstein et al., 1973; Goren et al., 2004). Girdling as a form of wounding may also enhance ethylene production (Autio and Greene, 1994) resulting in a promotion of ripening.

The effect of girdling on plant growth regulator balance and the effects of thinning and girdling on the allocation and production (photosynthesis) of carbohydrates will be discussed further.

1.2 Influence of thinning on fruit quality

Apple trees bear an abundance of flowers, which, even after poor pollination conditions, produce a surplus of fruit that the tree is unable to support at the desired quality. This situation has a number of disadvantages, of which two are most serious: (a) low and often unacceptable

market quality (Link, 2000), and (b) inhibition of flower bud induction, causing severe alternate bearing (Tromp, 2000). To overcome these shortcomings, flower or fruit thinning is an efficient method and has become necessary in modern fruit production (Bangerth, 2000).

Link (2000) stated that mean fruit weight was negatively correlated with crop load, but factors other than crop load in the year of thinning may also influence fruit size. Carbon-based compounds are the backbone of fruit growth, and the supply of carbon to individual fruitlets may be limiting during early fruit development by competition from other fruitlets or other sinks (Lakso et al., 1998). Therefore, the timing of thinning has a marked influence on fruit size.

Researchers in different fruit growing regions have documented the effect of early thinning on increasing fruit size (Denne, 1960; Westwood et al., 1967; Silbereisen, 1976; Wertheim et al., 1977; Bergh, 1990). In thinning trials done by Link (2000), mean fruit size was increased by up to 30% when thinning was performed between pink bud and full bloom as compared to thinning after the June drop (Northern hemisphere). Compared to un-thinned controls, blossom thinning increased cell number by 5-35% and cell size by 4-10%. The results also indicate response differences between cultivars (Link, 2000).

Fruit size can be viewed as the result of a combination of cell number, cell size and volume of intercellular space (Goffinet et al., 1995). Pearson and Robertson (1953) stated that the variation in fruit size is determined early in development by the amount of cell division, and that within one season cell volume in individual fruit of the same age was fairly uniform. The variations in fruit size were determined by cell number and, to some extent, by the amount of air space. Between seasons, however, the size of the cells may be the overriding factor in determining fruit size. Crop load in the year before thinning may also be a source of fruit size variation. Heavy cropping in the previous season reduces the cell number in flower receptacles when compared to those in normal cropping trees (Bergh, 1985).

Fruit size distribution usually corresponds to a normal distribution curve. Effective thinning treatments shift the curve from the lower size categories to the higher ones. Thinning may thus result in fewer kilograms of small and increased kilograms of large fruit (Link, 2000).

Only fruit well supplied with carbohydrates attain good colour and flavour (Walter, 1967). Fruit thinning decreases the percentage of green fruit and increases yellow ground

colour of yellow cultivars, and the extent of surface blush colour in red cultivars (Link, 2000). Fruit thinning is considerably more effective in improving fruit colour than picking over trees during an extended harvesting period, which is common practice in the fruit industry (Link, 1986).

When the growth rate of fruitlets is low, small cracks develop in the epidermis leading to fruit russeting. Hand thinning will usually increase the growth rate of fruitlets, and thus reduce the amount of fruit russeting (Link, 2000). The improvement in fruit size and colour by thinning are accompanied by higher contents of soluble solids and titratable acidity. Thinning therefore improves taste and also appearance of the fruit (Schumacher and Stadler, 1987).

1.3 The effect of girdling on plant growth regulators

Redistribution of assimilate supply between different plant organs appears to be the predominant effect of girdling, with reproductive growth being favoured over the growth of vegetative organs (Dann et al., 1984; Goren et al., 2004). Similarly, aging peach trees allocate more assimilates to reproductive than to vegetative growth (Chalmers and Van den Ende, 1975). Reduced leaf area per fruit, together with reductions in leaf size, limb circumference growth, lateral shoot length and internode length reflects this change on girdled trees. Leaves senesce and fruit mature more rapidly on older trees as is also the case on girdled trees (Dann et al., 1984). These effects on growth are likely to be controlled by plant regulators and girdling, like aging, may alter in the same way the balance between endogenous growth regulators, in a manner which favours reproductive development.

The changes that develop with time above the girdle indicate that girdling may diminish the supply of juvenile factors. Cytokinins and gibberellins generally retard senescence of leaves (Farquhar and Sharkey, 1982) and maturation of fruits (McGlasson et al., 1978). Girdling may reduce the activity of these two groups of hormones above the girdle. Lowered cytokinin and or gibberellin synthesis/ activation by the roots may be a secondary effect, resulting from diminished supply of a phloem-borne activator to the roots (Dann et al., 1984).

Auxin (Indoleacetic acid (IAA)) moves primarily in a basipetal direction in plants (Goldsmith, 1977) and is thought to be a principle factor in correlative growth control (Bangerth, 1989, 2000). Girdling would disrupt this basipetal signal. Since mainly these three

groups of plant growth regulators seem to be affected by girdling they will be discussed shortly.

1.3.1 Auxin

The enzymes necessary for the production of IAA are most active in young tissues, such as shoot meristems, young leaves and fruits (Salisbury and Ross, 1992). IAA is not usually translocated through the phloem sieve tubes or through xylem, but instead primarily through parenchyma cells in contact with vascular bundles. IAA transport is polar, occurring in stems in a basipetal direction and in an acropetal direction in roots. IAA transport also requires energy, as evidenced by the ability of ATP-synthesis inhibitors or the lack of oxygen to block it (Salisbury and Ross, 1992).

Dann et al. (1985) found a sharp increase in IAA concentration in the bark above the girdle in peach trees and a 75% reduction in the IAA concentration below the girdle. This would indicate the disruption of the basipetal movement of IAA due to girdling. This decrease in IAA concentration below the girdle severely affected the growth and cell division of the cambium below the girdle. Callus growth is confined almost entirely to the upper edge of the girdle (Dann et al., 1985).

Dominance or correlative phenomena are widespread in the plant kingdom (Goodwin et al., 1978). They are particularly common among fruit and between fruit and vegetative sinks. Plant growth regulators have been suggested as the dominance signals among fruit sinks as well as in apical dominance. However the regulatory mechanism for fruit must be more complex because not a uni- but a multidirectional signal has to be envisaged to explain the sometimes simultaneous apical, basal or acropetal effects occurring (Bangerth, 1989). The unidirectional polar transport of IAA (Salisbury and Ross, 1992) is obviously not suited to account for these effects. There is another distinction between the dominance of fruit/sinks and apical dominance: it is not the morphological position of the fruit/sinks that determines their degree of dominance or inhibition but, rather, their sequence of development relative to each other. Usually the earlier developed organ dominates over later developed ones (Bangerth, 1989).

There is some evidence to suggest that IAA may be involved in the transfer of the dominance signal.

- The rate of diffusible IAA from dominant apple fruit is higher than that from inhibited fruit on the same plant (Bangerth, 1989).
- Manipulating the dominance relationship is quickly reflected in an altered rate of IAA diffusion (Bangerth, 1989). This relationship also exists between fruits and vegetative sinks. For instance, when the shoot tip of a growing shoot on an apple tree is removed, nearby fruit show higher fruit set and start to export more IAA (Quinlan and Preston, 1971).
- The possible role of the IAA export rate in dominance also follows from the importance of seeds in the dominant fruits. Without the stimulative effects of hormone applications, parthenocarpic fruit set can be obtained in some species only when competition with vegetative sinks is eliminated (Carbonell and Garcia-Martinez, 1980). Even when phytohormones are used to induce parthenocarpic fruit set, the simultaneous presence of competing, seeded fruits restricts or even prevents their further development (Retamales and Bukovac, 1986). Competition for assimilates cannot be the sole reason for this, because a similar number of seeded fruits can be supported. This suggests a dominance effect of shoot tips and/or seeded fruit on the growth of parthenocarpic fruit. The rate of export of IAA from a fruit is also closely related to the presence and number of seeds (Sjut and Bangerth, 1984).

The most reasonable explanation for this is that the higher export of the first induced or otherwise dominating organ inhibits the IAA export from the later induced, inhibited organs. The results indicate that high IAA concentrations at the base of an excised segment or at junctions of dominant and inhibited organs inhibit polar IAA transport out of the subordinated organ. The physiological mechanism underlying this auto-inhibition of polar IAA is still unclear (Bangerth, 1989).

The assumption is thus made that polar IAA export is essential for a growing organ. If this IAA export does not take place or is inhibited, for instance because of the proposed auto-inhibition, the growth of the respective organ may be reduced or it may abscise (Bangerth, 1989).

Girdling disrupts the basipetal IAA hormone signal, and this nearly always stimulates the development of basal shoots due to the removal of apical dominance (Noel, 1970). Dann et al. (1985) found no reduction in IAA concentration of the shoot tip after girdling, indicating

that IAA was being produced but that its transport was disrupted by girdling. Cytokinin movement in the plant is influenced by IAA, and the disruption of basipetal movement of IAA will therefore also influence cytokinin concentrations in the plant (Morris, 1977).

1.3.2 Cytokinin

Cytokinin concentrations are the highest in young organs (seeds, fruits and leaves) but Salisbury and Ross (1992) concluded that cytokinins are primarily synthesized in root tips, and transported through the xylem to various plant organs. The major function of cytokinins is to promote cell division. It has also been shown that cytokinin retards the senescence of leaves (Kende, 1971), and increases the sink strength of an organ for carbohydrates and amino acids (Morris, 1977).

Zeatin riboside is the primary cytokinin in apple xylem sap (Cutting et al., 1991). Girdling reduced the zeatin riboside concentration in the shoots above the girdle in peach trees (Cutting and Lyne, 1993). This was probably due to reduced export from the roots, possibly as a result of reduced levels of carbohydrates in the roots or the disruption of the basipetal IAA signal from the shoots. This would explain the altered allocation of carbohydrates from vegetative growth to reproductive growth, and the subsequent increase in fruit sink strength, as shoot tips are unable to synthesize cytokinins whereas seeds and fruit do (Salisbury and Ross, 1992).

1.3.3 Gibberellin

Gibberellins are synthesized in seeds, young leaves and roots, and transport occurs through both xylem and phloem and is non-polar. Cytokinins and gibberellins are thought to interact in the control of shoot growth (Goodwin et al., 1978). The functions of gibberellins at the cellular level include cell elongation and expansion as well as stem elongation at the whole plant level (Brock and Kaufman, 1991). Cutting and Lyne (1993) found a reduction in gibberellin concentration in xylem sap of girdled peach branches. These reduced concentrations could be due to either reduced allocation of root produced gibberellins (as a result of the disruption of the basipetal auxin signal and/or reduced root growth as a result of reduced carbohydrate supply), or reduced localized synthesis, possibly as a result of altered cytokinin concentrations.

Girdling thus disrupts the basipetal transport of IAA to the roots, as well as reducing the carbohydrate flow to the roots, which in turn leads to a reduction in the synthesis of cytokinins and gibberellins by the roots. Gibberellins and cytokinins are transported in the xylem to the shoots (girdling does not disrupt their transport) where they increase the sink strength of growing shoots and also enhance shoot elongation. Reduced concentrations of cytokinins and gibberellins in shoots due to girdling led to a reduction in shoot sink strength. Therefore more of the carbohydrates produced by photosynthesis are allocated to the fruit, which in turn leads to increased fruit growth.

1.4 The effect of thinning and girdling on the production and allocation of assimilates

The production, partitioning and use of carbohydrates in apple follow specific seasonal patterns (Oliveira and Priestley, 1988). Stored reserves decline during early growth, with a minimum near bloom, and increase thereafter during the summer until leaf fall (Hansen and Grauslund, 1973). At the onset of new growth in the spring, these reserves are primarily used for respiration, while subsequent growth seems to depend primarily on current photosynthate production (Hansen and Grauslund, 1973). This would mean that the partitioning patterns of photosynthate between vegetative development and reproductive development early in the season would influence fruit development and size (Quinlan and Preston, 1971). The vegetative development of extension and bourse shoots seems to sometimes have priority over reproductive development in the early season, thus negatively affecting fruit set and fruit growth by cell division.

Source-sink relationships and the regulation of carbon allocation therefore determine yields. The growth of individual plant organs may be restricted by assimilate availability (source limitation) or by the organ's ability to utilize assimilates (sink limitation) (Patrick, 1988). The strength of a fruit sink depends on its size, the time of its initiation relative to other sinks, its location, and distance from the source (Bangerth and Ho, 1984). Wardlaw (1990) prioritized sink strength in the following order: seeds > fleshy fruit parts = shoot apices and leaves > cambium > roots > storage. Girdling reduces the sink strength of the shoot apices, therefore more assimilates will be available for fruit growth. Thinning reduces competition between fruitlets, therefore more assimilates are available for the remaining fruits. The effect of both these practices on assimilate partitioning leads to increased fruit size.

Yield at a given net carbon exchange rate depends on the photoassimilates available for export (source regulation) and on the capacity of consuming organs to import carbohydrates (sink regulation) (Daie, 1985). We must examine the processes that affect assimilate utilization and storage in the fruits as this will determine the sink strength of the fruits, as well as the effects of assimilate build-up on photosynthesis, to fully understand source and sink limitations on fruit growth.

1.5 Processes affecting sink strength

The potential capacity of a sink (sink strength) is a function of its size and activity (assimilate flux). Sink strength and sink activity have been characterized as changing with development, often not reaching their maximum potential because of reduced availability of resources (Ho, 1988). This suggests a closely regulated pattern of expression of the enzymes determining sink strength and activity with substrate availability as the sole limiting factor.

A growing apple is a sink organ that accumulates fructose and sucrose. Starch is also synthesized, but is degraded in later stages of fruit development (Hulme, 1958). The carbohydrates are derived from sorbitol and sucrose produced in leaf photosynthesis and transported to the fruit via the phloem (Hansen, 1970). Apple fruit growth rate may be determined in part by the ability to acquire sorbitol (Archbold, 1992). Three enzymes with important roles in the metabolism of imported sugars in apple fruit are sorbitol dehydrogenase (SDH), sorbitol oxidase (SOX) and acid invertase (Yamaki, 1995). SDH converts sorbitol to fructose, SOX converts sorbitol to glucose, and acid invertase converts sucrose to glucose and fructose. Since SDH exhibits significantly greater activity than SOX during the extended cell expansion phase and sorbitol is the primary imported sugar, SDH may play the most important role of the three enzymes (Archbold, 1999).

Apple fruit SDH activity on a per gram fresh weight basis is low during the cell division stage, increases to a maximum as the fruit enters the cell expansion phase, and then declines slowly until the fruit enters ripening when it increases again (Berlter, 1985). Archbold (1999) found that SDH activity might be modulated by the availability of sorbitol and other carbohydrates. Reduced sorbitol availability arising from high levels of competition between sinks as well as from adverse environmental conditions reducing leaf photosynthetic rates, reduce SDH activity. As a result, sink strength and activity are regulated to fit the

available resources, maintaining a balance between supply and utilization of carbohydrates (Geiger et al., 1996). Minchin et al. (1997) found that when assimilate supply to a fruit was increased by removing other nearby competing fruits, there was a three-fold increase in the amount of carbohydrates partitioned to the fruit within one day. They concluded that an increase in the ability of the fruit to utilize the available photosynthate through an increase in the expression of catabolic enzyme gave rise to their observation. All this supports the theory that carbohydrate metabolizing enzymes are induced by high levels of sorbitol and/or sucrose, providing a mechanism for carbohydrate supply to affect sink capacity.

1.6 Processes affecting source regulation.

The chloroplast is the site of photosynthetic carbon fixation, therefore the chloroplast envelope can be regarded as the ultimate barrier between source and sinks, and the regulation of metabolites across this barrier is of prime importance (Herold, 1980). The initial product of carbon dioxide (CO₂) fixation in C₃ plants is phosphoglycerate, which is then reduced to glyceraldehyde phosphate or triosephosphate. Triosephosphate, which is a substrate for several alternative pathways, is involved in a) regeneration of ribulose bisphosphate (required for CO₂ fixation), b) the photorespiration process (a substantial loss of fixed carbon), and c) sucrose or starch formation (carbon end-product formation).

Triosephosphate is the principal form by which carbon crosses the chloroplast envelope. Export of triosephosphate is strictly linked with counter-movement of inorganic phosphate (P_i) via the phosphate translocator (Heldt and Rapley, 1970). Triosephosphate is oxidized in the cytoplasm to phosphoglycerate or condensed to hexose phosphate, a precursor for sucrose, fructans and cell wall polysaccharides (Walker and Robinson, 1978). During periods of high net CO₂ fixation, triosephosphate still in the chloroplast is partially converted to starch and stored there. Starch reserves can be utilized at later times when photosynthetic rates are low (Daie, 1985).

Sorbitol is an early product of photosynthesis in apple leaves, accounting for about 80% of the total sugar, whereas sucrose makes up about 10% of the total soluble sugars. The enzyme sorbitol-6-phosphate dehydrogenase catalyzes the reaction in which sorbitol is formed from glucose-6 phosphate (Yamaki and Ishikawa, 1986). The activity of the enzyme is highest

in young leaves and gradually declines with leaf age, whereas sorbitol accumulation shows the reverse trend. This inverse correlation is attributed to a) export of sorbitol from young leaves and b) increased utilization of sorbitol *in situ* as a substrate (Yamaki and Ishikawa, 1986). The rate of sorbitol synthesis is a function of the carbon fixation rate, chemical partitioning of carbon between starch and sucrose/sorbitol and the rate of sorbitol export from the leaf (Daie, 1985).

Reduced sorbitol/sucrose synthesis results in less free phosphate available for exchange with triosephosphate across the chloroplast membrane, thereby favouring starch synthesis in the chloroplast (Geiger, 1979). Reaction rates for *in vivo* sucrose formation may be proportional to the cytoplasmic concentration of UDP-glucose, which in turn depends on the supplies of triosephosphate and ATP to regenerate UDP (Harbron et al., 1981).

Sorbitol/sucrose levels within the cytoplasm are influenced not only by export from the source leaf, but by compartmentation within the vacuole. Fondy and Geiger (1983) showed that sucrose is transiently stored in leaf vacuoles as a buffer against short-term changes in photosynthesis and export from the mesophyll cells. The mechanism and control of sucrose transport into vacuoles therefore constitutes a potentially important control point for carbon allocation within source leaves.

Increased carbohydrate levels in the cytoplasm lead to a decrease in photosynthetic rate. The chloroplast membrane is impermeable to sucrose and sorbitol (Heldt and Sauer, 1971), which cannot, therefore, act as messengers to the source chloroplast. Increased levels of sucrose in the cytoplasm are likely to lead to increased concentration of triosephosphate from a mass action effect, or from feedback inhibition of enzyme activity (Herold, 1980). High concentration of triosephosphate in the cytoplasm inhibits net export of triosephosphate from the chloroplast via the phosphate translocator. The increased ratio of PGA/P_i in the chloroplast favours starch synthesis in the chloroplast (Preiss and Levi, 1979). Accumulation of starch could influence the rate of CO_2 fixation directly through distortion of the thylakoid membrane or by interference with the path of light (Rackham, 1966) whilst both starch and sugar content may influence leaf resistance to diffusion of CO_2 (Rackham, 1966). Direct inhibition of reactions of the reductive phosphate pathway by triosephosphate is associated with low P_i concentrations inside the chloroplast (Herold, 1980). The photosynthesis reaction inside the chloroplast consumes P_i , and a lowered P_i concentration is likely to inhibit photosynthesis,

probably via a decreased ATP/ADP ratio (Robinson and Walker, 1979) or a direct effect of P_i concentration on RUBP carboxylase activity (Heldt et al., 1978). Low cytoplasmic P_i concentration is an outcome of high triosephosphate concentration, where rapid sucrose synthesis is not operating to bring about release of P_i in the cytoplasm (Walker and Herold, 1977). Inhibition of sucrose phosphate phosphatase by sucrose (Hawker, 1967) may lead to an enhanced level of sucrose phosphate and a lowering of P_i concentration in the cytoplasm. It is thus proposed (Herold, 1980) that P_i , triosephosphate and PGA, which move freely across the chloroplast envelope via the phosphate translocator, have a role in transferring the requirements of the sinks to the chloroplast (source).

Girdling causes the accumulation of carbohydrates throughout the canopy, this build-up of carbohydrates in the leaves causes a reduction in photosynthesis (Goren et al., 2004). Excess carbohydrates may interfere with photosynthesis through several mechanisms: (1) enlarged starch granules damaging the chloroplasts (Schaffer et al., 1986); (2) closure of stomata (Goldschmidt and Huber, 1992); (3) accumulation of phosphorylated intermediates and depletion of P_i (Krapp and Stitt, 1995); and (4) indirect action by repressing the expression of genes that encode proteins needed for photosynthesis (Krapp and Stitt, 1995).

1.7 Crop load effects on gas exchange

A reduction in sink strength, due to a reduction in crop load, causes a reduction in the net CO_2 assimilation rate (A) of the leaves. However, this effect could be masked by the presence of strong vegetative sinks as the effect of sink strength on photosynthesis becomes apparent only under conditions of source limitation (Flore and Lakso, 1989). This downregulation of A under low and no crop load has often been observed in field studies (Hansen, 1970; Fujii and Kennedy, 1985; Wünsche et al., 2000). Palmer et al. (1997) reported a positive and curvilinear response of A to crop load in New Zealand. However, the effect of crop load on photosynthesis is very dependent upon time and severity of fruit removal (Lakso, 1994). Thinning at a later stage has a more profound effect as there are less actively growing sinks available for alternative carbohydrate movement (Wünsche et al., 2000).

It has also been shown that defruiting reduces the stomatal conductance and carboxylation efficiency (Monselise and Lenz, 1980) in apple trees, probably caused by an

increase in starch concentrations in chloroplasts of non-fruiting trees (Wünsche et al., 2000). This can be interpreted as the result of interrupted utilization of carbon assimilates by the fruit, which eventually causes a decrease in A through a feedback mechanism (Guinn and Mauney, 1980). Increases in starch concentration of the leaf also cause an increase in non-radiative thermal dissipation (Pammenter et al., 1993), indicating a redistribution of energy away from photosynthesis and hence a reduction in photochemical efficiency, that is the conversion of light to chemical energy by the photochemical apparatus.

1.8 Discussion

Market preferences for larger fruit have forced producers to adopt cultural practices that will ensure bigger fruit even if this comes at the expense of reduced total yields. In order to obtain acceptable fruit size there must be an adequate supply of photosynthetic carbon products especially during the cell division stage of fruit growth. Competition between fruits and between fruit and vegetative growth may limit the carbon supply to the fruits at this critical period and thus limit the final fruit size. Adverse environmental conditions such as low light levels as well as high temperatures can limit carbon assimilation rates, thus negatively influencing carbon supply to the developing fruit. Thinning reduces the number of competing fruit and should ensure that the remaining fruit have an adequate supply of carbon from the source leaves.

Girdling reduces the sink strength of competing vegetative growth by altering the internal plant growth regulator balance of the tree, thus ensuring that more carbon is available for fruit growth. Sink strength of developing fruits is determined in part by the ability of the fruit to metabolize the carbon supplied by the source leaves. Evidence suggests that the imported carbon compounds regulate the activities of the enzymes responsible for the metabolism of imported carbon. Thus an increase in the supply of carbon early in the season will lead to an increase in the expression and activity of the enzymes, thus ensuring that the fruit is established as a strong sink early in the season.

Source leaves react to the demand from the sink organs and regulate photosynthesis accordingly. Maximum rate of photosynthesis is determined by prevailing environmental conditions. Once the light environment inside the trees is optimized there is very little the grower can do to increase the maximum rate of photosynthesis. Therefore, cultural practices to

increase fruit size should aim to establish the fruits as dominant sinks very early in the season. This ensures an adequate supply of carbon to the fruit at all times. Early thinning and girdling can achieve this by eliminating competition between fruitlets and between fruits and vegetative sinks early in the season when the adequate supply of carbon to the fruits is critical.

1.9 References

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2. Introduction and objectives

In the Western Cape region of South Africa, fruit production, and especially apple production, is one of the most important industries within the agricultural sector in terms of job creation and the earning of foreign income. In recent years, industry leaders have focused on the planting of new bi-coloured cultivars such as 'Royal Gala', 'Fuji', 'Braestar' and 'Cripps' Pink' in high-density orchards. Returns have not always been satisfactory for a number of reasons, some of which will be investigated in this study.

'Royal Gala' is the most valuable early-maturing apple cultivar in this region, and a highly prized commodity on the export market if excellent quality is supplied. The average fruit size distribution of 'Royal Gala' apples is generally unsatisfactory in the Western Cape, in contrast with other regions worldwide where the same cultivar achieves very good size. Despite past studies, the underlying physiological reasons for this are still poorly understood. What is certain is that orchard practices such as early fruit thinning and girdling or scoring can be successfully used to improve fruit size. Both manipulations lower either the vegetative sink strength (girdling/scoring) or the total reproductive sink strength (thinning), thereby stimulating individual fruit growth rates. However, the interactive effects of these practices on tree physiology, and especially its carbon balance, are poorly studied.

Climatic conditions in spring in the Western Cape, including light and temperature, may also contribute to poor rates of cell division, which to a large degree determine final fruit size. Respiration rate is sensitive to temperature, and high respiration rates during warm spring nights could reduce the amount of carbohydrate available for fruit growth. During daytime this effect may be balanced partially by fruit photosynthesis. The carbon balance of leaves and fruits during the initial growth stage has not been studied under South African conditions. The late season (from harvest until leaf senescence) is long and conducive to continued high rates of photosynthesis until shortly before leaf drop, as well as good carbohydrate reserve accumulation and bud development. Climatic conditions and soil water status can, however, still be stressful, and in the last decade autumn temperatures have been unusually high and the rains have come later than usual. In a region where winter rainfall begins around April/May and summer rainfall is low, some dams dry up during the late season, forcing growers to stop irrigating. Little is known about how short-term stomatal sensitivity of apple leaves to

atmospheric factors may change through the season in response to changes in environmental conditions and sink demand. Stomata may be regulated so as to favour critical processes, such as rapid fruit growth during early summer and the pre-harvest period (high carbon requirement and high water loss), or carbohydrate reserve accumulation and bud development post-harvest (lower carbon requirement but sensitive to water status).

Another problem facing apple producers in the Western Cape is the difficulty in achieving the correct balance between vegetative and reproductive growth. Currently available dwarfing rootstocks perform poorly under South African conditions due to inadequate winter chilling, poor bud break and canopy development, generally poor soil conditions, high soil temperatures and replant situations which restrict the growth of these rootstocks severely. Currently, semi-vigorous rootstocks such as MM 106 or MM 793 are used in high-density orchards (1000-1666 trees ha⁻¹). Their lack of precocity is a serious limitation and this makes management of mature high density orchards difficult. These orchards must achieve high early yields, high sustained yield and excellent fruit quality in order for the producer to survive financially. In addition, growth on these rootstocks is often too vigorous and an effective restriction of vegetative growth is required. Chemical growth retardants are an effective way of managing vigour, but the persistence of harsh chemicals in the tree and the environment has led to an international trend towards a more environmentally acceptable approach, and has reduced their use considerably. Numerous non-chemical techniques are available to control excessive vigour, including summer and root pruning, limb spreading, restricted fertiliser application and the control of irrigation. However, these techniques may be only marginally effective. There is now increasing interest in trunk or limb girdling or scoring as an effective and environmentally friendly cultural practice to control tree vigour, and it has been widely used in South African orchards in recent years. However, recommendations for the timing and severity of girdling are usually based on an empirical formula giving rise to mixed results. A new and milder chemical growth retardant, prohexadione-calcium (ProCa), is now also available for vegetative growth control of apple trees. At the same time, fruit thinning is usually required to regulate the crop load and optimise yields and fruit quality. The combined simultaneous use of these practices, which all affect source-sink relations and carbohydrate allocation to competing sinks, is poorly understood and has not been studied at a physiological level.

The objectives of this study were:

- To study the mechanistic relationships between various climatic/atmospheric factors and gas exchange (photosynthesis, respiration, transpiration) of leaves and developing fruit ('Royal Gala', 'Fuji' and 'Cripps' Pink') throughout the season and following harvest (but with a particular focus on the first 40 days after full bloom), under various manipulative source/sink altering treatments.
- To relate this information to fruit and shoot growth, and ascertain which factors during the growth season are likely responsible for poor fruit size. This will show the way towards developing practical recommendations for improving fruit size distributions.
- To gain a better understanding of the physiological interaction between girdling/scoring, fruit thinning, and the chemical growth retardant ProCa, with respect to carbohydrate partitioning, growth patterns, growth regulation and carry-over effects. This will lead to improved recommendations regarding the timing and severity of these cultural practices in order to increase fruit size in 'Royal Gala' and control tree vigour in 'Royal Gala', 'Fuji' and 'Cripps' Pink' apple trees, and thus ensure consistently high yields of high-quality fruit.

3. Season-long feedback inhibition of photosynthesis following early-season reductions in sink strength (scoring and thinning) in 'Royal Gala' apple trees

Abstract

The 'Royal Gala' apple (*Malus domestica* Borkh.) produces unsatisfactory fruit sizes in the Western Cape, South Africa. Girdling or scoring can reduce shoot growth via reductions in vegetative sink strength, whereas fruit thinning reduces the total fruit sink thus increasing individual fruit sink strength. Both practices, when applied during the first five weeks after full bloom, increase the proportional allocation of available carbohydrates to fruit growth. Reductions in sink demand can, however, affect carbon assimilation rates via end product feedback inhibition. This could be a transient or long-term effect, influenced by the degree of sink reduction and periods of carbon-supply limitation. The objective of this study was to investigate the individual and combined effects of early-season (13-40 days after full bloom, DAFB) scoring and various thinning intensities, giving a range of sink strength reductions, on gas exchange and underlying photosynthetic capacity throughout the season. At 20, 50 and 85 DAFB, photosynthetic light response curves were performed on bourse shoot leaves at leaf temperatures of 20, 25, 30 and 35°C, and A/c_i curves were measured at 25°C. Light-saturated net CO₂ assimilation rate (A_{max}) was generally reduced by scoring and thinning throughout the season, although there was no response to thinning at 20 DAFB. The response to scoring was temperature-dependent at 20 DAFB, with significant reductions in A_{max} only at 30°C and above. Dark respiration rates were not influenced by treatments but showed the expected increases with increasing temperature. The reductions in A_{max} due to scoring and thinning were attributable to both reductions in stomatal conductance, and reductions in maximum rate of carboxylation by rubisco ($V_{c_{max}}$) and maximum rate of electron transport (J_{max}). $V_{c_{max}}$ was significantly lower in the scored treatments at 85 DAFB. J_{max} increased substantially from 20 to 50 DAFB and was decreased significantly by scoring at 85 DAFB, and by thinning at 20 DAFB but not thereafter. Therefore, slight feedback inhibition was evident only during the early season in thinned trees but did not influence A_{max} , whereas small reductions in A_{max} thereafter were due to lower stomatal conductances. In contrast, scoring resulted in season-long reductions in stomatal conductance and gradual down-regulation of photosynthetic

capacity during the mid- and late-season probably due to a reduction in the shoot growth sink. This effect was attributable primarily to the strong response of de-fruited trees. No feedback inhibition during the middle part of the season (50 DAFB) might indicate source-limitations, even on trees with a reduced fruit sink strength, due to excessive vegetative growth.

Keywords: *Apple, crop load, feedback inhibition, photosynthesis, rubisco, scoring, sink strength, stomatal conductance, thinning.*

3.1 Introduction

The 'Royal Gala' apple tree (*Malus domestica* Borkh.) has an unsatisfactory fruit size distribution when grown in the Western Cape region of South Africa. This is attributed to excessive tree vigour in combination with unfavourably warm temperatures during winter and spring. Fruit size can be improved through cultural manipulations which shift the allocation of available carbohydrates from vegetative to reproductive growth (Corelli Grappadelli et al., 1994). Since dwarfing rootstocks do not offer satisfactory solutions to excessive tree vigour in this region, and the use of chemical growth retardants is not encouraged due to environmental concerns, growers are showing renewed interest in the use of girdling or scoring in combination with the usual fruit thinning program to achieve growth control and increase fruit size. Girdling can be defined as the removal of a strip of bark from around a trunk or limb, whilst scoring is a form of girdling in which a narrow cut is made around the circumference of the trunk. This blocks basipetal phloem transport of photosynthates and metabolites and accumulation of these products above the girdle (Goren et al., 2004).

In deciduous fruit trees, carbohydrates must not only be produced but also allocated efficiently to fruit and new flower buds (Forshey and Elfving, 1989). Source-sink relationships and the regulation of carbon allocation therefore determine growth of individual organs. This may be restricted by assimilate availability (source limitation) or by the organ's ability to utilise assimilates (sink limitation) (Patrick, 1988). In the hierarchy of sinks fruits and shoots are equal (Wardlaw, 1990), but since shoots develop prior to fruits they compete more efficiently for carbohydrates during the early part of the season (Bangerth and Ho, 1984). Girdling at this stage reduces the sink strength of the shoot apices, making more assimilates

available for fruit growth. This has been found in various crops, including apples and pears (Dennis, 1968), peaches and nectarines (Dann et al., 1984), grapevines (Winkler et al., 1974), olives (Lavee et al., 1983) and several other species (Grierson et al., 1982). On the other hand, fruit thinning reduces competition between fruitlets, making more assimilates available for the remaining fruits (Denne, 1960; Westwood et al., 1967; Bergh, 1990). The effect of both these practices on assimilate allocation patterns leads to increased fruit size.

In addition to affecting carbohydrate allocation, the reductions in sink strength brought about by thinning and girdling can in themselves influence carbohydrate production of the leaves. Photosynthesis and stomatal conductance in apple leaves are regulated in the short-term primarily by light, but also to some degree by temperature and leaf-to-air vapour pressure deficit (VPD), soil and plant water status, and mineral nutrition (Farquhar and Sharkey, 1982; Jones et al., 1985; Schulze, 1986; Lakso, 1994). However, the effect of these environmental variables also depends strongly on the source:sink balance of the tree (Flore and Lakso, 1989; Lakso, 1994). Apple stomata, and thus photosynthesis, are known to be regulated over the longer-term primarily by photosynthetic demand for CO₂, as governed by current sink strength of growing vegetative and reproductive organs.

The photosynthetic rate of apple leaves is maximal shortly after full expansion, but declines only slowly over the season if the leaf remains healthy and well exposed (Fujii and Kennedy, 1985). The rate of decline can also be reduced by the presence of strong sinks (Rom and Ferree, 1986). A reduction in the fruit sink size, due to a reduction in crop load or complete crop removal, has been shown to cause reductions in stomatal conductance and carboxylation efficiency (Monselise and Lenz, 1980; Wünsche et al., 2000) through feedback mechanisms (Guinn and Mauney, 1980; Krapp and Stitt, 1995). Feedback inhibition of photosynthesis has also been measured after girdling (Goldschmidt and Huber, 1992; Berüter et al., 1997; Li et al., 1996). To be effective, these manipulations are carried out during the early period of fruit and shoot growth two to four weeks after full bloom, when carbohydrate demand of the rapidly growing crop and canopy is high and useable carbon reserves have been depleted (Lakso et al., 1999). It is possible that positive effects on fruit growth are to be found only for a moderate degree of sink strength reduction; stronger reductions such as heavy thinning in combination with girdling may lead to strong feedback inhibition (Schechter et al., 1994a,b; Urban et al., 2004) and carbon supply limitations. This could be exacerbated if

environmental conditions are not optimal for photosynthesis (Bepete and Lakso, 1998). Leaf photosynthetic rates shows a parabolic response to temperature, with a peak at 25-30°C, thereafter dropping off rapidly due to an exponential increase in dark respiration with increasing temperature (Palmer et al., 2003). In the Western Cape, temperatures often reach 30°C during spring and this could account for a carbohydrate supply limitation during the cell division phase.

Growers require more accurate guidelines with respect to the combined use of these manipulations and the intensity thereof, in order to optimise both vegetative growth control and fruit size and yield. The objectives of this study were to investigate the effects of, and potential interaction between scoring and thinning, with respect to shoot and fruit growth and yield, as well as their effects on gas exchange at increasing leaf temperatures, and underlying photosynthetic capacity. We hypothesised that moderate to strong decreases in sink strength would reduce net leaf CO₂ assimilation rate via reductions in the maximum rate of carboxylation by rubisco (V_{cmax}) and the light-saturated electron transport rate (J_{max}) (increased non-stomatal limitation) of the leaves, as well as reductions in stomatal conductance (g_s) (increased stomatal limitation).

3.2 Materials and methods

3.2.1 Plant material and orchard management

The study was conducted in the Elgin region (34°10'S, 19°05'E, 350m above sea level), during the 2000-2001 season. An eight-year-old commercial 'Royal Gala' orchard on seedling rootstock was used, spaced 4.5 x 1.5 m and trained to a central leader. Full bloom was on 20 October 2000 and harvest on 7-14 February 2001 (110-117 days after full bloom, DAFB).

The trial was laid out as a factorial design with scoring (S), done on 2 and 27 November 2000 (13 and 38 DAFB) or no scoring (C), and hand thinning, done on 2 and 9 November 2000 (13 and 20 DAFB). Scoring was done with a citrus pliers (Cape Agricultural Products, Somerset West, South Africa), cutting a complete circle around the trunk, through the phloem (no removal of the phloem), below the bottom scaffold branches. The thinning treatments (with abbreviations) were: 1. Control (C), all fruit left; 2. Thinned to two fruit per bearing position (2F); 3. Thinned to one fruit per bearing position (1F); 4. Thinned to one fruit

every second bearing position (1/2F); 5. All fruit removed (0F). A randomised complete block design with 10 single tree replications was used. Standard commercial orchard management practices were applied, with the exception that no chemical thinning was done on the trial trees.

3.2.2 Gas exchange measurements

Measurements of gas exchange were taken using a portable photosynthesis system (LI-6400, Li-Cor, Lincoln, Nebraska, USA). The system uses the equations derived by von Caemmerer and Farquhar (1981) to calculate net CO₂ assimilation rate, transpiration rate and stomatal conductance.

On three dates (20, 50 and 85 DAFB) A/c_i (leaf net CO₂ assimilation rate, A , as a function of intercellular CO₂ concentration, c_i) and photosynthetic light response curves were performed on bourse shoot leaves in full sunlight. Treatments used for gas exchange measurements and their abbreviations were: 1. Un-thinned control (scored and un-scored), (SC and CC), 2. One fruit every second bearing position (scored and un-scored), (S1/2F and C1/2F) and 3. De-fruited scored trees (S0F). This selection was expected to represent both extremes of sink strength (CC and S0F) with the other three treatments in-between. Gas exchange measurements were performed on five of the 10 replicates.

3.2.2.1 Photosynthetic light response

Photosynthetic light response curves were generated at leaf temperatures of 20°, 25°, 30°, and 40°C (20 DAFB), 20°, 25° and 30°C (50 DAFB) and 20°, 25°, 30° and 35°C (85 DAFB), controlled electronically by the instrument. Irradiances of 2000, 1500, 1000, 800, 600, 400, 200, 100, 50 and 0 $\mu\text{mol m}^{-2} \text{s}^{-1}$ photosynthetic photon flux (PPF) were provided by an internal red/blue LED light source (LI-6400-02B Li-Cor, Lincoln, Nebraska, USA). Cuvette CO₂ concentration was controlled at 380 $\mu\text{mol mol}^{-1}$ using the LI-6400 CO₂ injection system and compressed CO₂-cylinders. Leaf-to-air vapour pressure deficit (VPD) was controlled at 2.0-2.7 kPa, by manipulating the flow rate through the desiccant.

Response curves of A against PPF were fitted individually using non-linear regression (Statistica 5.5, Statsoft Inc., Tulsa, OK, USA) and the monomolecular function $y=a(1-e^{-b \cdot cx})$ given by Causston and Dale (1990) (R^2 values for the fits obtained by the model were in the range 0.90-0.99 for the 10 data points). The strong feature of this model lies in the fact that it flattens out at high values, thus giving more realistic estimates of the light-saturated rate of net CO₂ assimilation (A_{\max}), compared to Michaelis-Menton-type functions which tend to overestimate this value (Causston and Dale, 1990). In this function, the coefficient “a” represents the light-saturated rate of net CO₂ assimilation (A_{\max}) and stomatal conductance (g_s) was calculated by the instrument and reported at the highest light intensity (PPF = 2000 $\mu\text{mol m}^{-2} \text{s}^{-1}$). Values for dark respiration (R_d) were taken as net CO₂ exchange rate at 0 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPF.

3.2.2.2 A/c_i response

A/c_i curves were generated at leaf temperatures of 25°C, controlled electronically by the instrument, and an irradiance level of 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PFD. Cuvette CO₂ concentrations were sequentially set at 380, 300, 200, 150, 100, 50, 1200, 700, 500 and again at 380 $\mu\text{mol mol}^{-1}$ to test for drift. VPD was controlled at 2.0-2.5 kPa.

The PhotosynAssistant program (v. 1.1.2, Dundee Scientific, Dundee, UK) was used for the analysis of the A/c_i curves. The program uses the mechanistic model proposed by Farquhar et al. (1980), as subsequently modified by Harley et al. (1992). An iterative procedure is used to make estimations of the maximum rate of carboxylation by rubisco ($V_{c_{\max}}$) and the PFD-saturated rate of electron transport (J_{\max}) from the A/c_i curves (Harley et al., 1992).

3.2.3 Fruit and shoot growth

The lengths of one randomly selected bourse and extension shoot on a tagged branch on both sides of the tree were measured three times (20, 40 and 85 DAFB). Fruit diameter of a tagged fruit on both sides of the tree were measured weekly and the average fruit growth rate calculated in mm day^{-1} for the cell division stage (0-53 DAFB) and cell enlargement stages

(54-102 DAFB). Day 53 was retrospectively chosen based on analysis of the seasonal fruit growth curves.

Trees were selectively harvested on 7 February 2001 (Harvest 1) and random sub-samples of 10 fruit per tree were analysed for fruit diameter, fruit mass and number of seeds. On 14 February 2001 trees were strip harvested (Harvest 2) and a random sub-sample of 20 fruit per tree analysed as before. Total yield per tree was recorded and expressed as yield cm^{-1} trunk circumference.

3.2.4 Statistical analysis

Five replicates of photosynthetic light response curves were obtained at each leaf temperature. Five replicates of A/c_i curves were obtained for each treatment. All data were analysed using SAS (v. 6.12, SAS Institute, Cary, NC, USA). Contrasts between scored (SC, S1/2F and S0F) and un-scored (CC and C1/2F) and between thinned (S1/2F and C1/2F) and un-thinned (SC and CC) treatments were used to test differences between treatments at each temperature and date. The program was also used to test for a linear or quadratic temperature response at each date.

Fruit and shoot growth data were analysed by two-way ANOVA (SAS v. 6.12) to test the significance ($Pr \leq 0.05$) of thinning and scoring effects and their interactions. Average of three measurement dates were used for shoot lengths. Multiple comparison tests (Tukey, $Pr \leq 0.05$) were also performed.

3.3 Results

3.3.1 Photosynthetic light response

At each measurement date A_{\max} exhibited a quadratic response to increasing leaf temperatures (Table 1), with a temperature optimum of 22-28°C (Fig. 1). A_{\max} of the scored trees was significantly reduced at leaf temperatures higher than 30°C compared to un-scored trees early in the season (20 DAFB) (Fig. 1a). The contrast between scored and un-scored treatments and a significant treatment x temperature interaction indicate the faster reduction in A_{\max} of scored treatments with an increase in temperature above 30°C (Table 1). During this period there

were no differences in A_{\max} between thinned and un-thinned treatments (Fig. 1d, Table 1). Low ambient temperatures during the 50 DAFB measurement period resulted in maximum leaf temperatures of only 30°C being achieved. During this period A_{\max} of the scored treatment was significantly lower than that of the un-scored treatment (no interaction between treatment and temperature) (Fig. 1b, Table 1). A_{\max} of thinned treatments was also significantly lower than that of un-thinned treatments during this period (Fig. 1e, Table 1). The same pattern was repeated later in the season (85 DAFB) (Fig. 1c, f, Table 1).

Light-saturated stomatal conductance (g_s) decreased linearly with increasing leaf temperature at 20 and 85 DAFB, and exhibited a quadratic response to increasing leaf temperature at 50 DAFB (Fig. 2, Table 1). g_s was significantly lower in scored treatments compared with un-scored treatments throughout the season (Fig. 2a, b, c, Table 1). Only at 50 DAFB, were g_s of thinned treatments significantly lower than that of un-thinned treatments (Fig. 2d, e, f, Table 1).

Dark respiration rate (R_d) increased linearly, 20 and 85 DAFB, and quadratic, 50 DAFB with increasing leaf temperature, with no significant differences between treatments, at any time during the season (Fig. 3, Table 1). R_d was reduced at 50 and 85 DAFB compared to 20 DAFB (Fig. 3).

3.3.2 A/c_i response

There was a slight decline in $V_{c_{\max}}$ throughout the season in the scored treatments while the un-scored treatments remained relatively constant (Fig. 4a). Only at 85 DAFB was there a significant difference between scored and un-scored treatments (Table 2). However, J_{\max} increased after 20 DAFB for all the treatments (Fig. 4b, c). Early in the season (20 DAFB), J_{\max} of the thinned treatments was significantly lower than that of the un-thinned treatments (Fig. 4b, Table 2). Late in the season (85 DAFB), J_{\max} of the scored treatments was significantly lower than that of the un-scored treatments (Fig. 4c, Table 2). 85 DAFB A_{\max} of de-fruited scored trees (SOF) were significantly reduced compared to un-scored control trees (CC) due to a significant reduction in $V_{c_{\max}}$, J_{\max} and g_s (Table 3).

3.3.3 Fruit and shoot growth

Scoring (Fig. 5a) significantly reduced the length of bourse shoots, while all thinning treatments led to increased bourse shoot growth compared to the control. However thinning to one fruit per bearing position showed a significantly stronger response compared to thinning to two fruit per bearing position, with severe thinning and fruit removal in between (Fig. 5b). The significant thinning x scoring interaction on extension shoot length was due to a particularly strong scoring-induced decrease in extension shoot length in de-fruited trees relative to the other thinning treatments (Fig. 5c).

Individual fruit growth rate was significantly higher in the scored treatments during the first 53 DAFB (Fig. 6a). During the latter part of the season (53-102 DAFB) there were no significant differences in fruit growth rates between treatments (Fig. 6a). Consequently, fruit from scored trees were significantly larger than fruit from control trees on both harvest dates (Fig. 6b). Decreasing crop load also resulted in increased average fruit mass on both harvest dates (Fig. 6c).

Although there was a significant difference between the number of fruit per tree (data not shown), yields did not differ significantly between treatments thinned to different crop loads. Total yield expressed in kg cm^{-1} trunk circumference were significantly higher on scored trees compared to control trees (Fig. 7).

3.4 Discussion

Fruit size is determined by the combination of cell number, cell size and percentage intercellular space (Goffinet et al., 1995). Pearson and Robertson (1953) stated that the variation in fruit size is determined early in development by the number of cell divisions (cell number), and that within one season cell volume in individual fruit of the same age is fairly uniform. In order to maximise fruit size, carbon allocation to the fruit during this period must be optimised. In this study scoring during the cell division stage of fruit growth resulted in a significant increase in fruit growth rate. This increase in fruit growth rate culminated in increase fruit size at harvest. It would appear as if scoring allows for the preferential allocation of carbohydrates to the fruit. In this study, fruit size was significantly improved by thinning to

one fruit per cluster or one fruit every second cluster. The effect of crop load on fruit growth rate and size in apple is well documented (Forshey and Elfving, 1989; Palmer et al., 1997; Link, 2000; Wünsche et al., 2000). However the combination of fruit thinning with scoring seems to have an additive effect on fruit growth rate and final fruit size.

In this study the optimum temperature range for photosynthesis was between 20 and 25°C, 20 and 50 DAFB and between 25 and 30°C, 85 DAFB (Fig. 2). This upward shift was likely due to differential stomatal responses to temperature (Fig. 3), but may also have been related to the increase in average maximum air temperature later in the season. Photosynthetic acclimation to seasonal temperature variation has also been observed in other temperate fruit species e.g. *Prunus armeniaca* (Lange et al., 1974). Slatyer and Ferrar (1977) found that this acclimation could occur in two weeks or less. At temperatures higher than the optimum, the ratio CO_2/O_2 decreases and this decreases the carboxylation efficiency of rubisco, with a resulting increase in photorespiration and thus a decrease in carbon fixation (Berry and Björkman, 1980). The increase in respiratory production of CO_2 with increasing temperature also decreases A_{max} at higher temperatures. There was a greater reduction in A_{max} in treatments with reduced sink strength induced by scoring at temperatures higher than 30°C during the early spring (Fig. 2). This was largely due to a decrease in g_s at higher temperatures (Fig. 3) on the scored treatment. A similar stronger reduction in A_{max} at higher temperatures has also been observed in an earlier study after harvest of 'Braestar' apple trees (Chapter 9). In that case, the reduction in A_{max} post-harvest was due to both increases in stomatal and non-stomatal limitations.

The partitioning pattern of photosynthate between vegetative development and reproductive development early in the season, when fruit growth is mainly due to cell division, has a pronounced influence on fruit development and size (Corelli Grappadelli et al., 1994). Cell division takes place primarily during the first 40 days after full bloom (DAFB), followed by cell enlargement (Bergh, 1990). In order to maximise fruit size, it is imperative to optimise carbon allocation to the fruit during this period. The increase in R_d with an increase in temperature (Lakso, 1994) (Fig. 5) would mean that significant carbohydrate shortages could occur in trees during source limited periods early in the season, especially under warm weather conditions.

The vegetative development of extension and bourse shoots apparently has priority over reproductive development in the early season, thus negatively affecting fruit set and fruit growth by cell division (Wardlaw, 1990). In this study, scoring reduced the average length of bourse shoots, while extension shoot lengths were reduced on scored trees thinned to one fruit per bearing position (S1F), scored control trees (SC) and scored de-fruited trees (S0F), therefore allowing more of the available assimilates to be partitioned to the developing fruit during the early cell division stage of fruit development. The effect of increased availability of photosynthate to the developing fruit culminated in an increased fruit growth rate, during the cell division stage, and ultimately bigger fruit and increased yields on scored trees. This means that scoring shifted the balance from vegetative to reproductive growth, by decreasing the strength of the vegetative sink during the first 40 DAFB. Moderate thinning (to one fruit per bearing position) stimulated extension shoot growth, but strong thinning (to one fruit every second cluster) and complete fruit removal led to decreased shoot growth on scored trees, but not on control trees. Whilst thinning can optimise fruit size, there is a compromise necessary with yield. Crop load will reflect the genetically determined size potential of the cultivar and the varietal fruit size response to thinning (Wünsche and Ferguson, 2005). In order to improve fruit size of small-fruited cultivars such as 'Royal Gala', early thinning is critical, but reducing fruit: shoot competition early in the season can be just as beneficial. There was a particularly strong scoring-induced decrease in extension shoot growth in de-fruited trees, while extension shoot length increased with a decrease in crop load on control trees. The length of bourse shoots was also reduced on control trees compared to trees thinned to one fruit per bearing position and one fruit every second bearing position. According to Wünsche et al. (2000), reducing the number of fruit per tree increases shoot growth by increasing the number of active shoots and their growth rate. In this study a similar trend was observed with a decrease in fruit numbers until a moderate thinning level (one fruit per bearing position) is reached. Further decrease in fruit numbers (one fruit every second bearing position and complete fruit removal) decreases extension shoot length on scored trees.

A reduction in sink strength due to a reduction in crop load causes a reduction in A of the leaves. However, this effect could be masked by the presence of strong vegetative sinks as the effect of sink strength on photosynthesis becomes apparent only under conditions of sink limitation (Flore and Lakso, 1989). This down-regulation of A under low and no crop load has

been observed in field studies (Hansen, 1970; Fujii and Kennedy, 1985; Wünsche et al., 2000). However, early in the season this effect is less clear due to strong vegetative growth. By scoring the trees the vegetative sink strength was reduced and the effect of crop load on photosynthesis could be studied. At 20 DAFB J_{\max} of the scored treatment with the lowest crop load was lower than the rest of the treatments, indicating reduced sink demand and resulted in down regulation of the light harvesting system of photosynthesis. This trend is also seen in A_{\max} and g_s , since a combination of J_{\max} and $V_{c_{\max}}$ directly influence these parameters. Reduction in A_{\max} due to scoring induced feedback inhibition has also been measured on citrus trees (Goldschmidt and Huber, 1992; Li et al., 1996). Palmer et al. (1997) reported a positive and curvilinear response of A to crop load in New Zealand. However, the effect of crop load on photosynthesis is very dependent upon time and severity of fruit removal. Thinning at a later stage has a more profound effect, as there are fewer actively growing alternative sinks available for carbohydrate movement (Wünsche et al., 2000). In this study no significant differences in A_{\max} were observed between thinned and un-thinned treatments 20 DAFB, indicating that at this stage vegetative growth acted as a strong sink for carbohydrates.

De-fruiting reduces the stomatal conductance and carboxylation efficiency (stomatal and non-stomatal limitations) in apple trees (Monselise and Lenz, 1980), probably caused by an increase in starch concentrations in chloroplasts of non-fruiting trees (Wünsche et al., 2000). This can be interpreted as the result of interrupted utilization of carbon assimilates by the fruit, which eventually causes a decrease in A through a feedback mechanism (Guinn and Mauney, 1980; Krapp and Stitt, 1995). Increases in starch concentration of the leaf also cause an increase in non-radiative thermal dissipation (Pammenter et al., 1993), indicating a redistribution of energy away from photosynthesis and hence a reduction in photochemical efficiency, that is the conversion of light to chemical energy by the photochemical apparatus.

There were no significant differences between photosynthetic efficiency parameters at 50 DAFB (Table 2), indicating that at this stage of the season there may have been a shortage in the carbon supply even in the treatments with the light crop load, as there was no negative feedback inhibition of photosynthesis. There was an increase in J_{\max} and A_{\max} throughout the season and a slight decrease or stability in $V_{c_{\max}}$ (Fig. 1, 2 and 4). Since A_{\max} is directly influenced by J_{\max} and $V_{c_{\max}}$ the increase in J_{\max} had to account for the increase in A_{\max} . At 85 DAFB there was a reduction in the photosynthetic capacity of the lower cropping and scored

trees indicating that photosynthesis was demand limited during this period. The cessation of shoot growth as well as the fact that extension shoots are exporting carbohydrates during this period meant that there was an abundance of photosynthate available in the tree.

In order to achieve optimum yields and fruit size, photosynthesis should be slightly demand limited throughout the season, and care must be taken to control the vegetative sinks in order to partition as much as possible of the photosynthate to the fruits. With the international trend to move away from harsh chemical growth retardants, scoring offers an environmentally safe alternative to dealing with tree vigour. The effect of scoring on return bloom as well as the long-term effect on assimilate partitioning, however, warrant further investigation.

3.5 References

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Table 1. Probability values of light-saturated net CO₂ assimilation rate (A_{\max}), stomatal conductance (g_s) and dark respiration rate (R_d) at 20, 50 and 85 days after full bloom (DAFB) for differences between treatments and different leaf temperatures, as well as for the contrasts between scored and un-scored and thinned and un-thinned treatments, and for the linear and quadratic temperature responses.

Variable		20 DAFB	50 DAFB	85 DAFB
A_{\max} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	Treatment	0.0032	0.0003	0.0001
	Temperature	0.0001	0.0195	0.0001
	Trt*Temp	0.0001	0.9133	0.1813
	Contrasts			
	Scored vs un-scored	0.0052	0.0024	0.0001
	Thinned vs un-thinned	0.6588	0.0403	0.0008
	Temperature response			
	Linear	0.0001	0.0408	0.0001
	Quadratic	0.0001	0.0486	0.0001
	g_s ($\text{mmol m}^{-2} \text{s}^{-1}$)	Treatment	0.0125	0.0001
Temperature		0.0001	0.0001	0.0001
Trt*Temp		0.4142	0.6271	0.2393
Contrasts				
Scored vs un-scored		0.0222	0.0058	0.0001
Thinned vs un-thinned		0.1841	0.0462	0.1737
Temperature response				
Linear		0.0001	0.0001	0.0001
Quadratic		0.3109	0.0092	0.1538
R_d ($\mu\text{mol m}^{-2} \text{s}^{-1}$)		Treatment	0.8404	0.3536
	Temperature	0.0001	0.0001	0.0001
	Trt*Temp	0.8272	0.9131	0.4434
	Contrasts			
	Scored vs un-scored	0.9078	0.4947	0.2462
	Thinned vs un-thinned	0.7413	0.2995	0.1963
	Temperature response			
	Linear	0.0001	0.0001	0.0001
	Quadratic	0.6585	0.0013	0.4615

Table 2. Probability values of the maximum rate of carboxylation by Rubisco (V_{cmax}) and light-saturated rate of electron transport (J_{max}) at 20, 50 and 85 DAFB at a leaf temperature of 25°C for the differences between treatments and the contrasts between scored and un-scored and thinned and un-thinned treatments.

Variable	Pr>F	20 DAFB	50 DAFB	85 DAFB
V_{cmax} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	Treatment	0.9343	0.3600	0.0073
	Contrasts			
	Scored vs un-scored	0.6895	0.1190	0.0021
	Thinned vs un-thinned	0.9618	0.2690	0.4804
J_{max} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	Treatment	0.0388	0.6227	0.0008
	Contrasts			
	Scored vs un-scored	0.1689	0.2275	0.0004
	Thinned vs un-thinned	0.0283	0.4164	0.6745

Table 3. Late-season gas exchange capacity (data taken from A/c_i curves at 85 DAFB) of the measured treatments. Means with the same letter within columns are not significantly different (Tukey $Pr < 0.05$).

	Treatment					Pr > F
	CC	SC	C1/2F	S1/2F	S0F	
$V_{c_{max}}$ ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	60.8 a	58.4 ab	63.8 a	53.8 ab	49.5 b	0.0057
J_{max} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	160.2 a	155.6 a	166.0 a	150.0 ab	135.4 b	0.0020
A_{max} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	18.0 a	17.6 a	19.1 a	15.9 ab	14.2 b	0.0037
g_s ($\text{mmol m}^{-2} \text{s}^{-1}$)	0.364 ab	0.384 ab	0.408 a	0.318 ab	0.268 b	0.0261

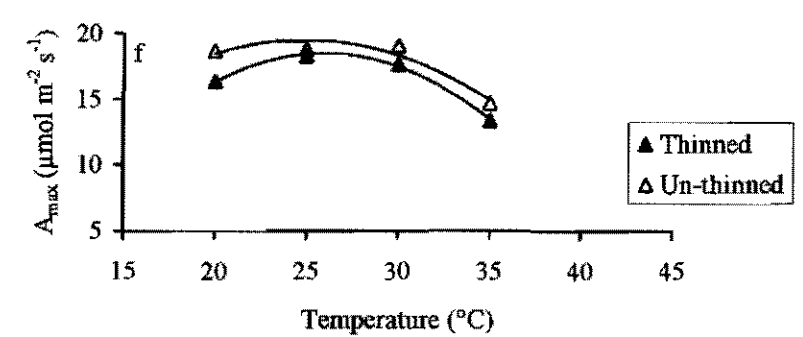
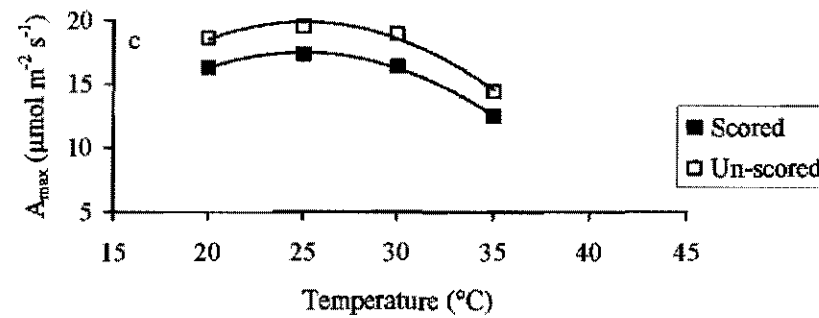
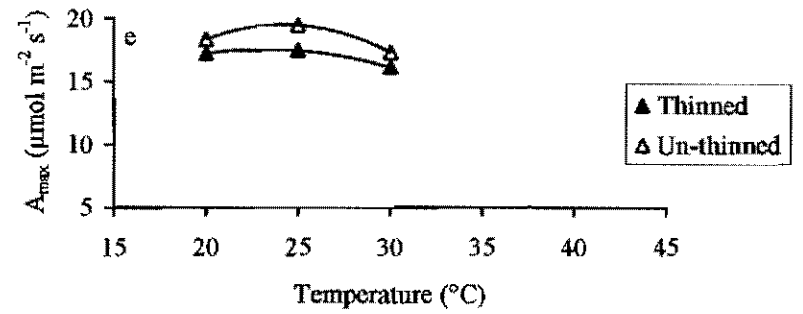
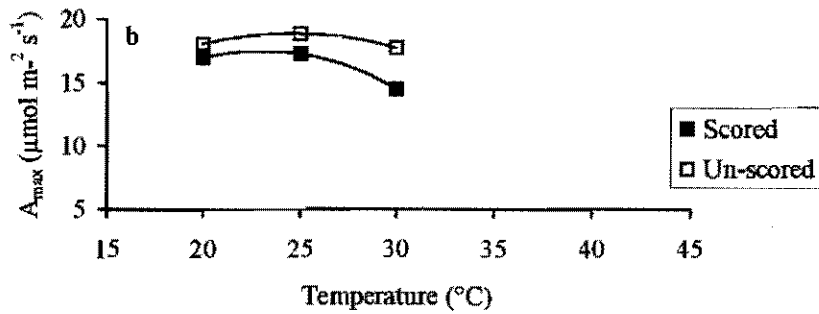
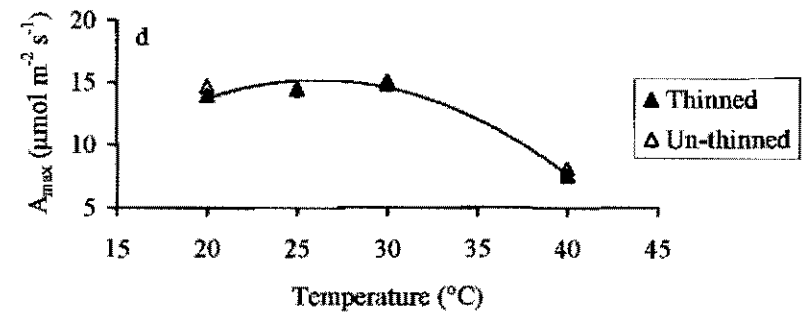
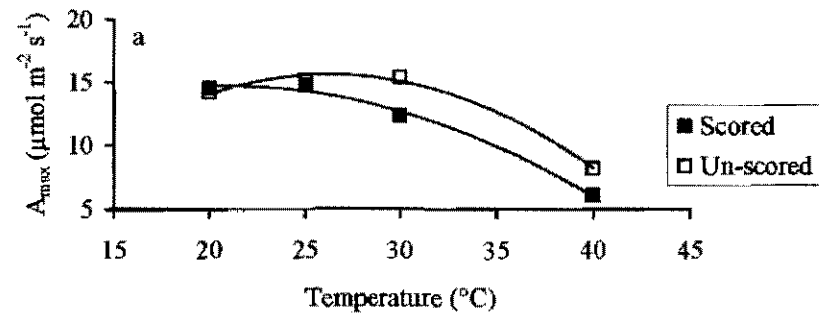


Figure 1. Maximum light-saturated net CO₂ assimilation rate (A_{max}) at different leaf temperatures for the scored and un-scored treatments at (a) 20 DAFB, (b) 50 DAFB and (c) 85 DAFB, and the thinned and un-thinned treatments at (d) 20 DAFB, (e) 50 DAFB and (f) 85 DAFB.

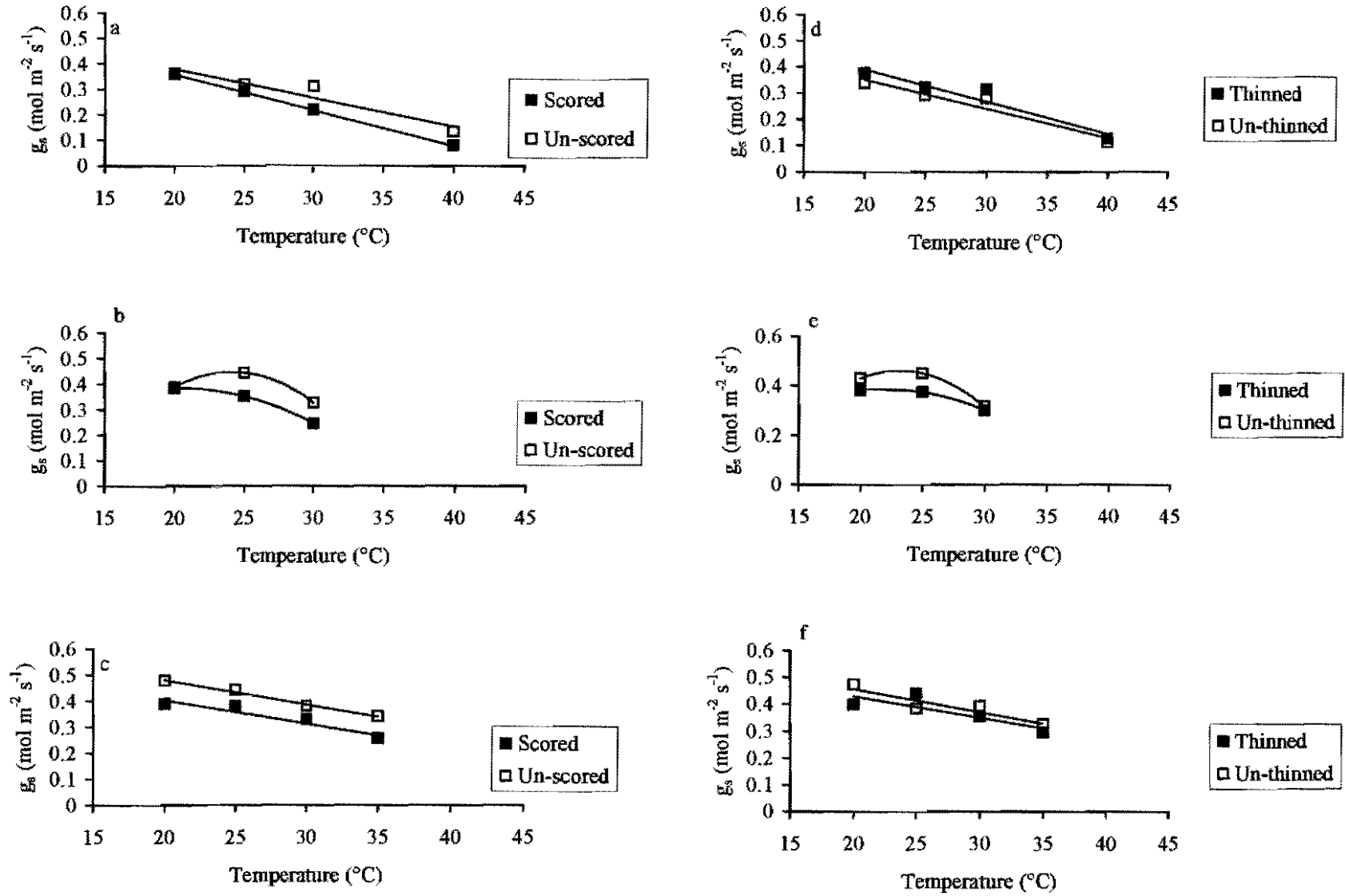


Figure 2. Light-saturated stomatal conductance (g_s) at different leaf temperatures for the scored and un-scored treatments at (a) 20 DAFB, (b) 50 DAFB and (c) 85 DAFB and the thinned and un-thinned treatments at (d) 20 DAFB, (e) 50 DAFB and (f) 85 DAFB.

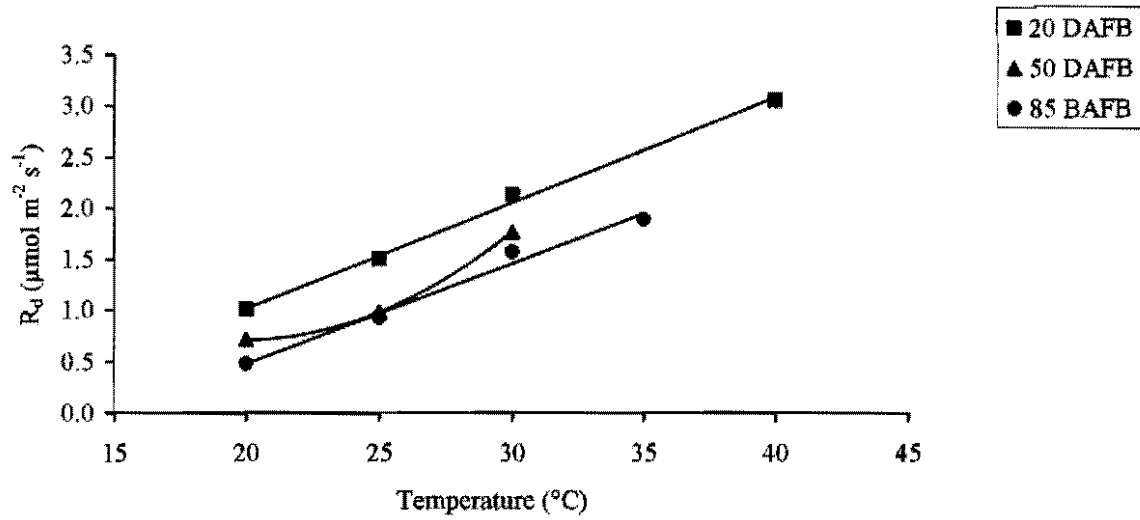


Figure 3. Dark respiration (R_d) at different leaf temperatures at 20 DAFB, 50 DAFB and 85 DAFB.

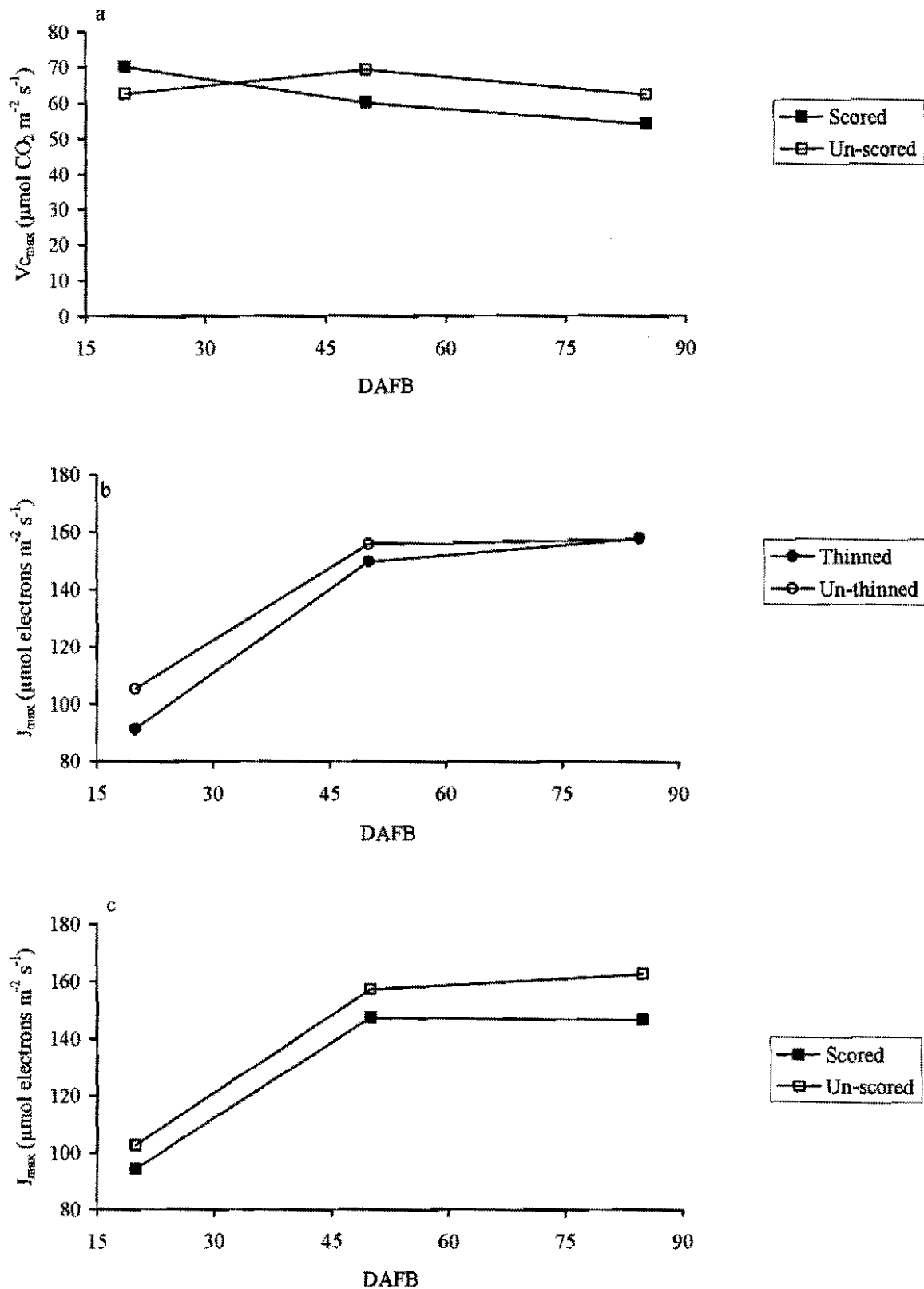


Figure 4. (a) The maximum rate of carboxylation by rubisco ($V_{C_{max}}$) for the scored and un-scored treatments, (b) maximum rate of electron transport (J_{max}) for the thinned and un-thinned treatments and (c) J_{max} for the scored and un-scored treatments.

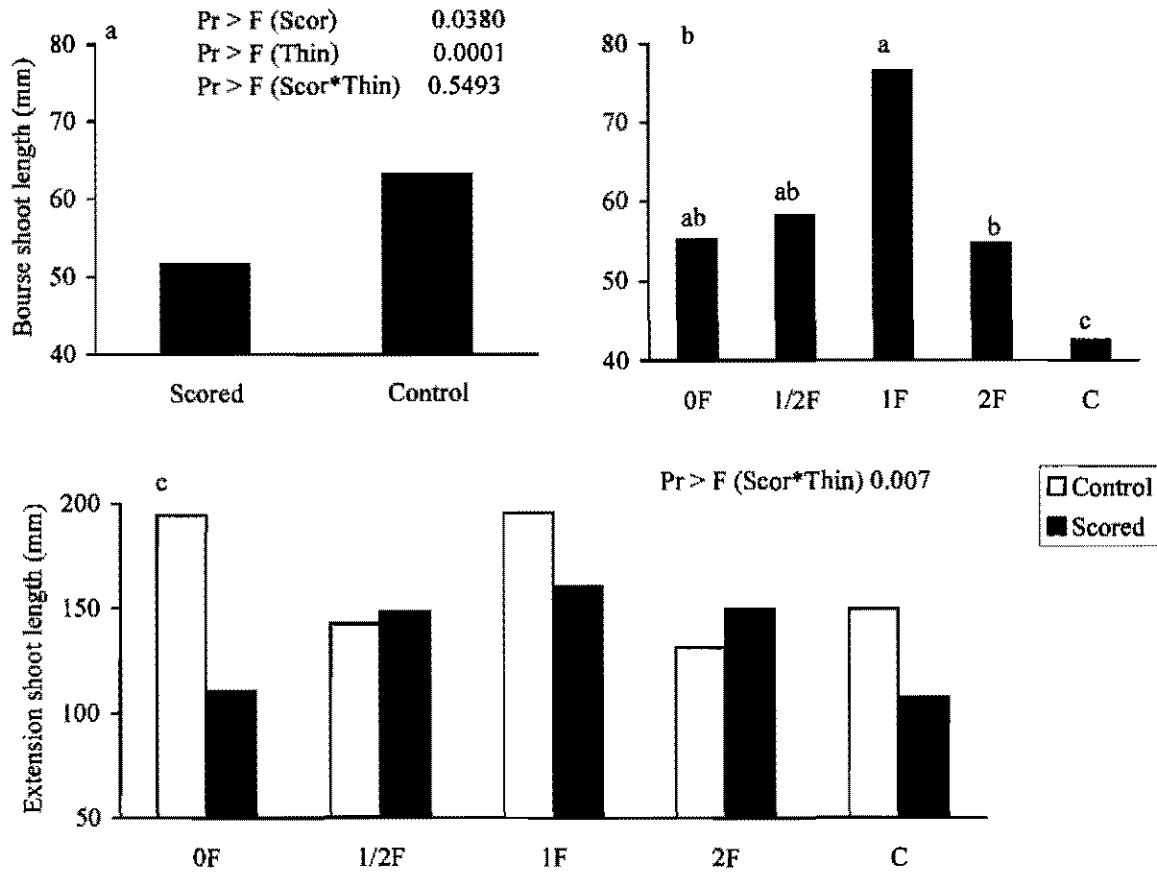


Figure 5. Shoot length averaged over three measurement times. (a) bourse shoot length of control and girdled treatments, (b) bourse shoot length at different crop loads, (c) extension shoot length of girdled and control treatments at different crop loads. C = control, all fruit left; 2F = thinned to two fruit per bearing position; 1F = thinned to one fruit per bearing position; 1/2F = thinned to one fruit every second bearing position; 0F = all fruit removed. Means of bars capped with the same letter are not significantly different (Tukey Pr < 0.05).

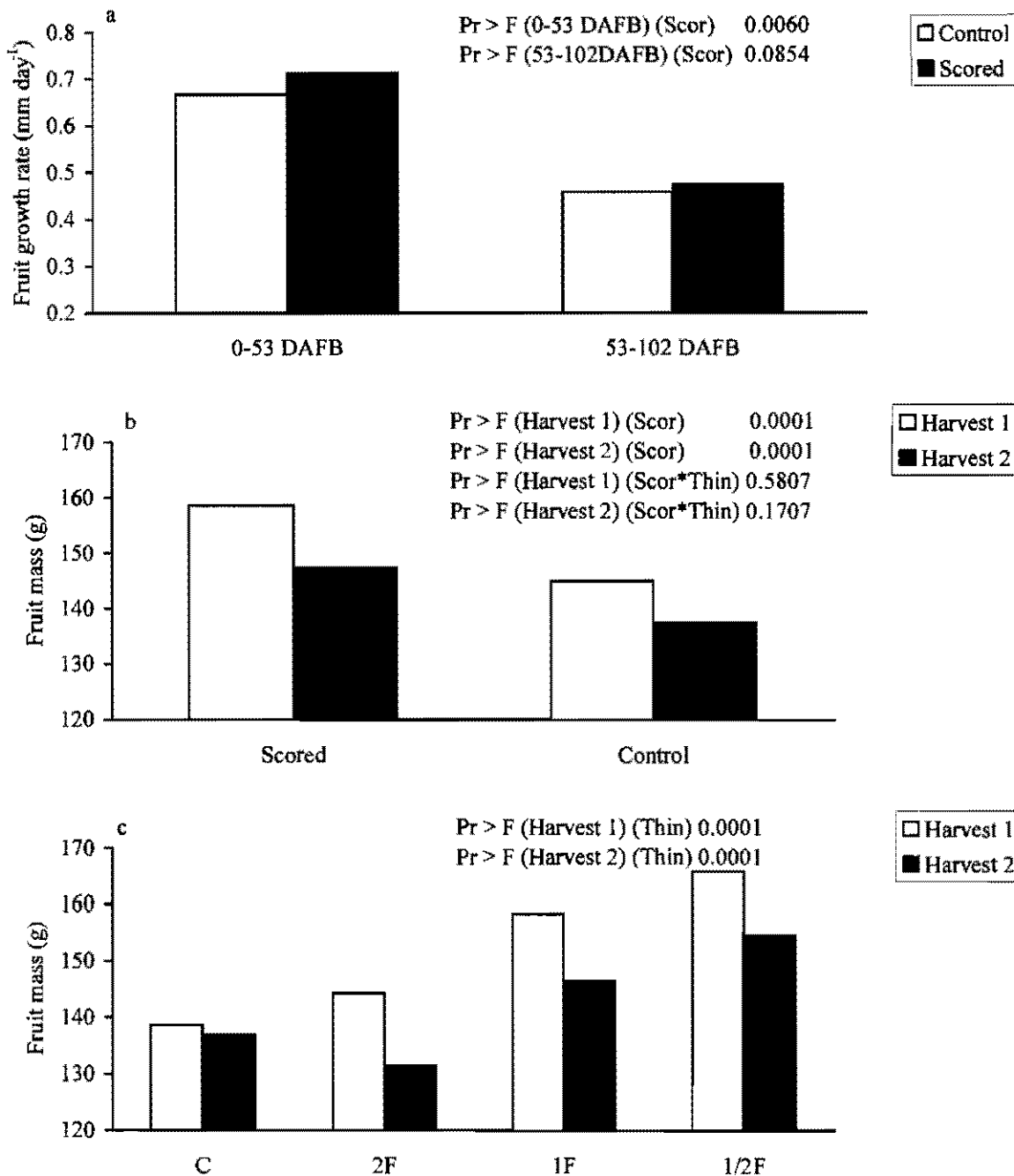


Figure 6. (a) fruit growth rate (mm day⁻¹) of scored and control treatments for two stages during the season; (b) fruit fresh mass (g) of scored and control treatments and (c) fruit fresh mass (g) of the thinning treatments, C = control, all fruit left; 2F = thinned to two fruit per bearing position; 1F = thinned to one fruit per bearing position; 1/2F = thinned to one fruit every second bearing position. Measurements were taken at harvest 1 (7 February 2001) and harvest 2 (14 February 2001).

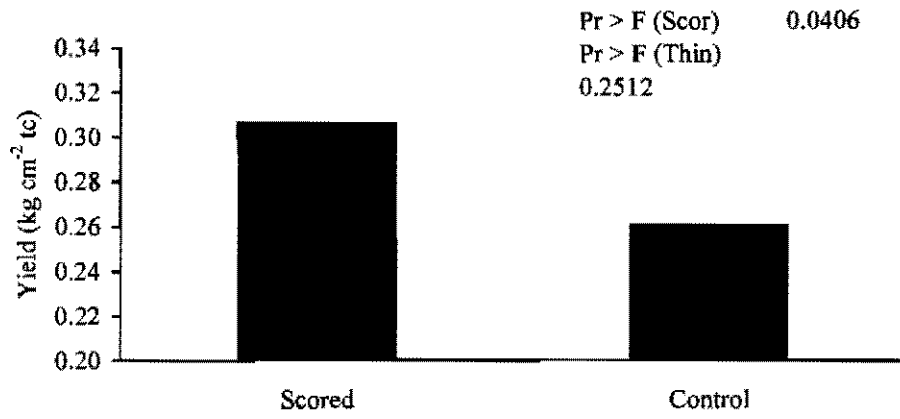


Figure 7. Total yield (kg cm⁻¹ trunk circumference(tc)) for the scored and control treatments.

4. Shoot growth and photosynthetic response following scoring, spraying of prohexadione-calcium and thinning on 'Royal Gala', 'Fuji' and 'Cripps' Pink' apple trees.

Abstract

Girdling or scoring and the gibberellin inhibitor prohexadione-calcium (ProCa) are widely used in orchards to control tree vigour. In this study, the individual and interactive effects of the growth retarding treatments scoring and ProCa and fruit thinning to various crop loads were investigated on 'Royal Gala', 'Fuji' and 'Cripps' Pink' during the 2001/02 and 2002/03 seasons with respect to shoot growth and photosynthetic responses. There were no interactive effects of treatments on light saturated net CO₂ assimilation rate (A_{max}). Scoring early in the season reduced A_{max} , this reduction in carbon availability led to earlier cessation of shoot growth as well as shorter shoots, which meant that more carbohydrates could be allocated to fruit growth. Later in the season, reduced fruit numbers led to a decrease in A_{max} . ProCa decreased final extension shoot length in all three cultivars during both seasons. Scoring reduced final extension shoot length in 'Fuji' and 'Cripps' Pink' during both seasons. Neither ProCa nor scoring affected the final bourse shoot length on 'Royal Gala' during both seasons. In both 'Fuji' and 'Cripps' Pink', scoring and ProCa reduced final bourse shoot length during 2001/02. Extension shoots were generally more sensitive to scoring and ProCa than bourse shoots. ProCa seems to be a more effective way of controlling shoot growth than scoring, with 'Fuji' and 'Cripps' Pink' being more sensitive to the application of ProCa than 'Royal Gala'. The apparent inhibiting effects of scoring on shoot growth were likely attributed to the bigger crop load on scored trees rather than a direct effect of scoring on shoot growth. The combined use of scoring and ProCa holds potential for decreasing shoot growth and increasing fruit growth.

Keywords. *Fruit sink, photosynthesis, prohexadione-calcium, scoring, shoot growth, vegetative sink strength, vigour.*

4.1 Introduction

Internationally there is a tendency to move away from harsh chemical growth retardants towards a more environmentally acceptable approach to control vigour of fruit trees. In the Western Cape region of South Africa, semi-vigorous rootstocks such as MM 106 or MM 793 are used in high-density orchards (1000-1666 trees ha⁻¹). Their lack of precocity are a serious limitation and this makes management of mature high density orchards difficult (Robinson, 2003). Currently available dwarfing rootstocks perform poorly under South African conditions due to inadequate winter chilling, causing poor bud break and canopy development, generally poor soil conditions, high soil temperatures and replant situations which restrict the growth of these rootstocks severely (Webster and Wertheim, 2003). Excessive vegetative growth is a major concern to the producer because it competes with fruit growth (Forshey and Elfving, 1989). Therefore a number of non-chemical ways are used to control excessive vigour (semi-vigorous rootstocks) in these orchards, including summer and root pruning, girdling or scoring, limb spreading, restricted fertiliser application and the control of irrigation (Greene, 2003). Used alone these techniques may be only marginally effective. An effective restriction of vegetative growth is desirable in vigorous apple cultivars in order to achieve a balance between vegetative and reproductive development (Goren et al., 2004).

Girdling or scoring is an effective technique to reduce vegetative growth, promote flowering, improve fruit set, increase fruit size and advance maturity in apples and pears (Dennis, 1968), peaches and nectarines (Dann et al., 1984), grapevines (Winkler et al., 1974), olives (Lavee et al., 1983) and several other species (Grierson et al., 1982). A girdled tree is one in which the phloem is completely severed, either by a narrow incision (scoring) or by the removal from the trunk of a more or less wide cylinder of bark without damage to the underlying tissue (Noel, 1970), thus blocking basipetal phloem transport and allowing for improved availability of metabolites for developing organs above the girdle.

Gibberellins (GA) are plant growth regulators associated with stem elongation (Owens and Stover, 1999) and prohexadione-calcium (ProCa; BAS-125, (3-oxido-4-propionyl-5-oxo-3-cyclohexene-carboxylate)) is a product, with a low persistency in the plant and soil, developed to act as a GA biosynthesis inhibitor, which can be applied by spraying (Rademacher, 1995). ProCa has been found to be an effective inhibitor of shoot growth in

apple trees (Miller, 2002). Simulated carbohydrate supply and demand curves for apple trees through the season show that carbon supply for fruit development can be potentially limiting two to four weeks after full bloom, when carbohydrate demand of the rapidly growing crop is high (Lakso et al., 1999). At this stage, the vegetative development of extension and bourse shoots appears to have priority over reproductive development, thus negatively affecting fruit set and fruit growth by cell division (Bepete and Lakso, 1998). Scoring, fruit thinning and the spraying of ProCa at this stage reduce the number of fruit sinks and total vegetative sink strength, thereby ensuring an adequate supply of carbohydrates for the remaining fruit. However, the interactions between these growth retarding cultural practices and fruit thinning are poorly understood.

The objective of this study was to investigate the effects of, and potential interaction between the growth retarding treatments scoring and ProCa and fruit thinning to various crop loads, with respect to shoot growth and the photosynthetic response. We hypothesised that a decrease in total fruit sink strength (thinning) and a decrease in vegetative sink strength due to scoring or the use of ProCa would reduce photosynthesis and have an additive effect on reducing shoot growth when applied in combination. Data on fruit growth, fruit quality and bud development will be presented in Chapter 5.

4.2 Materials and methods

4.2.1 Plant material and treatments

This study was conducted on commercial 'Royal Gala', 'Fuji' and 'Cripps' Pink' apple orchards (Nooitgedacht Farm) in the Koue Bokkeveld (Ceres) region, (33°13'S, 19°02'E, 975 m above sea level) (Western Cape, South Africa) during 2001/02 and 2002/03. Trees on M793 rootstock were planted in 1996 spaced 4.5 x 1.25 m and trained to a central leader. Orchard management practices were as for commercial orchards in the region.

The trial was laid out as a randomised complete block design arranged as a split plot with ten blocks and two trees per replication. Main treatments used were an un-scored control (C), trunk scoring (S) and the application of the growth retardant prohexadione-calcium (ProCa) (100 mg kg⁻¹) (referred to as the CSP treatments). Sub-treatments were fruit thinning by hand to various crop loads. Scoring was done with a girdling tool (Cape Agricultural

Products, Somerset West, South Africa), cutting a complete circle around the trunk, through the phloem (no removal of the phloem), below the bottom scaffold branches. Dates of full bloom, spraying of ProCa, scoring and hand thinning for 'Royal Gala', 'Fuji' and 'Cripps' Pink' for both seasons are presented in Table 1. 'Royal Gala' was scored twice during the season, as this is the commercial practice in the region.

For all cultivars a commercial chemical thinning program was applied (Table 2, 3, 4) after which crop loads were adjusted by hand thinning. In 'Royal Gala' an average tree in the orchard had 14 branches on the central leader. The number of fruit required per branch was calculated for projected yields of 75, 50, 30 and 15 t ha⁻¹ (Table 5) assuming an average fruit size of 145g and 1778 trees per hectare.

In 'Fuji' an average tree in the orchard had 12 branches on the central leader. The number of fruit required per branch was calculated for projected yields of 75, 50, 30 and 15 t ha⁻¹ (Table 5) assuming an average fruit size of 165g and 1778 trees per hectare.

In 'Cripps' Pink' an average tree in the orchard had 16 branches on the central leader. The number of fruit required per branch was calculated for projected yields of 75, 50, 30 and 15 t ha⁻¹ (Table 5) assuming an average fruit size of 160g and 1778 trees per hectare.

4.2.2 Shoot growth measurements

The lengths of two (one on each side of the tree) tagged bourse and extension shoots were measured at different times during the season. Shoot length data were fitted individually using non-linear regression (Statistica 5.5, Statsoft Inc., Tulsa, OK, USA) and the monomolecular function $y=a(1-e^{-bx})$ given by Causston and Dale (1990). This function described shoot growth accurately ($R^2 = 0.90-0.95$) since it flattens, thus describing shoot growth cessation effectively. The co-efficient 'a', represents the maximum value (100%) of final shoot length. Values for 95% of the final shoot length were then entered and by solving for x the number of days needed to reach this value were calculated.

4.2.3 Gas exchange measurements

Measurements of gas exchange were taken using a portable photosynthesis system (LI-6400, Li-Cor, Lincoln, Nebraska, USA). The system uses the equations derived by von Caemmerer and Farquhar (1981) to calculate net CO₂ assimilation rate (A) and stomatal conductance (g_s).

In 'Royal Gala' leaves light saturated net CO₂ assimilation rate, (A_{max}) and g_s were measured at ambient temperature and 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ photosynthetic photon flux (PPF) on three dates (31, 46 and 110 DAFB). Light was provided by an internal red/blue LED light source (LI-6400-02B, Li-Cor, Lincoln, Nebraska, USA). Cuvette CO₂ concentration was controlled at 380 $\mu\text{mol mol}^{-1}$ using the LI-6400 CO₂ injection system and compressed CO₂-cylinders. Leaf-to-air vapour pressure deficit (VPD) was controlled at 2.0-2.7 kPa, by manipulating the flow rate through the desiccant. In 'Fuji' and 'Cripps' Pink' A_{max} and g_s were measured on two dates (110 and 151 DAFB). Measurements were done on bourse shoot leaves in full sunlight during 2001/02, using five of the ten replicates.

Treatments used for gas exchange measurements were scored trees at a high crop-load (58, 71 and 64 t ha⁻¹ for 'Royal Gala', 'Fuji' and 'Cripps' Pink', respectively), medium (27, 41 and 31 t ha⁻¹ for 'Royal Gala', 'Fuji' and 'Cripps' Pink', respectively) and de-fruited trees and un-scored trees at a high crop load and de-fruited trees.

4.2.4 Statistical analysis

All data were analysed by split plot ANOVA (SAS Enterprise Guide release 1.3) to test the significance ($Pr \leq 0.05$) of individual treatment effects and their interactions. Multiple comparison tests (Tukey, $Pr \leq 0.05$) were also performed on means.

4.3 Results

There was no significant interaction of scoring and thinning on light saturated net CO₂ assimilation rate (A_{max}) and stomatal conductance (g_s) at any time during the season on any of the cultivars studied (Tables 6, 7). In 'Royal Gala', A_{max} was significantly lower in scored trees compared to un-scored trees 31 and 46 days after full bloom (DAFB), while there was no

significant difference at 110 DAFB (Table 6). Only at 110 DAFB was A_{\max} of trees with a high crop load significantly higher than A_{\max} of de-fruited trees. Stomatal conductance (g_s) followed a similar pattern, but was significantly lower in scored trees than in un-scored trees only at 46 DAFB (Table 6). There were no significant differences at any time during the season in A_{\max} or g_s between scored trees thinned to a high or medium crop load or de-fruited trees (Table 8).

In 'Fuji' there were no significant differences in A_{\max} between scored and un-scored trees 110 and 151 DAFB (Table 7). Scored trees had significant higher g_s than un-scored trees 151 DAFB (Table 7). A_{\max} of the de-fruited trees was significantly lower than that of the trees carrying a high crop load on both dates, while only at 151 DAFB was g_s of the de-fruited trees significantly lower than that of the trees carrying a high crop load (Table 7). At 110 DAFB, A_{\max} of the scored trees carrying a medium crop load significantly higher than that of de-fruited trees, whilst there were no significant treatment differences in A_{\max} at 151 DAFB or in g_s 110 and 151 DAFB (Table 9).

In 'Cripps' Pink' there were no significant differences in A_{\max} or g_s between scored and un-scored trees 110 and 151 DAFB (Table 7). A_{\max} of the de-fruited trees was significantly lower than that of the trees carrying a high crop load 110 and 151 DAFB with g_s following the same pattern (Table 7). At 110 and 151 DAFB, A_{\max} of the scored trees carrying a high crop load were significantly higher than that of de-fruited trees, with g_s following the same pattern (Table 9).

In 'Royal Gala' during 2001/02, extension shoots of trees on the un-scored (control) treatment took significantly longer to reach 95% of final extension shoot length than the scored treatment, which in turn took significantly longer than the ProCa treatment (Table 10, Fig. 1a). During 2002/03, the un-scored and scored treatments took significantly longer than the ProCa treatment to reach 95% of final extension shoot length (Table 10, Fig. 1b). There were no significant CSP treatment or CSP*Thin interaction effects on the time it took to reach 95% of final bourse shoot length during 2001/02 and 2002/03 (Table 10). The expected increase in the number of days it took to reach 95% of final shoot length in reaction to a reduced crop load was observed in bourse shoots (2001/02) and in extension shoots (2002/03) (Table 10).

During 2001/02, final extension shoot length was stimulated more by decreasing crop load in the un-scored (control) treatment compared to the scored and ProCa treatments as indicated by a significant CSP*Thin ($P > F$ 0.0290) interaction (Table 11, Fig. 2a). During 2002/03, extension shoots were significantly longer on the un-scored and scored treatments compared to ProCa treatment (Fig. 2b). There were no significant CSP treatment or CSP*Thin interaction effects on final bourse shoot length during 2001/02 and 2002/03 (Table 11). The expected increase in final shoot length in reaction to a decreasing crop load was observed in bourse shoots (2001/02) and extension shoots (2002/03) (Table 11).

In 'Fuji' during 2001/02, there was a significant CSP*Thin ($P > F$ 0.0247) interaction in the number of days needed to reach 95% of final bourse shoot length (Table 10). There was an increase in the number of days with a decrease in crop load in the scored treatment, while the number of days remained relatively constant at different crop loads in the un-scored and ProCa treatments, with the exception that there was a sharp rise in the number of days at zero crop load in the ProCa treatment (Fig. 3a). Extension shoots of the scored and un-scored treatments took significantly longer than those of the ProCa treatment to reach 95% of final extension shoot length (Table 10, Fig. 3b). During 2002/03, extension shoots of the un-scored treatment took significantly longer to reach 95% of final extension shoot length than scored and ProCa treatments (Table 10, Fig. 3c). There were no effects of thinning, probably due to the low crop loads in all thinning treatments during this season.

During 2001/02, extension shoots were significantly longer on the un-scored treatment than on the scored and ProCa treatments (Table 11, Fig. 4a). During 2002/03, extension shoots were significantly longer on the un-scored treatment than on the scored treatment, which were in turn significantly longer than those on the ProCa treatment (Table 11, Fig. 4b). During 2001/02, bourse shoots were significantly longer on the un-scored treatment than on the scored and ProCa treatments (Table 11, Fig. 4c). Thinning only increased the number of days needed to reach 95% of extension shoot growth, and final bourse shoot length during 2001/02 (Table 10, 11). During 2002/03, there were no effects of thinning, probably due to the low crop loads in all thinning treatments during this season.

In 'Cripps' Pink' during 2001/02, there was a significant CSP*Thin ($P > F$ 0.0036) interaction in the number of days needed to reach 95% of final bourse shoot length (Table 10). There was an increase in the number of days with a decrease in crop load in scored and ProCa

treatments, while the number of days remained relatively constant at different crop loads in the un-scored treatment (Fig. 5a). Extension shoots of the ProCa treatment reached 95% of final shoot length significantly sooner than those of the un-scored and scored treatments in both seasons (Table 10, Fig. 5b, c). During 2002/03, there were no effects of thinning on growth days, probably due to the low crop loads in all thinning treatments during this season.

During 2001/02, extension shoots were significantly longer on the un-scored treatment than on the scored and ProCa treatments (Table 11, Fig. 6a). During 2002/03, extension shoots were significantly longer on the un-scored treatment than on the scored treatment, which were in turn significantly longer than on the ProCa treatment (Table 11, Fig. 6b). During 2001/02, bourse shoots were significantly longer on the un-scored treatment than on the scored and ProCa treatments (Table 11, Fig. 6c). During 2002/03, there was a significant CSP*Thin ($P > F 0.0005$) interaction on final bourse shoot length due to an increase in final bourse shoot length with decreasing crop loads on un-scored trees, whereas final bourse shoot length remained constant with a decrease in crop load in the scored and ProCa treatments, with the exception that at zero crop load final bourse shoot length increased in the ProCa treatment (Table 11, Fig. 6d).

4.4 Discussion

The accumulation of assimilates (carbohydrates) above the girdle and particularly within the leaves is often associated with a reduction in the net CO_2 assimilation rate (A) apparently through an end-product feedback mechanism (Goren et al., 2004). A reduction in crop load can also lead to a reduction in A . However, the effect of crop load on A is dependent on the time and severity of fruitlet removal (Wünsche et al., 2000). It seems that the later the thinning occurs the greater the effect since fewer actively growing sinks are available for alternative carbohydrate allocation. In the first season (2001/02) of this study, scoring reduced A_{max} only early in the season (31 and 46 DAFB) in 'Royal Gala' trees. Fruit thinning did not have an effect on A_{max} of 'Royal Gala' early in the season. After shoot growth cessation (measured at 110 DAFB and thereafter) the effect of reduced fruit numbers on A_{max} became apparent in all cultivars studied. In 'Royal Gala' reduced fruit numbers reduced the final extension shoot lengths in the scored treatment (Fig. 2a). This reduction in sink strength on the scored

treatment was accompanied by reduced photosynthetic rate. In 'Fuji' the number of days needed to reach 95% of final bourse shoot length was reduced in the de-fruited scored trees compared to scored trees carrying a medium crop load (41 and 51 t ha⁻¹) (Fig. 3a) and final bourse and extension shoot length were also reduced on the scored treatment (Fig 4a, c). This reduction in sink strength led to a reduction in A_{\max} of the de-fruited scored trees 110 and 151 DAFB. In 'Cripps' Pink' shoot growth responses followed the same pattern as in 'Fuji', and A_{\max} was likewise reduced in the scored de-fruited trees. Pooled scored and un-scored data also showed a reduction in A_{\max} on de-fruited trees compared to trees carrying a high crop load. Later in the season it appeared as though the score was completely healed in all cultivars, as there were no significant differences in A_{\max} between un-scored and scored treatments. From this it can be concluded that scoring early in the season reduced A , probably through a feedback mechanism. This reduction in carbon availability led to earlier cessation of shoot growth as well as shorter shoots, which meant that more carbohydrates could be allocated to fruit growth (see Chapter 5). This was also observed in an earlier study (Chapter 3).

De-fruiting reduces the stomatal conductance and carboxylation efficiency (stomatal and non-stomatal limitations) in apple trees (Monselise and Lenz, 1980, Chapter 3). There are a number of theories explaining this reduction in A (Goren et al., 2004). This down-regulation of A under low and no crop load has been observed in field studies (Hansen, 1970; Fujii and Kennedy, 1985; Wünsche et al., 2000). However, early in the season this effect is less clear due to strong vegetative growth, but it has been measured following harvest (Chapter 9). In this study, the decline in A_{\max} due to de-fruiting only became apparent after shoot growth cessation. In all three cultivars the decline in photosynthesis late in the season was at least partially due to a stomatal limitation, although not as strongly in 'Royal Gala'. The reduction in sink strength later in the season due to reduced fruit numbers led to a decrease in A_{\max} that is caused by stomatal and probably also non-stomatal limitations (Chapter 3).

Non-chemical ways of restricting vegetative growth of apple trees are only marginally effective when used alone (Greene, 2003). Prohexadione-calcium holds great potential for effective control of vegetative growth on apple trees (Greene, 2003). In this study ProCa decreased final extension shoot length in all three cultivars during both seasons. The number of days needed to reach 95% of final shoot length was also reduced in all cultivars during both seasons by applying ProCa. This is consistent with results found by Miller (2002). Scoring

reduced final extension shoot length in 'Fuji' and 'Cripps' Pink' during both seasons. The reduction in final extension shoot length of 'Fuji' during 2002/03 can be partly attributed to a higher crop load on scored trees (Chapter 5). Thinning increased final extension shoot lengths of 'Royal Gala' during both seasons and of 'Cripps' Pink' during 2002/03. Thinning increased final bourse shoot length of 'Royal Gala' and 'Fuji' during 2001/02 and of 'Cripps' Pink' during 2002/03. This reduction in shoot growth with an increase in crop load has been widely documented (Quinlan and Preston, 1968; Wünsche and Palmer, 1997; Wünsche and Ferguson, 2005). During 2001/02, scoring reduced final extension shoot length on 'Royal Gala' at low (27 ton ha⁻¹) and no crop load, however during 2002/03 this was not the case. A reduction in average shoot length can have added benefits such as better fruit quality (blush colour development), improved yields, better pest control through better spray penetration and improved light penetration leading to better return bloom (Greene, 1999). Excessive vegetative growth is also a major concern for the producer because it competes with fruit growth (Forshey and Elfving, 1989).

The supply of carbon to the fruits may be limiting during the first 40 DAFB, when fruit growth is mainly due to cell division, and there is strong competition between fruitlets and vegetative sinks (Lakso et al., 1998). At this stage, the vegetative development appears to have priority over reproductive development, thus negatively affecting fruit set and fruit growth by cell division (Bepete and Lakso, 1998). Neither ProCa nor scoring affected the final bourse shoot length or the number of days needed to reach 95% of final bourse shoot length on 'Royal Gala' during both seasons. In both 'Fuji' and 'Cripps' Pink' scoring and ProCa reduced final bourse shoot length during 2001/02. During 2002/03 final bourse shoot length remained constant with a reduction in crop load on 'Cripps' Pink' trees scored or sprayed with ProCa. However, at zero crop load final bourse shoot length on trees sprayed with ProCa increased compared to that of the control treatment.

Extension shoots are generally more sensitive to both scoring and ProCa than bourse shoots. ProCa seems to be a more effective way of controlling shoot growth than scoring, with 'Fuji' and 'Cripps' Pink' being more sensitive to the application of ProCa than 'Royal Gala'. This varying response between different cultivars have also been observed in pears (*Pyrus communis* L.) (Smit et al., 2005). The apparent inhibiting effect of scoring on shoot growth is more likely an effect of a bigger crop load on scored trees than a direct effect of scoring on

shoot growth, this is especially true in the second year of scoring. The possible combined use of girdling/scoring and ProCa holds potential for decreasing shoot growth and increasing fruit growth, and warrants further investigation.

4.5 References

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Table 1. Dates of full bloom, spraying of ProCa, scoring and hand thinning for 'Royal Gala', 'Fuji' and 'Cripps' Pink' during 2001/02 and 2002/03.

'Royal Gala'		
	2001/02	2002/03
Full bloom	12 October 2001	28 September 2002
Spraying of ProCa	1 November 2001 (20 DAFB)	18 October 2002 (20 DAFB)
Scoring	7 November 2001 (26 DAFB) 27 November 2002 (46 DAFB)	28 October 2002 (30 DAFB) 18 November 2002 (51 DAFB)
Hand thinning	11 November 2001 (30 DAFB) 11 December 2001 (60 DAFB)	19 November 2002 (52 DAFB)
'Fuji'		
Full bloom	11 October 2001	26 September 2002
Spraying of ProCa	1 November 2001 (21 DAFB)	18 October 2002 (22 DAFB)
Scoring	13 November 2001 (33 DAFB)	14 November 2002 (49 DAFB)
Hand thinning	4 December 2001 (54 DAFB)	5 December 2002 (70 DAFB)
'Cripps Pink'		
Full bloom	12 October 2001	26 September 2002
Spraying of ProCa	1 November 2001 (20 DAFB)	18 October 2002 (22 DAFB)
Scoring	14 November 2001 (33 DAFB)	15 November 2002 (50 DAFB)
Hand thinning	4 December 2001 (53 DAFB)	15 November 2002 (50 DAFB)

Table 2. Chemical thinning program applied to 'Royal Gala' during 2001/02 and 2002/03 (DAFB = Days after full bloom).

2001/02			
Timing	Product	Active ingredient	Rate
4 DAFB	Planofix	2-(1-naphthyl)acetic acid (45 g l ⁻¹)	11 ml 100 l ⁻¹ H ₂ O
	Servin 850 XLR Tankmix	Carbaryl 480 g l ⁻¹	150 ml 100 l ⁻¹ H ₂ O
14 DAFB	Servin 850 WP	Carbaryl 850 g kg ⁻¹	90 g 100 l ⁻¹ H ₂ O
2002/03			
2 DAFB	Planofix	2-(1-naphthyl)acetic acid (45 g l ⁻¹)	11 ml 100 l ⁻¹ H ₂ O
9 DAFB	Planofix	2-(1-naphthyl)acetic acid (45 g l ⁻¹)	11 ml 100 l ⁻¹ H ₂ O
	Servin 850 WP Tankmix	Carbaryl 850 g kg ⁻¹	90 g 100 l ⁻¹ H ₂ O
16 DAFB	Servin 850 WP	Carbaryl 850 g kg ⁻¹	90 g 100 l ⁻¹ H ₂ O
24 DAFB	Servin 850 WP	Carbaryl 850 g kg ⁻¹	90 g 100 l ⁻¹ H ₂ O

Table 3. Chemical thinning program applied to 'Fuji' during 2001/02 and 2002/03.

2001/02			
Timing	Product	Active ingredient	Rate
5 DAFB	Planofix	2-(1-naphthyl)acetic acid (45 g l ⁻¹)	11 ml 100 l ⁻¹ H ₂ O
	Servin 850 XLR Tankmix	Carbaryl 480 g l ⁻¹	150 ml 100 l ⁻¹ H ₂ O
13 DAFB	Planofix	2-(1-naphthyl)acetic acid (45 g l ⁻¹)	11 ml 100 l ⁻¹ H ₂ O
	Servin 850 WP Tankmix	Carbaryl 850 g kg ⁻¹	90 g 100 l ⁻¹ H ₂ O
2002/03			
Full bloom	Planofix	2-(1-naphthyl)acetic acid (45 g l ⁻¹)	15 ml 100 l ⁻¹ H ₂ O
12 DAFB	Planofix	2-(1-naphthyl)acetic acid (45 g l ⁻¹)	15 ml 100 l ⁻¹ H ₂ O
	Servin 850 WP Tankmix	Carbaryl 850 g kg ⁻¹	90 g 100 l ⁻¹ H ₂ O
16 DAFB	Servin 850 WP	Carbaryl 850 g kg ⁻¹	90 g 100 l ⁻¹ H ₂ O
20 DAFB	Servin 850 WP	Carbaryl 850 g kg ⁻¹	90 g 100 l ⁻¹ H ₂ O

Table 4. Chemical thinning program applied to 'Cripps' Pink' during 2001/02 and 2002/03.

2001/02			
Timing	Product	Active ingredient	Rate
7 DAFB	Servin 850 WP	Carbaryl 850 g kg ⁻¹	90 g 100 l ⁻¹ H ₂ O
19 DAFB	Servin 850 WP	Carbaryl 850 g kg ⁻¹	90 g 100 l ⁻¹ H ₂ O
2002/03			
11 DAFB	Servin 850 WP	Carbaryl 850 g kg ⁻¹	90 g 100 l ⁻¹ H ₂ O
18 DAFB	Servin 850 WP	Carbaryl 850 g kg ⁻¹	90 g 100 l ⁻¹ H ₂ O
26 DAFB	Servin 850 WP	Carbaryl 850 g kg ⁻¹	90 g 100 l ⁻¹ H ₂ O

Table 5. Number of fruit required on an average branch in the 'Royal Gala', 'Fuji' and 'Cripps' Pink' orchards to achieve target yields for the different hand thinning treatments and actual yields achieved during 2001/02 and 2002/03.

Thinning treatment target yields	Required fruit per branch	2001/02 Actual yield (t ha ⁻¹)	2002/03 Actual yield (t ha ⁻¹)
'Royal Gala'			
75 t ha ⁻¹	20	58.3	29.6
50 t ha ⁻¹	14	40.1	30.6
30 t ha ⁻¹	8	36.6	23.4
15 t ha ⁻¹	4	27.3	22.8
'Fuji'			
75 t ha ⁻¹	20	70.9	7.0
50 t ha ⁻¹	14	54.3	12.3
30 t ha ⁻¹	8	50.7	13.9
15 t ha ⁻¹	4	40.9	11.9
'Cripps' Pink'			
75 t ha ⁻¹	18	64.2	50.4
50 t ha ⁻¹	12	50.5	55.8
30 t ha ⁻¹	7	46.3	30.8
15 t ha ⁻¹	4	30.6	26.1

Table 6. Light saturated net CO₂ assimilation rate (A_{\max}) and stomatal conductance (g_s) of 'Royal Gala' following scoring and thinning treatments and their interaction. Means with the same letter within columns are not significantly different (Tukey Pr < 0.05).

	31 DAFB	46 DAFB	110 DAFB
A_{\max} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)			
Un-scored	15.4 a	16.2 a	17.3 a
Scored	13.2 b	13.7 b	16.6 a
Pr > F (Scor)	0.0032	0.0066	0.3284
High crop load	14.9 a	14.9 a	18.7 a
De-fruited	13.7 a	14.9 a	15.3 b
Pr > F (Thin)	0.2226	0.9888	0.0035
Pr > F (Scor*Thin)	0.6422	0.1352	0.4060
g_s ($\text{mmol m}^{-2} \text{s}^{-1}$)			
Un-scored	0.23 a	0.23 a	0.32 a
Scored	0.18 a	0.19 b	0.31 a
Pr > F (Scor)	0.0684	0.0073	0.7068
High crop load	0.21 a	0.20 a	0.36 a
De-fruited	0.20 a	0.21 a	0.26 b
Pr > F (Thin)	0.8207	0.7101	0.0096
Pr > F (Scor*Thin)	0.8496	0.0947	0.4711

Table 7. Light saturated net CO₂ assimilation rate, (A_{max}) and stomatal conductance (g_s) of 'Fuji' and 'Cripps' Pink' following scoring and thinning treatments and their interaction. Means with the same letter within columns are not significantly different (Tukey Pr < 0.05).

	'Fuji'		'Cripps' Pink'	
	110 DAFB	151 DAFB	110 DAFB	151 DAFB
A_{max} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)				
Un-scored	18.2 a	16.7 a	15.2 a	13.9 a
Scored	17.4 a	17.4 a	13.8 a	14.1 a
Pr > F (Scor)	0.3357	0.1559	0.2594	0.6784
High crop load	19.2 a	18.4 a	16.4 a	15.9 a
De-fruited	16.4 b	15.6 b	12.6 b	12.2 b
Pr > F (Thin)	0.0084	0.0030	0.0063	0.0052
Pr > F (Scor*Thin)	0.9906	0.1615	0.6684	0.5839
g_s ($\text{mmol m}^{-2} \text{s}^{-1}$)				
Un-scored	0.32 a	0.26 b	0.26 a	0.25 a
Scored	0.28 a	0.30 a	0.22 a	0.26 a
Pr > F (Scor)	0.1832	0.0015	0.2026	0.2055
High crop load	0.33 a	0.32 a	0.29 a	0.51 a
De-fruited	0.28 a	0.24 b	0.28 b	0.30 b
Pr > F (Thin)	0.0616	0.0146	0.0100	0.0055
Pr > F (Scor*Thin)	0.4522	0.7921	0.2935	0.3619

Table 8. Light saturated net CO₂ assimilation rate (A_{\max}) and stomatal conductance (g_s) of 'Royal Gala' following thinning treatments on scored trees.

	31 DAFB	46 DAFB	110 DAFB
A_{\max} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)			
High crop load	14.1	14.2	18.7
Medium crop load	14.1	15.2	14.2
De-fruited	12.3	13.1	14.5
Pr > F	0.5495	0.2487	0.0787
g_s ($\text{mmol m}^{-2} \text{s}^{-1}$)			
High crop load	0.19	0.20	0.37
Medium crop load	0.20	0.22	0.28
De-fruited	0.18	0.17	0.25
Pr > F	0.8683	0.2987	0.1878

Table 9. Light saturated net CO₂ assimilation rate (A_{\max}) and stomatal conductance (g_s) of 'Fuji' and 'Cripps' Pink' following thinning treatments on scored trees. Means with the same letter within columns are not significantly different (Tukey $Pr < 0.05$).

	'Fuji'		'Cripps' Pink'	
	110 DAFB	151 DAFB	110 DAFB	151 DAFB
A_{\max} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)				
High crop load	18.8 ab	18.3 a	16.0 a	16.2 a
Medium crop load	19.2 a	18.1 a	14.2 ab	13.7 ab
De-fruited	16.0 b	16.5 a	11.6 b	12.0 b
Pr > F	0.0269	0.0586	0.022	0.0093
g_s ($\text{mmol m}^{-2} \text{s}^{-1}$)				
High crop load	0.32 a	0.33 a	0.28 a	0.32 a
Medium crop load	0.33 a	0.29 a	0.23 ab	0.24 ab
De-fruited	0.25 a	0.26 a	0.17 b	0.20 b
Pr > F	0.1252	0.1585	0.0403	0.0239

Table 10. Probability values ($Pr > F$) following scoring, spraying of prohexadione-calcium (CSP treatments) and thinning treatments and their interactions on the number of days needed to reach 95% of final bourse and extension shoot length on 'Royal Gala', 'Fuji' and 'Cripps' Pink' during 2001/02 and 2002/03.

	'Royal Gala' 2001/02		'Royal Gala' 2002/03	
	Bourse	Extension	Bourse	Extension
CSP	0.7381	0.0012	0.2773	0.0010
Thin	<.0001	0.3990	0.2158	0.0139
CSP*Thin	0.4263	0.1364	0.5009	0.0771
	'Fuji' 2001/02		'Fuji' 2002/03	
	Bourse	Extension	Bourse	Extension
CSP	0.0114	0.0181	0.3082	0.0277
Thin	0.2299	0.0293	0.1567	0.8266
CSP*Thin	0.0247	0.0832	0.9264	0.5561
	'Cripps' Pink' 2001/02		'Cripps' Pink' 2002/03	
	Bourse	Extension	Bourse	Extension
CSP	0.2352	0.0270	0.0636	<.0001
Thin	0.0039	0.1116	0.2841	0.2030
CSP*Thin	0.0036	0.6124	0.1853	0.8469

Table 11. Probability values (Pr > F) following scoring, spraying of prohexadione-calcium (CSP treatments) and thinning treatments and their interactions on final bourse and extension shoot length on 'Royal Gala, 'Fuji' and 'Cripps' Pink' during 2001/02 and 2002/03.

	'Royal Gala'			
	Bourse 2001/02	Extension 2001/02	Bourse 2002/03	Extension 2002/03
CSP	0.0859	<.0001	0.0612	<.0001
Thin	<.0001	0.1823	0.0061	0.0061
CSP*Thin	0.2117	0.0290	0.0786	0.0786
	'Fuji'			
	Bourse 2001/02	Extension 2001/02	Bourse 2002/03	Extension 2002/03
CSP	0.0024	<.0001	0.3166	<.0001
Thin	0.0133	0.4319	0.1802	0.4165
CSP*Thin	0.3668	0.9323	0.9245	0.1264
	'Cripps' Pink'			
	Bourse 2001/02	Extension 2001/02	Bourse 2002/03	Extension 2002/03
CSP	0.0006	<.0001	0.0220	<.0001
Thin	0.2358	0.2167	0.0095	0.0250
CSP*Thin	0.3569	0.6196	0.0005	0.1528

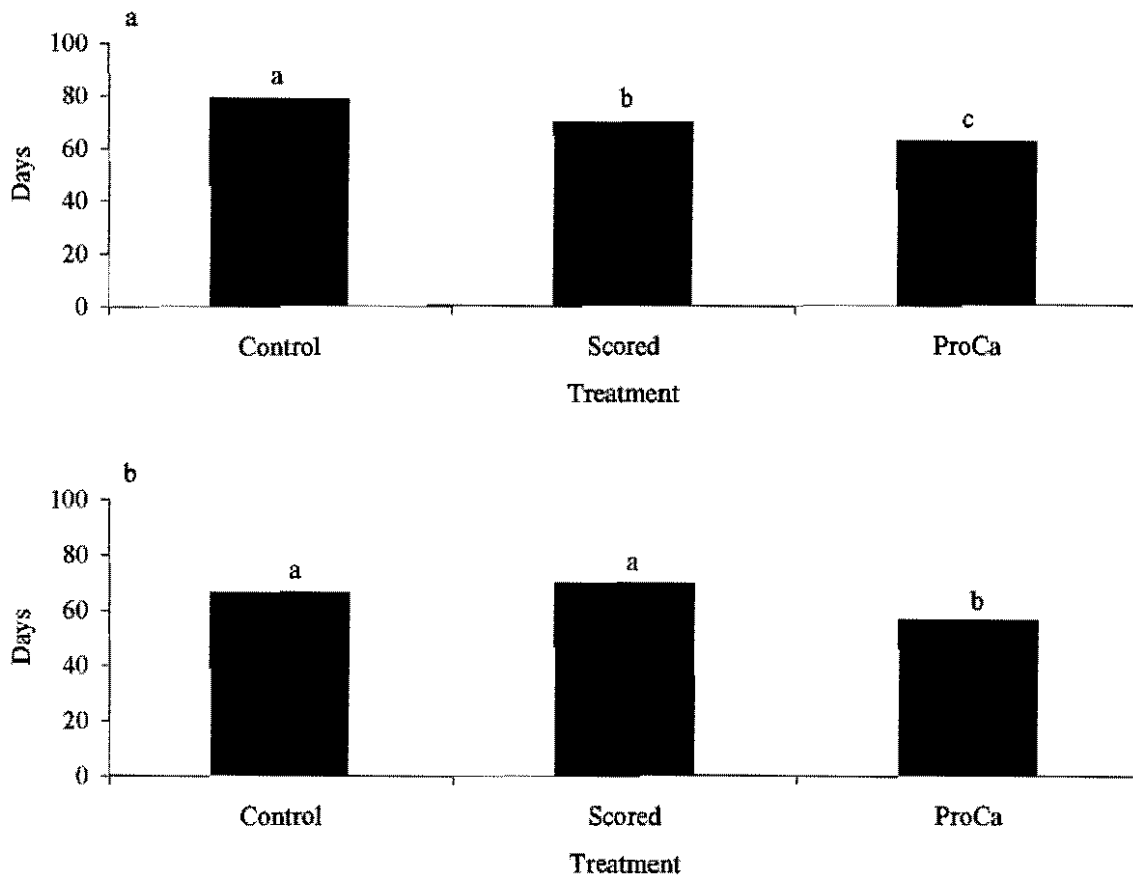


Fig. 1. Number of days to reach 95% of final extension shoot length following scoring, spraying of prohexadione-calcium (CSP treatments) of 'Royal Gala' during (a) 2001/02 and (b) 2002/03. Means with the same letter are not significantly different (Tukey Pr < 0.05).

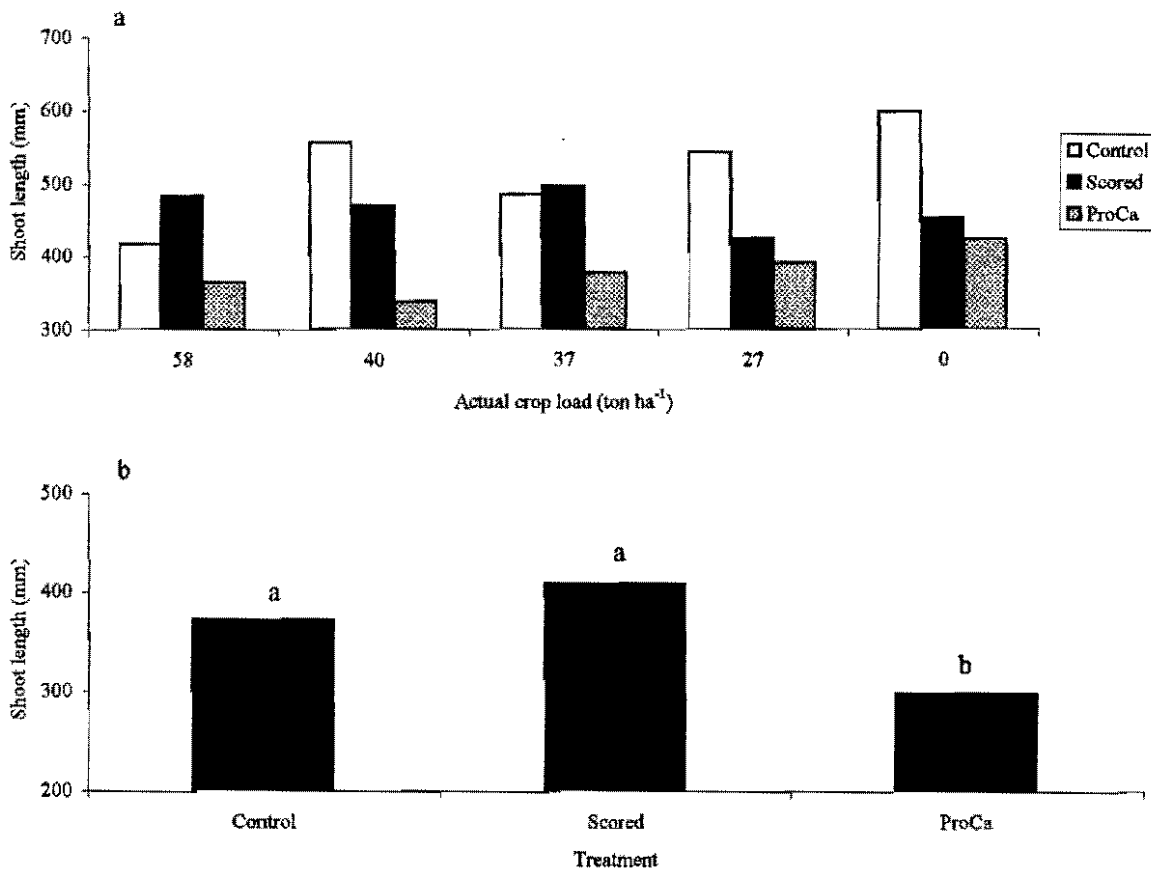


Fig. 2. Final extension shoot lengths of 'Royal Gala' following scoring, spraying of prohexadione-calcium (CSP treatments) and thinning treatments during (a) 2001/02 and (b) following scoring, spraying of prohexadione-calcium (CSP treatments) during 2002/03. Means with the same letter are not significantly different (Tukey Pr < 0.05).

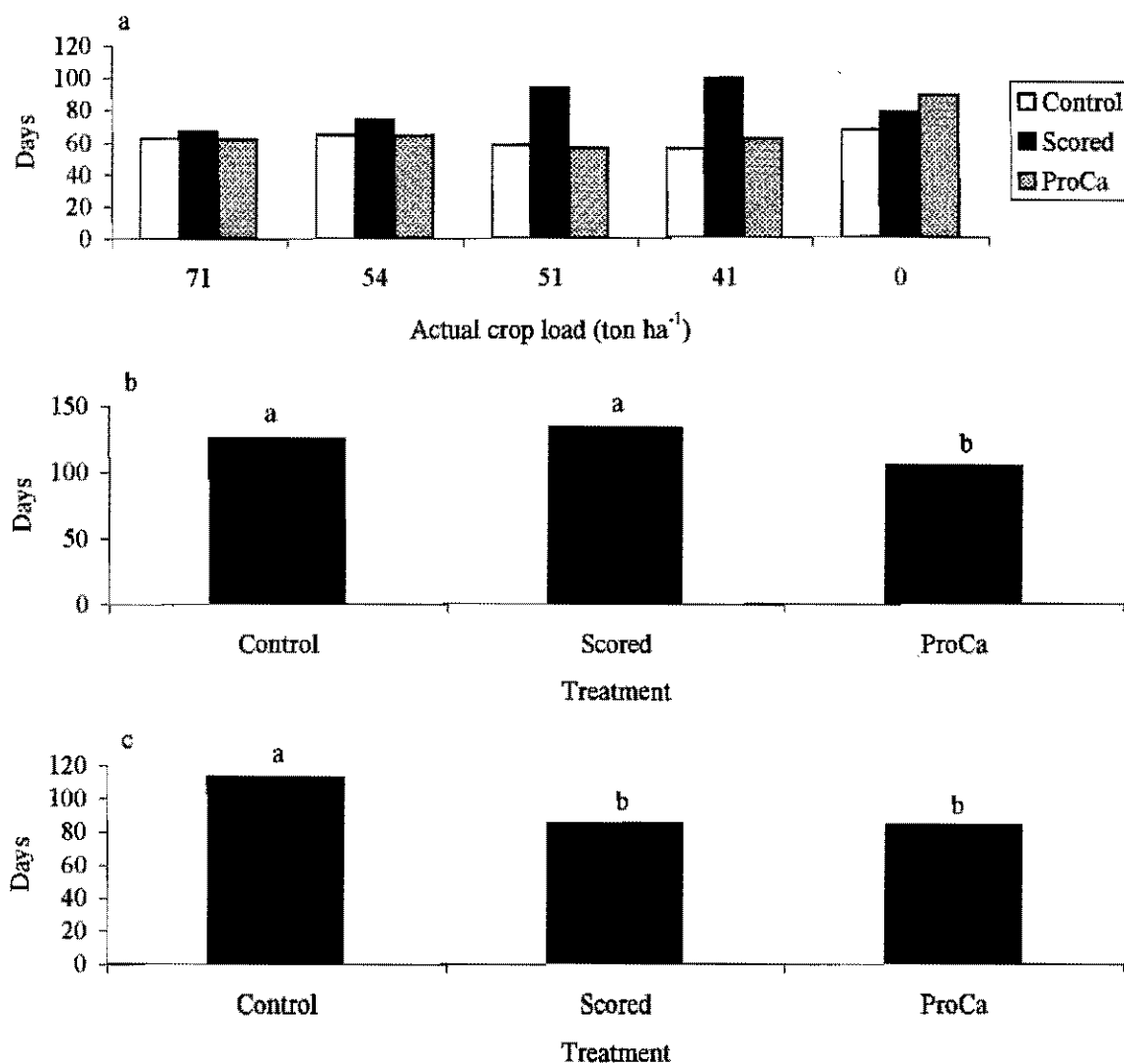


Fig. 3. (a) Number of days to reach 95% of final bourse shoot length following scoring, spraying of prohexadione-calcium (CSP treatments) and thinning treatments of 'Fuji' during 2001/02, (b) number of days to reaching 95% of final extension shoot length during 2001/02 and (c) number of days to reaching 95% of final extension shoot length following scoring, spraying of prohexadione-calcium (CSP treatments) during 2002/03. Means with the same letter are not significantly different (Tukey Pr < 0.05).

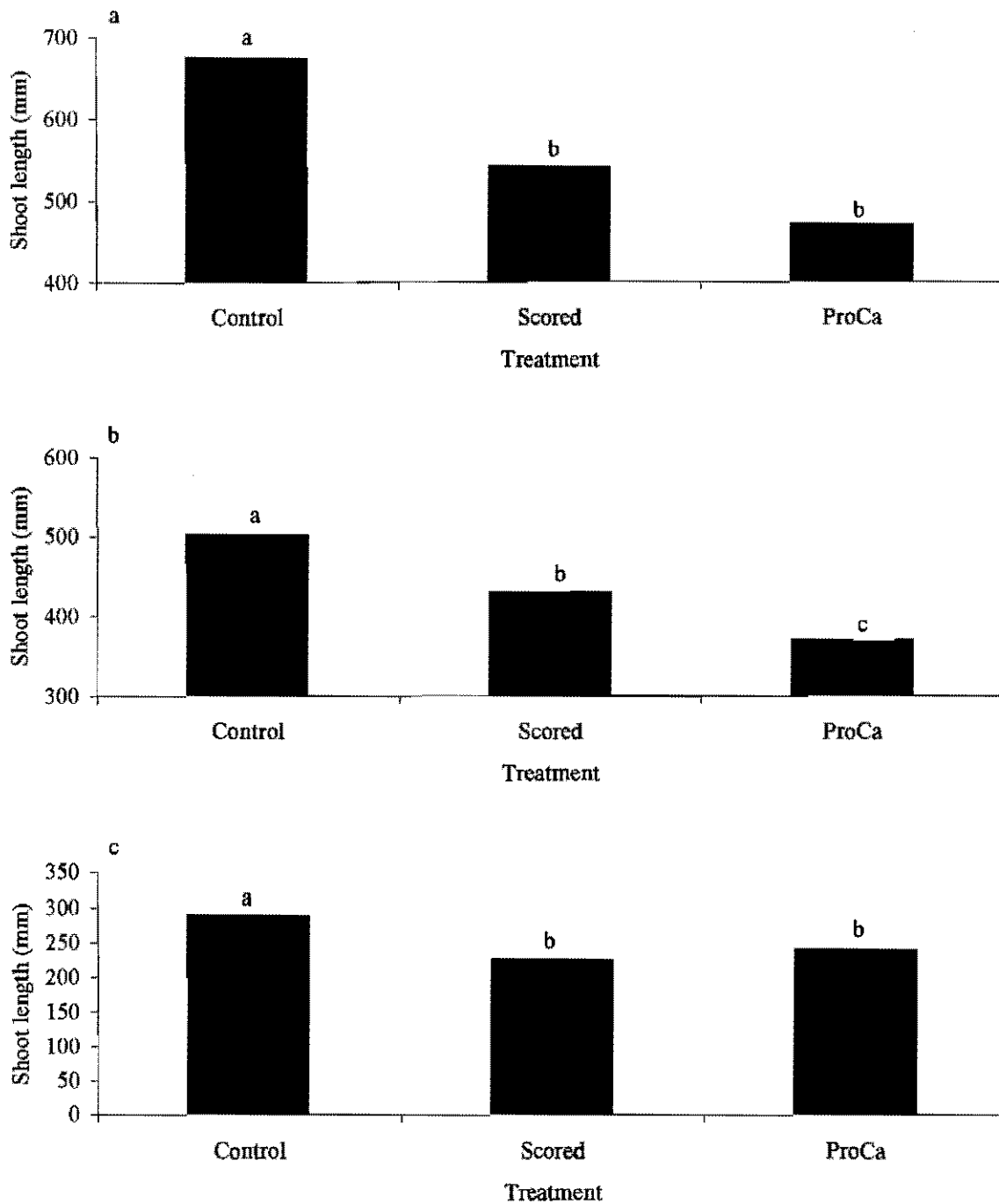


Fig. 4. Final extension shoot length of 'Fuji' following scoring, spraying of prohexadione-calcium (CSP treatments) during (a) 2001/02 season, (b) during 2002/03 and (c) final bourse shoot length following scoring, spraying of prohexadione-calcium (CSP treatments) during 2001/02. Means with the same letter are not significantly different (Tukey Pr < 0.05).

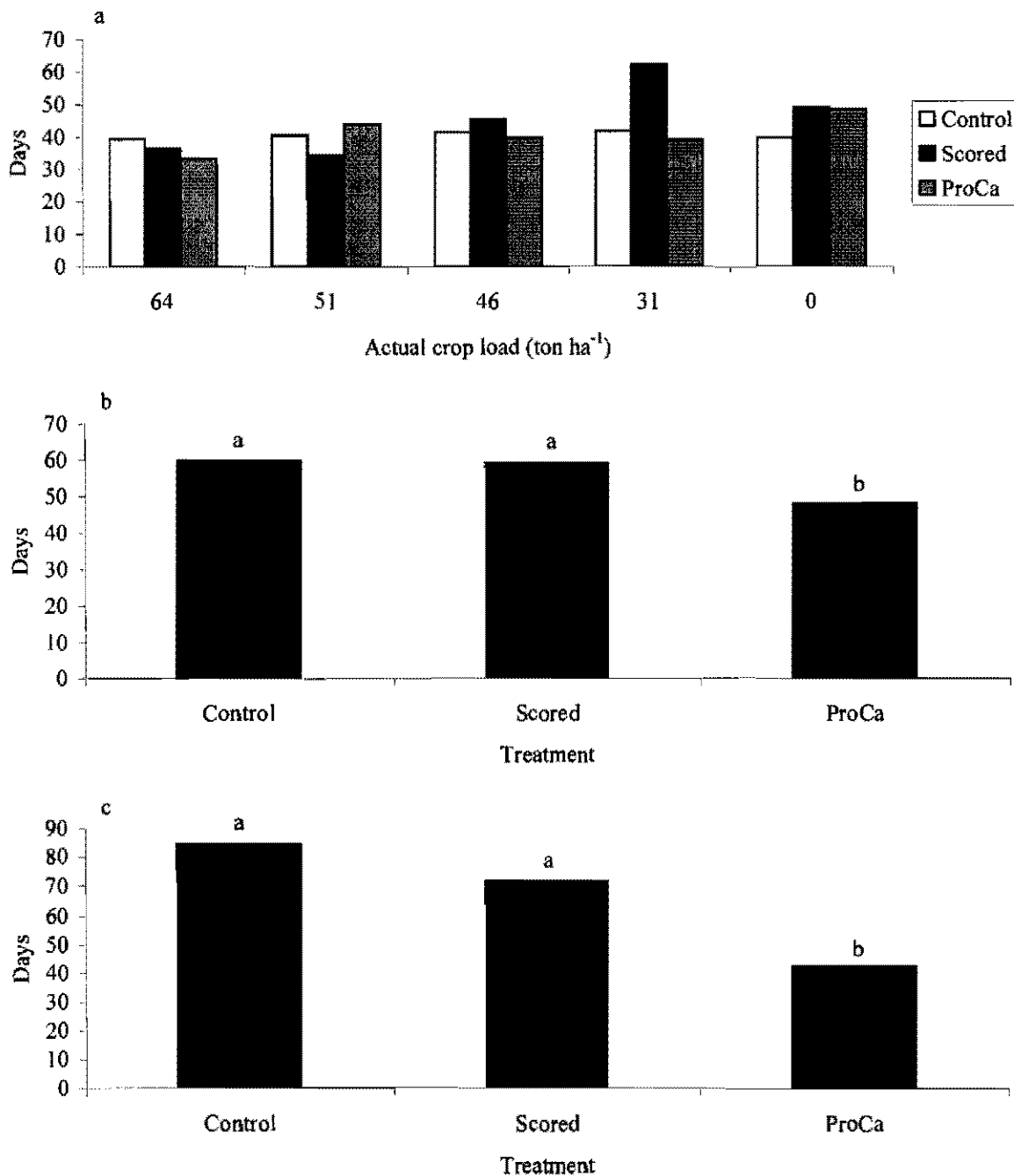


Fig. 5. (a) Number of days to reach 95% of final bourse shoot length following scoring, spraying of prohexadione-calcium (CSP treatments) and thinning treatments of 'Cripps' Pink' during 2001/02, (b) number of days to reach 95% of final extension shoot length for the CSP treatments during 2001/02 and (c) number of days to reach 95% of final extension shoot length for the CSP treatments during 2002/03. Means with the same letter are not significantly different (Tukey $P < 0.05$).

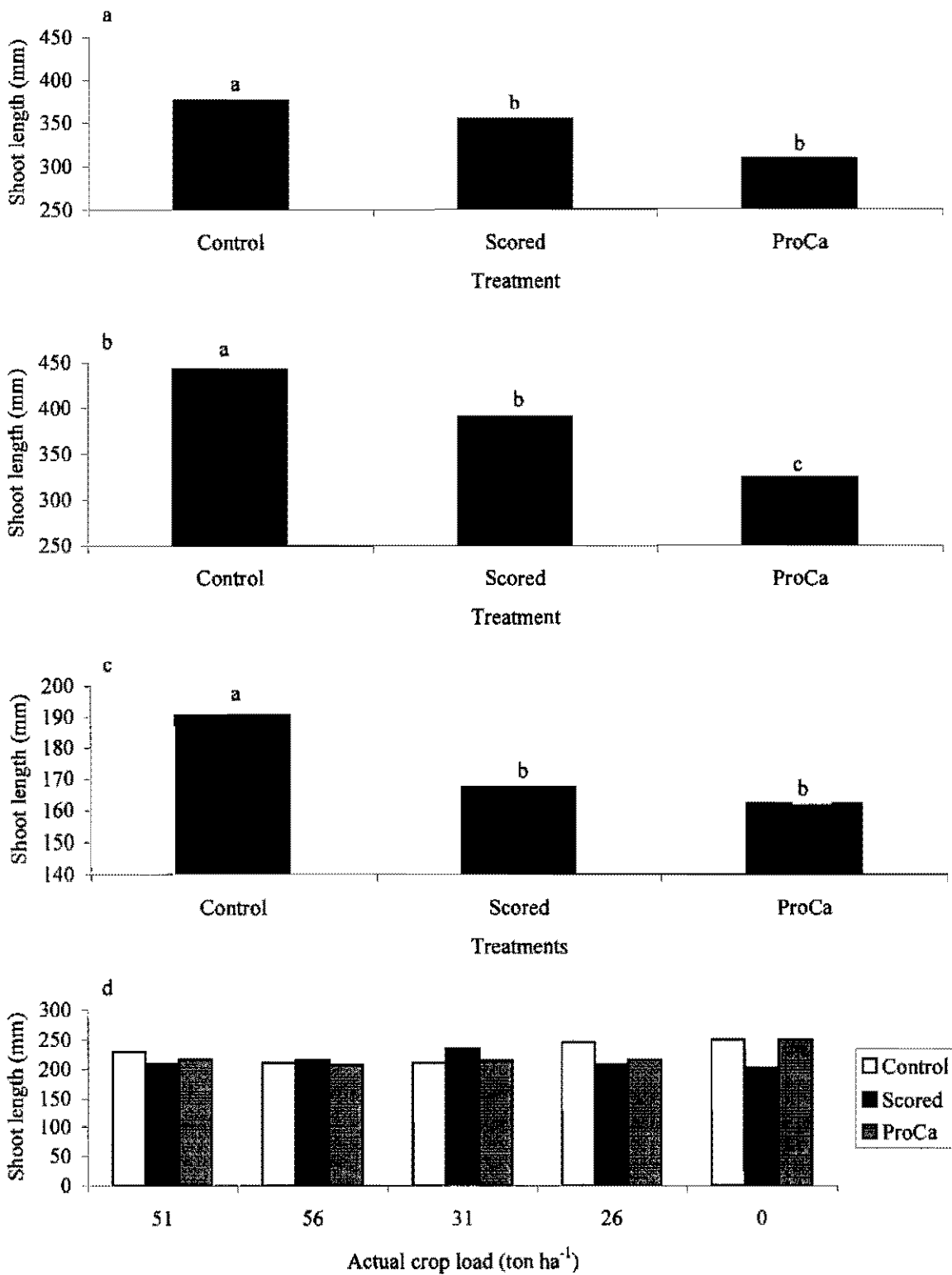


Fig. 6. Final extension shoot length of 'Cripps' Pink' following scoring, spraying of prohexadione-calcium (CSP treatments) during (a) 2001/02, (b) CSP treatments during 2002/03, final bourse shoot length for the (c) CSP treatments during 2001/02 and (d) CSP and thinning treatments during the 2002/03 season. Means with the same letter are not significantly different (Tukey Pr < 0.05).

5. Fruit growth, quality and return bloom as affected by scoring, prohexadione-calcium and thinning on 'Royal Gala', 'Fuji' and 'Cripps' Pink' apple trees.

Abstract

In the Western Cape region modern high-density orchard planting systems must achieve high early yields, high sustained yield and excellent fruit quality in order for the producer to survive financially. This can be done by managing crop load and effectively restricting vegetative growth in vigorous apple cultivars in order to achieve a balance between vegetative and reproductive development. The objective of this study was to investigate the effects of, and potential interaction between scoring, the use of a growth retardant prohexadione-calcium (ProCa) and fruit thinning, with respect to fruit growth and quality and return bloom on 'Royal Gala', 'Fuji' and 'Cripps' Pink' apple orchards in the Koue Bokkeveld (Ceres) region during 2001/02 and 2002/03. Scoring improved reproductive bud development in 'Royal Gala', 'Fuji' and 'Cripps' Pink' on all bearing structures. Scoring led to increased fruit growth rates during the first 40 days after full bloom (DAFB), and culminated in better fruit size at harvest. Scoring improved the total soluble solids concentration (TSS) of 'Fuji' and 'Cripps' Pink' compared to control and ProCa treatments during 2002/03. In 'Cripps' Pink' fruit firmness and colour were also improved in scored trees compared to control trees during 2002/03. ProCa did not improve fruit firmness compared to control trees in 'Cripps' Pink'. However, fruit from the ProCa treatment showed better colour development than fruit from the control treatment during 2002/03. This improvement in fruit colour was probably due to increased light penetration into the canopy resulting from reduced shoot growth. Reproductive bud development was enhanced on scored trees with 'Fuji' being the most responsive cultivar, leading to consistently high yields on scored trees. ProCa inhibited shoot growth effectively, but no evidence was found for improved carbon allocation to fruits or reproductive buds.

Keywords. Apple, carbohydrate supply, fruit growth, fruit quality, return bloom, scoring.

5.1 Introduction

In modern fruit culture and particularly within the present global marketing system, total yield is less important than the number of saleable first class fruit. Thus producers must manage fruit production in order to optimise quality and produce fruit with specific quality attributes (Wünsche and Ferguson, 2005). South African producers are being forced to alter their cultural practices to increase fruit size and improve quality attributes such as blush colour development in order to stay competitive on the international markets. Modern high-density orchard planting systems must achieve high early yields, high sustained yield and excellent fruit quality in order for the producer to survive financially (Robinson, 2003). This can be done by managing crop load and effectively restricting vegetative growth in vigorous apple cultivars in order to achieve a balance between vegetative and reproductive development (Goren et al., 2004; Wünsche and Ferguson, 2005).

Scoring and the spraying of prohexadione-calcium (ProCa; BAS-125, (3-oxido-4-propionyl-5-oxo-3-cyclohexene-carboxylate)) are two growth-retarding practices that hold great potential to control vegetative growth on apple trees (Chapter 4). This reduction in vigour can have added benefits such as better blush colour development and improved fruit quality, improved yields, better pest control through better spray penetration and improved light penetration leading to better return bloom (Greene, 1999). Excessive vegetative growth is also a major concern to the producer because it competes with fruit growth (Forshey and Elfving, 1989).

Early fruit thinning is known to increase fruit size (Denne, 1960; Westwood et al., 1967; Bergh, 1990), improve ground and blush colour (Link, 2000), and increase the percentage total soluble solids and titratable acid. For these techniques, producers require more accurate guidelines with respect to the timing and intensity of the manipulation, particularly when they are used in combination. An improved understanding of carbohydrate assimilation and allocation patterns is needed for this purpose.

Growth retarding practices such as scoring and ProCa, and thinning, early in the season, reduce the number of fruit sinks and total vegetative sink strength, thereby ensuring an adequate supply of carbohydrates for the remaining fruit. Fruit size is determined by the combination of cell number, cell size and percentage intercellular space (Goffinet et al., 1995). Pearson and Robertson (1953) stated that the variation in fruit size is determined early in development by the number of cell divisions (cell number), and that within one season cell

volume in individual fruit of the same age is fairly uniform. Cell division takes place primarily during the first 40 days after full bloom (DAFB), followed by cell enlargement (Bergh, 1990). In order to maximise fruit size, it is critical to optimise carbon allocation to the fruit during this period. In an earlier study, scoring reduced shoot growth and it appeared as if assimilates were preferentially allocated to fruit as fruit size was consistently bigger on scored trees. This was largely due to a faster fruit growth rate during the cell division stage of fruit development (Chapter 3).

The objective of this study was to investigate the effects of, and potential interaction between scoring, the use of a growth retardant (ProCa) and thinning, with respect to fruit growth and quality and return bloom. We hypothesised that a decrease in total fruit sink strength (thinning) would lead to increased individual fruit growth, that a decrease in vegetative sink strength due to scoring, or the use of ProCa would lead to increased fruit size and quality due to preferential carbon allocation to the fruit, and that these manipulations would have an additive effect when applied in combination.

5.2 Materials and methods

This study was conducted on the same commercial 'Royal Gala', 'Fuji' and 'Cripps' Pink' apple orchards (Nooitgedacht Farm) in the Koue Bokkeveld (Ceres) region, (33°13'S, 19°2'E, 975 m above sea level) (Western Cape, South Africa) as described in Chapter 4. A commercial chemical thinning program was followed on all cultivars after which crop loads were adjusted by hand thinning (Chapter 4).

Fruit diameter of two (one on each side of the tree) tagged fruit was measured weekly and the relative fruit growth rate calculated in mm day^{-1} for the cell division stage and cell enlargement stages (Table 1, 2, 3). These stages were retrospectively identified based on analysis of the seasonal fruit growth curves. At harvest, random sub-samples of 20 fruit per tree were analysed for fruit mass, fruit firmness and number of seeds, and total soluble solids concentration (TSS) was measured only during 2002/03. Fruit flesh firmness was determined on peeled, opposite cheeks of the fruit using a penetrometer fitted with an 11 mm tip (Southtrade, FT 327, Alphonsine, Italy). Slices cut from each side of the fruits were juiced together and a TSS reading taken using a refractometer (PR-100 9501, ATAGO CO. Ltd., Tokyo, Japan). For 'Cripps' Pink' fruit colour was assessed using the Pink Lady™ blush

colour chart (Australian Apple and Pear Growers' Association, values 1-12 where 12=dark red).

During winter following both seasons, microscopic bud analysis was performed on long shoots (>100 mm), short shoots (30-100 mm) old spurs and new spurs to determine the percentage reproductive buds. Two buds per structure were collected from every tree and the samples pooled, so that statistical analysis was not possible.

All other data were analysed by a split plot ANOVA (SAS Enterprise Guide release 1.3) to test the significance ($Pr \leq 0.05$) of individual treatment effects and their interactions. Multiple comparison tests (Tukey, $Pr \leq 0.05$) were also performed.

5.3 Results

Since responses to fruit thinning *per se* are well documented in the literature and the primary focus of this study was on responses to scoring and ProCa at different crop loads, only results for interactions between the CSP (control, scoring or ProCa) and thinning treatments, or main effects of CSP treatments will be presented, with a few exceptions where thinning effects will be mentioned.

Fruit from scored 'Royal Gala' trees were significantly larger than fruit from the other CSP treatments on both harvest dates during 2001/02 (Fig. 1a). At harvest 1 (2002/03), fruit from scored trees were significantly smaller compared to the other CSP treatments (Fig. 1b). A significant CSP*Thin interaction for fruit mass at harvest 2 indicated variable responses to scoring and ProCa at different crop loads (Fig. 1c). There was no significant CSP treatment or CSP*Thin interactions on fruit mass of 'Fuji' during both seasons (Table 4). During 2001/02, 'Cripps' Pink' fruit from the scored treatment were significantly larger compared to other CSP treatments (Fig. 2a). During 2002/03, fruit from the scored treatment were significantly smaller compared to other CSP treatments at harvest 1 (Fig. 2b).

There were no significant CSP treatment differences in TSS of 'Royal Gala' fruit at both harvest dates during 2002/03 (Table 4). TSS of 'Fuji' fruit from the scored treatment was significantly higher compared to the other CSP treatments during 2002/03 (Fig. 3a). At harvest 1 (2002/03), TSS of 'Cripps' Pink' fruit from the scored treatment was significantly higher than that of the ProCa treatment, and at harvest 2, TSS of fruit from the scored treatment was significantly higher compared to both the control and ProCa treatments (Fig. 3b). During 2001/02, fruit firmness of 'Cripps' Pink' fruit from the scored treatment was significantly

higher compared to other CGP treatments (Fig. 3c). At harvest 2 (2002/03), blush colour development of the control treatment was significantly less compared to the scored and ProCa treatments (Fig. 3d).

In 'Royal Gala' during 2001/02 for the period 24-45 DAFB, there was a significant CSP*Thin interaction on the fruit growth rate (Table 5) due to a greater increase in fruit growth rates on scored and ProCa treatments at intermediate crop loads compared to the control treatment (Fig. 4a). Throughout the season, the expected increase in fruit growth rate with a decrease in crop load was observed (Table 5). During 2002/03 for the period 45-100 DAFB (Fig. 4b), negative effects of scoring on fruit growth rate were found at higher crop but not at lower crop load, leading to a significant CSP*Thin interaction (Fig. 4b).

In 'Fuji' during the period 33-54 DAFB (2001/02), fruit growth rate was significantly faster on the scored treatment compared to the other CSP treatments (Table 5, Fig. 5a). During the period 104-165 DAFB, fruit growth rate of the scored treatment was significantly lower compared to the other CSP treatments (Fig. 5a). The expected increase in fruit growth rate in reaction to thinning was observed from 54 DAFB onwards (Table 5). During 2002/03, there were no significant differences in fruit growth rates between CSP treatments (Table 5).

In 'Cripps' Pink' during the period 33-54 DAFB (2001/02), fruit growth rate was significantly increased by scoring and ProCa compared to the control treatment (Table 5, Fig. 5b). During the period 103-165 DAFB, fruit growth rate of the ProCa treatment was significantly lower than on the control treatment (Fig. 5b). The expected increase in fruit growth rate in reaction to thinning was observed from 54 DAFB onwards (Table 5). During 2002/03, there were no significant differences in fruit growth rates between CSP treatments (Table 5), but increased fruit growth rates in reaction to thinning were observed for the period 46-102 DAFB (Table 5).

Both scoring and decreasing crop loads favoured reproductive bud development on 'Royal Gala' during 2001/2002 (Fig. 6). During 2002/03 there was no apparent difference in reproductive bud development between treatments (Fig. 7). In 'Fuji' during 2001/02 (Fig. 8), reproductive bud development was poor on the control and ProCa treatments (<20%), while the scored treatment had 40-60% reproductive bud development. During 2002/03, reproductive bud development on long and short shoots was best on the scored treatment (Fig. 9a, b). In 'Cripps' Pink' (2001/2002), scoring favoured reproductive bud development (Fig. 10). During 2002/2003 reproductive bud development was similar across all treatments (Fig. 11).

5.4 Discussion

Increasing the yield of marketable fruit is one of the main objectives in fruit production. Girdling/scoring often increases yield in various tree crops (Goren et al., 2004). In this study, yield of scored trees was significantly improved in all cultivars during the second season (data not shown). Greene and Lord (1983) also observed an increase in yields of 'Golden Delicious' after a second year of girdling. The increase in yield in year two in this study was due to better reproductive bud development after the first year of scoring in 'Royal Gala', 'Fuji' and 'Cripps' Pink' on all bearing structures. It would appear as if 'Fuji' trees are more responsive to a scoring treatment in this respect than 'Royal Gala' and 'Cripps' Pink'. Li et al. (1996) also reported a five-fold increase in reproductive bud development on girdled 'Red Fuji' trees.

The promotion of flowering by girdling may be due to an increase in carbohydrates beyond a threshold level in the canopy of girdled trees (Goldschmidt et al., 1985). However, girdling also interferes with the transport of auxins and other plant growth regulators (Cutting and Lyne, 1993). The increase in flower bud formation can thus not solely be attributed to an increase in carbohydrate levels in the canopy of the girdled tree (Goren et al., 2004). Although the carbohydrate requirement for flower bud formation may be small compared to that of fruit sinks, the proportion of available carbohydrates is likely to be limited in heavy cropping trees with relatively low source/sink ratios (Wünsche and Ferguson, 2005). This may particularly be the case in the early season when the specific cost of fruit growth is relatively high due to the start of accumulation of energy-demanding metabolites such as starch and lipids (Walton et al., 1999). The rich source of gibberellins in apple seeds and their translocation into the plant can inhibit reproductive bud development, especially in heavy cropping trees (Bangerth, 1993, 2000). In this study, extension shoot lengths were significantly reduced in the scored and ProCa treatment (Chapter 4) which could have led to better light penetration and thus reproductive bud development in these treatments (Greene, 1999). ProCa acts as a GA biosynthesis inhibitor (Rademacher, 1995), thus reducing gibberellin levels in the plant. However, in spite of this and better light penetration in the ProCa treatment, better reproductive bud development was not found with this treatment. Net CO₂ assimilation rates were reduced on 'Royal Gala' trees following scoring due to a feedback inhibition (Chapter 3, 4), indicating high levels of carbohydrates in the canopy. It thus remains unclear whether this increase in reproductive bud development is due to higher carbohydrate levels or interference with plant growth regulator translocation.

The positive effect of girdling on fruit size has been reported for a number of fruit crops (Goren et al., 2004). In this study, scoring increased fruit size during 2001/02 on 'Royal Gala' and 'Cripps' Pink' but not on 'Fuji'. During 2002/03 fruit size was significantly smaller on scored 'Royal Gala' and 'Cripps' Pink' trees, and this was likely due to a significant increase in yield of scored trees. Despite having significantly higher yields during 2002/03, fruit size was not negatively affected on scored 'Fuji' trees. There were no significant differences in fruit size between the ProCa and control treatments during both seasons.

Whilst fruit size can be optimised with thinning there is a compromise necessary with yield. The level of cropping reflects the genetically determined size potential of the cultivar and the cultivar fruit size response to thinning. For small-fruited cultivars such as 'Royal Gala', early thinning will be beneficial to increase mean fruit size. Thus if fruit numbers are adjusted earlier, higher cropping levels may be possible without reducing mean fruit size (Wünsche and Ferguson, 2005). Effective thinning may impart a considerable shift in the proportion of harvested crop from small to large fruit (Link, 2000). In this study, scoring had a similar effect, increasing average fruit size at higher cropping levels compared to control trees. This effect was most pronounced on 'Fuji' during the second season, where despite carrying a five time higher crop load than control trees, there were no significant decreases in average fruit size on scored trees.

The growth rate of the developing fruit depends not only on whole-tree assimilate production but also on how successfully the fruit can compete with other sinks (Wünsche and Ferguson, 2005). Fruit size at harvest can be viewed as the result of a combination of cell number, determined during the early developmental stage of cell division, cell size and volume of intercellular space (Goffinet et al., 1995). High crop densities and excessive vegetative growth during the early growth period of fruit cell division may cause a deficit in carbohydrate availability to the developing fruit which can lead to decreased fruit growth rate and reduced final fruit size (Lakso, 1994). In this study, both scoring and ProCa reduced final extension shoot length in 'Fuji' and 'Cripps' Pink' (Chapter 4). However, only scoring increased fruit growth rate in these cultivars during the cell division period (0-40 DAFB). Although ProCa reduced shoot growth effectively in 'Fuji', the carbon allocation patterns were unaffected. The increase in fruit growth rate due to thinning was observed later in the season (> 50 DAFB) and has also been well documented in the literature (Forshey and Elfving, 1989). During the second season, the heavier crop load associated with better reproductive bud development on the scored trees led to a reduction in fruit growth rate on these trees. Whilst scoring was not as

effective as ProCa in reducing shoot growth (Chapter 4), preferential carbon allocation to fruit and reproductive bud development on scored trees led to increased yields of larger marketable fruit. Apple fruit growth rate may be determined in part by the ability to acquire sorbitol (Archbold, 1992). Archbold (1999) found that SDH activity might be modulated by the availability of sorbitol and other carbohydrates. Reduced sorbitol availability arising from high levels of competition between sinks as well as from adverse environmental conditions reducing leaf photosynthetic rates, reduce SDH activity, thus negatively fruit sink strength. The reduction in shoot growth observed on scored trees is thus a combination of preferential carbon allocation to fruits, due to the fruit being established as a strong sink early in the season and heavier crop loads on trees scored the previous year.

Increased carbohydrate supply to fruit generally results in bigger fruit with favourable characteristics such as higher firmness, higher TSS and improved skin colour (Greer et al., 2002), but may store less well due to lower calcium levels in the fruit (Link, 2000). In this study, scoring improved the TSS of 'Fuji' and 'Cripps' Pink' fruit compared to control and trees sprayed with ProCa during 2002/03, indicating high carbohydrate supply to the fruits. In 'Cripps' Pink' fruit firmness and colour were also improved in scored trees compared to control trees during 2002/03. ProCa did not improve fruit firmness compared to control trees in 'Cripps' Pink', but fruit from the ProCa treatment showed better colour development than fruit from the control treatment during 2002/03. As there was no evidence of improved carbohydrate supply to the fruit in the ProCa treatment, this improvement in fruit colour was probably due to less shoot growth and better light penetration into the canopy of these trees. Elfving et al. (1991) have also found increased TSS and fruit firmness on 'McIntosh' apples after girdling as late as three weeks before harvest. The improvement of fruit size and colour due to better carbohydrate supply is often associated with higher contents of soluble solids and titratable acidity, and may therefore improve taste and appearance of fruit (Schumacher and Stadler, 1987).

In conclusion, scoring improved the carbohydrate supply to the fruits, which in turn led to increased fruit growth rates during the first 40 DAFB, and culminated in better fruit size at harvest. Reproductive bud development was also enhanced on scored trees, with 'Fuji' being the most responsive cultivar. ProCa inhibited shoot growth effectively, but no evidence was found of improved carbon allocation to fruits or reproductive buds. The possible use of girdling/scoring in combination with ProCa holds great potential for restricting vegetative

growth and allowing for better light penetration into the trees, as well as improving year-on-year marketable yield due to preferential carbon allocation to fruit and reproductive buds.

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Table 1. Dates of full bloom, spraying of ProCa, scoring, hand thinning, cell division stage, cell enlargement stages, shoot measurements, first and second harvest of 'Royal Gala' during the 2001/02 and 2002/03 seasons.

	2001/02	2002/03
Full bloom	12 October 2001	28 September 2002
Spraying of ProCa	1 November 2001 (20 DAFB)	18 October 2002 (20 DAFB)
Scoring	7 November 2001 (26 DAFB) 27 November 2002 (46 DAFB)	28 October 2002 (30 DAFB) 18 November 2002 (51 DAFB)
Hand thinning	11 November 2001 (30 DAFB) 11 December 2001 (60 DAFB)	19 November 2002 (52 DAFB)
Cell division stage	0-45 DAFB	0-44 DAFB
Cell enlargement stages	45-102 DAFB	44-72 and 72-121 DAFB
First harvest	7 February 2002 (118 DAFB)	4 February 2003 (129 DAFB)
Second harvest	12 February 2002 (123 DAFB)	18 February 2003 (143 DAFB)
Bud analysis	August 2002	August 2003

Table 2. Dates of full bloom, spraying of ProCa, scoring, hand thinning, cell division stage, cell enlargement stages, shoot measurements and harvest of 'Fuji' during the 2001/02 and 2002/03 seasons.

	2001/02	2002/03
Full bloom	11 October 2001	26 September 2002
Spraying of ProCa	1 November 2001 (21 DAFB)	18 October 2002 (22 DAFB)
Scoring	13 November 2001 (33 DAFB)	14 November 2002 (49 DAFB)
Hand thinning	4 December 2001 (54 DAFB)	5 December 2002 (70 DAFB)
Cell division stage	0-54 DAFB	0-46 DAFB
Cell enlargement stages	54-104 and 104-165 DAFB	46-81, 81-123 and 123-165 DAFB
Harvest	5 April 2002 (175 DAFB)	27 March 2003 (182 DAFB)

Table 3. Dates of full bloom, spraying of ProCa, scoring, hand thinning, cell division stage, cell enlargement stages, shoot measurements, first and second harvest of 'Cripps' Pink' during the 2001/02 and 2002/03 seasons.

	2001/02	2002/03
Full bloom	12 October 2001	26 September 2002
Spraying of ProCa	1 November 2001 (20 DAFB)	18 October 2002 (22 DAFB)
Scoring	14 November 2001 (33 DAFB)	15 November 2002 (50 DAFB)
Hand thinning	4 December 2001 (53 DAFB)	15 November 2002 (50 DAFB)
Cell division stage	0-54 DAFB	0-53 DAFB
Cell enlargement stages	54-103 and 103-178 DAFB	53-74, 74-123 and 123-186 DAFB
First harvest	26 April 2002 (196 DAFB)	8 April 2003 (194 DAFB)
Second harvest		15 April 2003 (201 DAFB)

Table 4. Probability values ($P > F$) following scoring and the spraying of prohexadione-calcium (CSP treatments) and thinning treatments and their interactions on fruit quality parameters of 'Royal Gala', 'Fuji' and 'Cripps' Pink' during 2001/02 and 2002/03.

'Royal Gala'	2001/02 Harvest 1			2001/02 Harvest 2		
	Mass (g)	Firmness (kg)	Seed no.	Mass (g)	Firmness (kg)	Seed no.
CSP	<i>0.0025</i>	<i>0.7907</i>	<i>0.0010</i>	<i>0.0005</i>	<i>0.0378</i>	<i>0.0007</i>
Thin	<i><.0001</i>	<i>0.5722</i>	<i>0.0005</i>	<i><.0001</i>	<i>0.0057</i>	<i>0.2915</i>
CSP*Thin	<i>0.1674</i>	<i>0.9197</i>	<i>0.2606</i>	<i>0.3562</i>	<i>0.4284</i>	<i>0.9227</i>
	2002/03 Harvest 1		2002/03 Harvest 2			
	Mass (g)	TSS (%)	Mass (g)	TSS (%)		
CSP	<i>0.0351</i>	<i>0.3421</i>	<i>0.0198</i>	<i>0.5237</i>		
Thin	<i>0.0020</i>	<i>0.6637</i>	<i>0.0365</i>	<i>0.0248</i>		
CSP*Thin	<i>0.1796</i>	<i>0.6949</i>	<i>0.0207</i>	<i>0.3725</i>		
'Fuji'	2001/02		2002/03			
	Mass (g)		Mass (g)	TSS (%)		
CSP	<i>0.3202</i>		<i>0.8294</i>	<i>0.0025</i>		
Thin	<i><.0001</i>		<i><.0001</i>	<i>0.3799</i>		
CSP*Thin	<i>0.1453</i>		<i>0.3467</i>	<i>0.7461</i>		
'Cripps' Pink'	2001/02					
	Mass (g)	Firmness (kg)				
CSP	<i>0.0080</i>	<i>0.0095</i>				
Thin	<i><.0001</i>	<i>0.0100</i>				
CSP*Thin	<i>0.4316</i>	<i>0.1992</i>				
	2002/03 Harvest 1			2002/03 Harvest 2		
	Mass (g)	TSS (%)	Blush	Mass (g)	TSS (%)	Blush
CSP	<i>0.0249</i>	<i>0.0316</i>	<i>0.0806</i>	<i>0.2906</i>	<i>0.0106</i>	<i>0.0072</i>
Thin	<i><.0001</i>	<i>0.5190</i>	<i>0.3158</i>	<i><.0001</i>	<i>0.0119</i>	<i>0.0751</i>
CSP*Thin	<i>0.2437</i>	<i>0.3427</i>	<i>0.6938</i>	<i>0.4180</i>	<i>0.0748</i>	<i>0.7679</i>

Table 5. Probability values ($Pr > F$) following scoring and the spraying of prohexadione-calcium (CSP treatments) and thinning treatments and their interactions on fruit growth rate (mm day^{-1}) on 'Royal Gala', 'Fuji' and 'Cripps' Pink' during 2001/02 and 2002/03.

'Royal Gala'		2001/02			
		0-24 DAFB	24-45 DAFB	45-102 DAFB	
CSP	0.3943	<i>0.0024</i>	<i>0.0453</i>		
Thin	<i>0.0237</i>	<i>0.0271</i>	<i>0.0017</i>		
CSP*Thin	0.2285	<i>0.0182</i>	0.4430		
		2002/03			
		0-23 DAFB	23-45 DAFB	45-100 DAFB	100-121 DAFB
CSP	0.5869	0.6553	0.1238	0.2162	
Thin	0.1024	0.0503	0.4195	0.2661	
CSP*Thin	0.5668	0.4384	0.0207	0.8133	
'Fuji'		2001/02			
		0-33 DAFB	33-54 DAFB	54-104 DAFB	104-165 DAFB
CSP	0.1554	<i><.0001</i>	0.9443	<i>0.0172</i>	
Thin	0.6673	0.8251	<i><.0001</i>	<i><.0001</i>	
CSP*Thin	0.4489	0.8510	0.2905	0.6907	
		2002/03			
		0-32 DAFB	32-46 DAFB	46-102 DAFB	102-165 DAFB
CSP	0.3335	0.5992	0.6849	0.9629	
Thin	0.5074	0.3673	0.3934	0.1495	
CSP*Thin	0.3893	0.5897	0.8263	0.1254	
'Cripps' Pink'		2001/02			
		0-33 DAFB	33-54 DAFB	54-103 DAFB	103-165 DAFB
CSP	0.1586	<i>0.0002</i>	0.7744	<i>0.0011</i>	
Thin	0.3259	0.9494	<i><.0001</i>	<i><.0001</i>	
CSP*Thin	0.7627	0.9589	0.9183	0.9339	
		2002/03			
		0-46 DAFB	46-102 DAFB	102-186 DAFB	
CSP	0.1218	0.7464	0.4408		
Thin	0.3160	<i>0.0042</i>	0.3444		
CSP*Thin	0.2392	0.4386	0.7124		

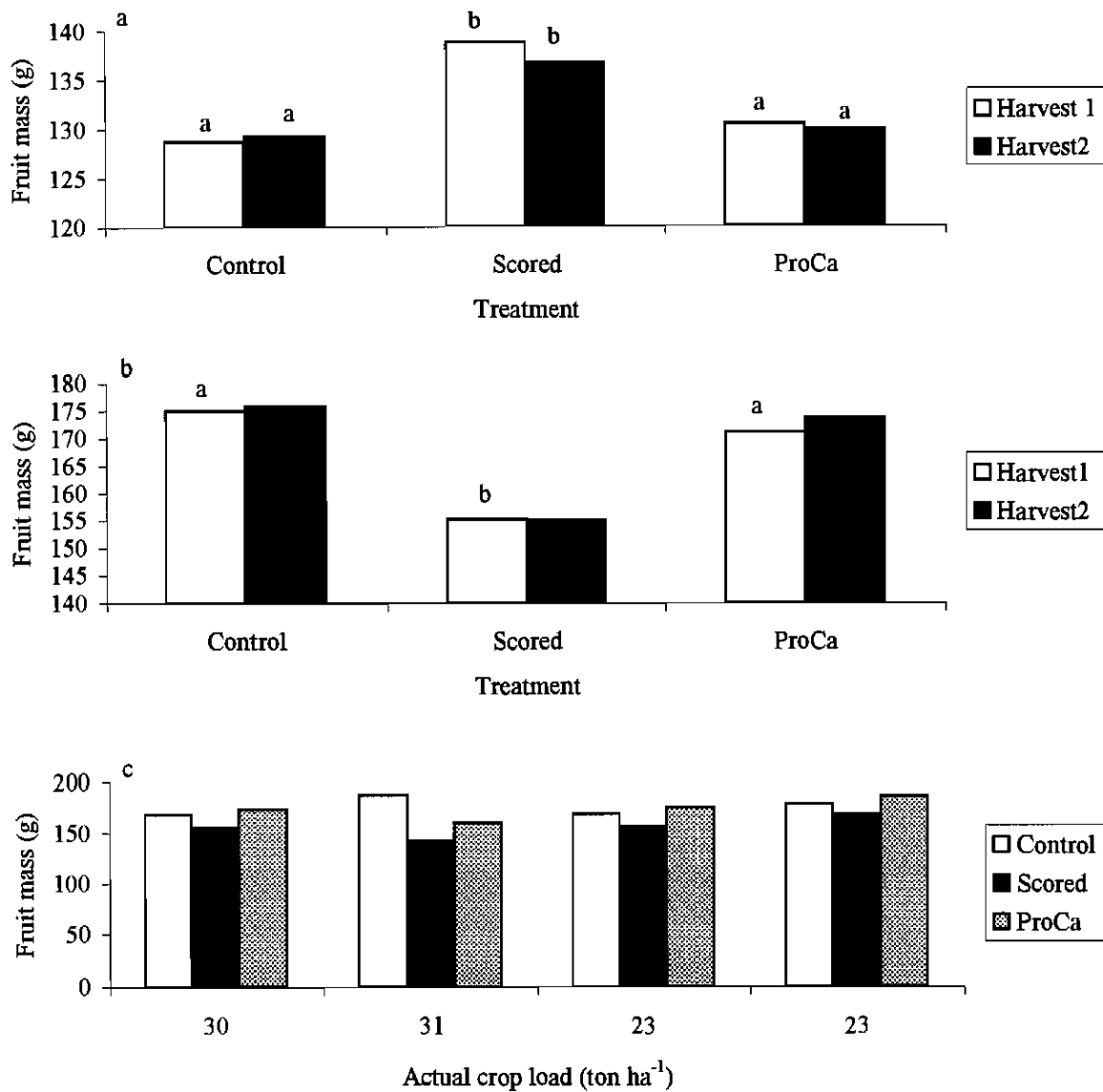


Fig. 1. Fruit mass of 'Royal Gala' for harvests 1 and 2 following scoring, spraying of prohexadione-calcium (CSP treatments) during (a) 2001/02, (b) the CSP treatments and (c) the CSP and thinning treatments for harvest 2 during 2002/03. Means with the same letter within graphs are not significantly different (Tukey Pr < 0.05).

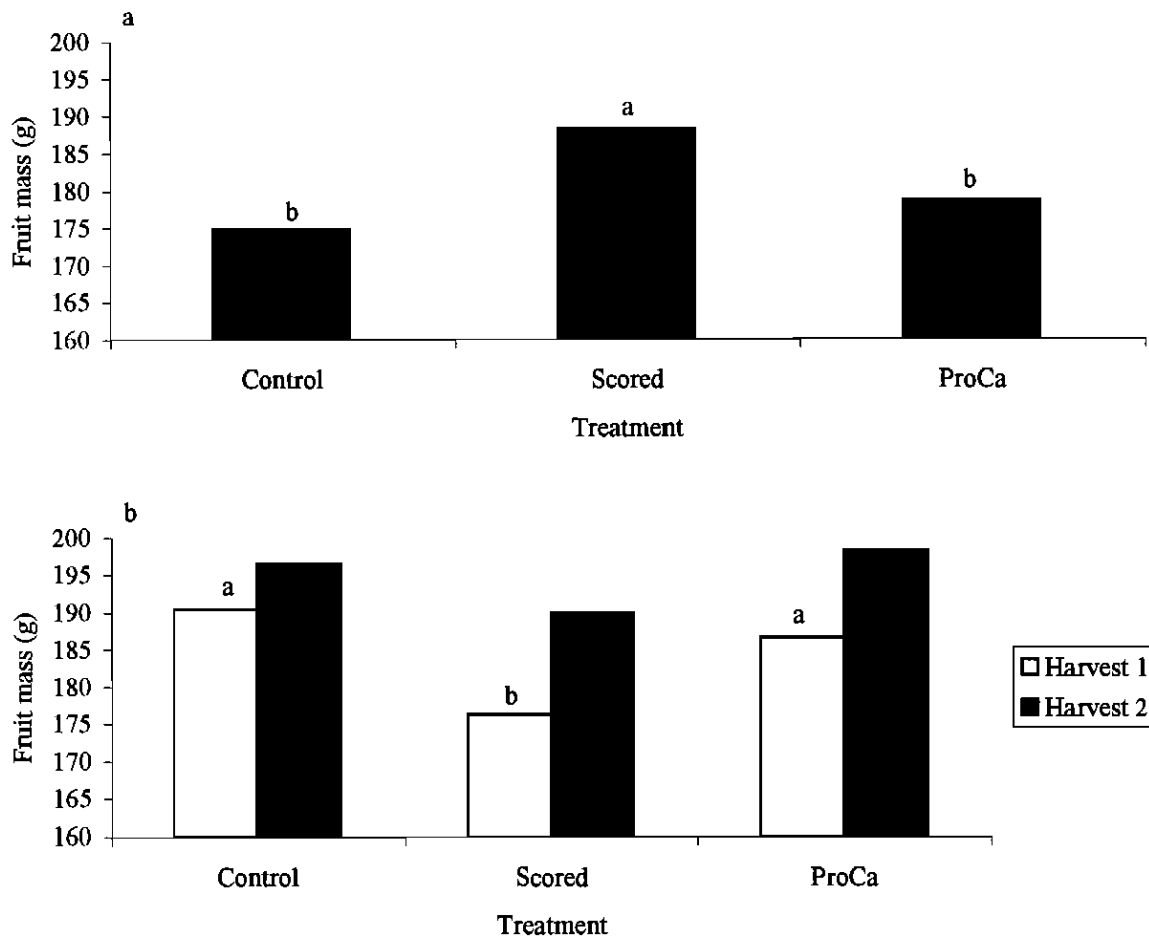


Fig. 2. Fruit mass of 'Cripps' Pink' following scoring, spraying of prohexadione-calcium (CSP treatments) during (a) 2001/02 and (b) the CSP treatments for harvest 1 and 2 during 2002/03. Means with the same letter within graphs are not significantly different (Tukey $P < 0.05$).

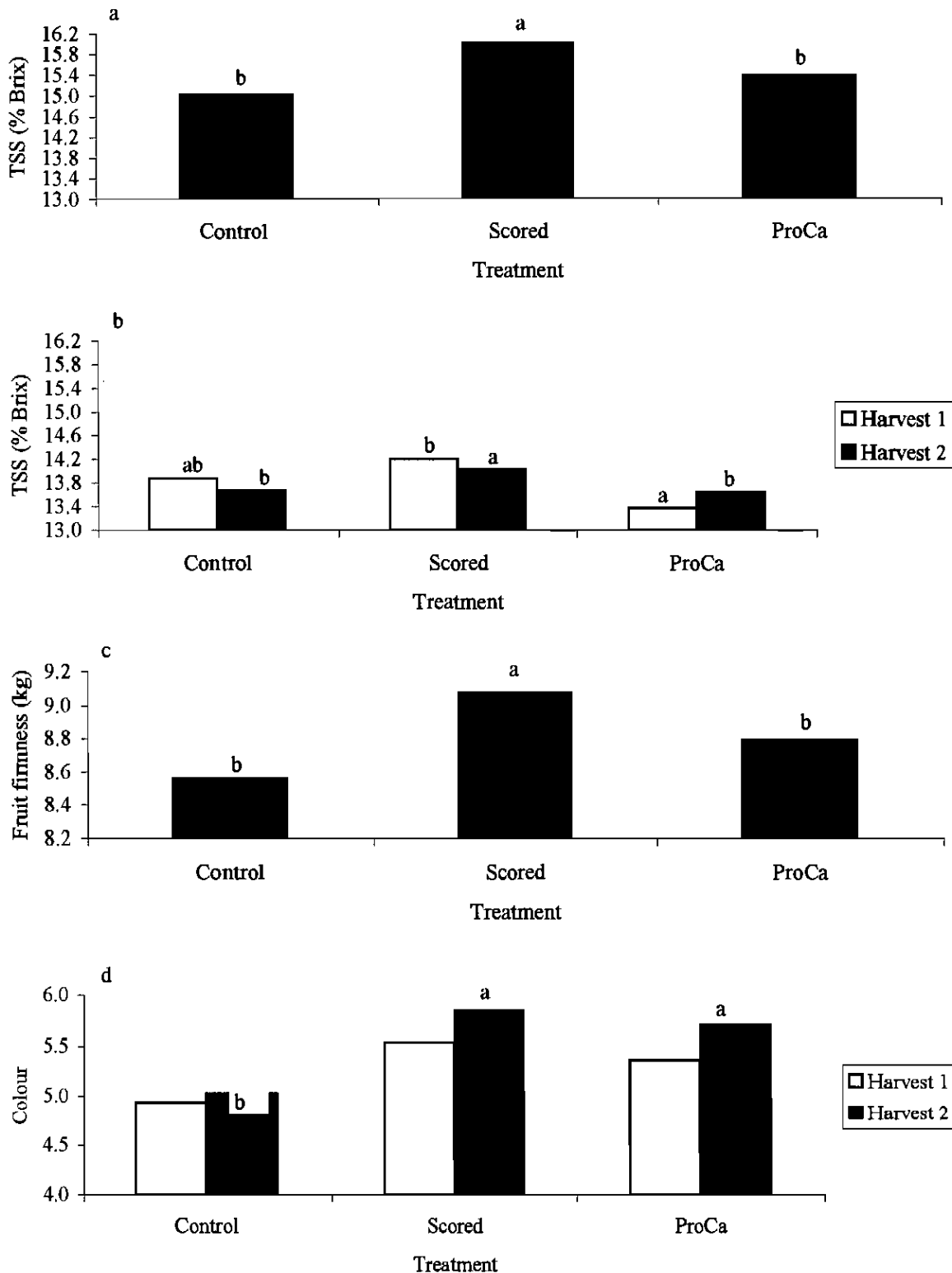


Fig. 3. (a) Percentage TSS for 'Fuji' following scoring, spraying of prohexadione-calcium (CSP treatments) during 2002/03, (b) percentage TSS for harvest 1 and 2 for the CSP treatments of 'Cripps' Pink', (c) fruit firmness for the CSP treatments during 2001/02, and (d) colour development for the CSP treatments (harvest 2) during 2002/03. Means with the same letter within graphs are not significantly different (Tukey $Pr < 0.05$).

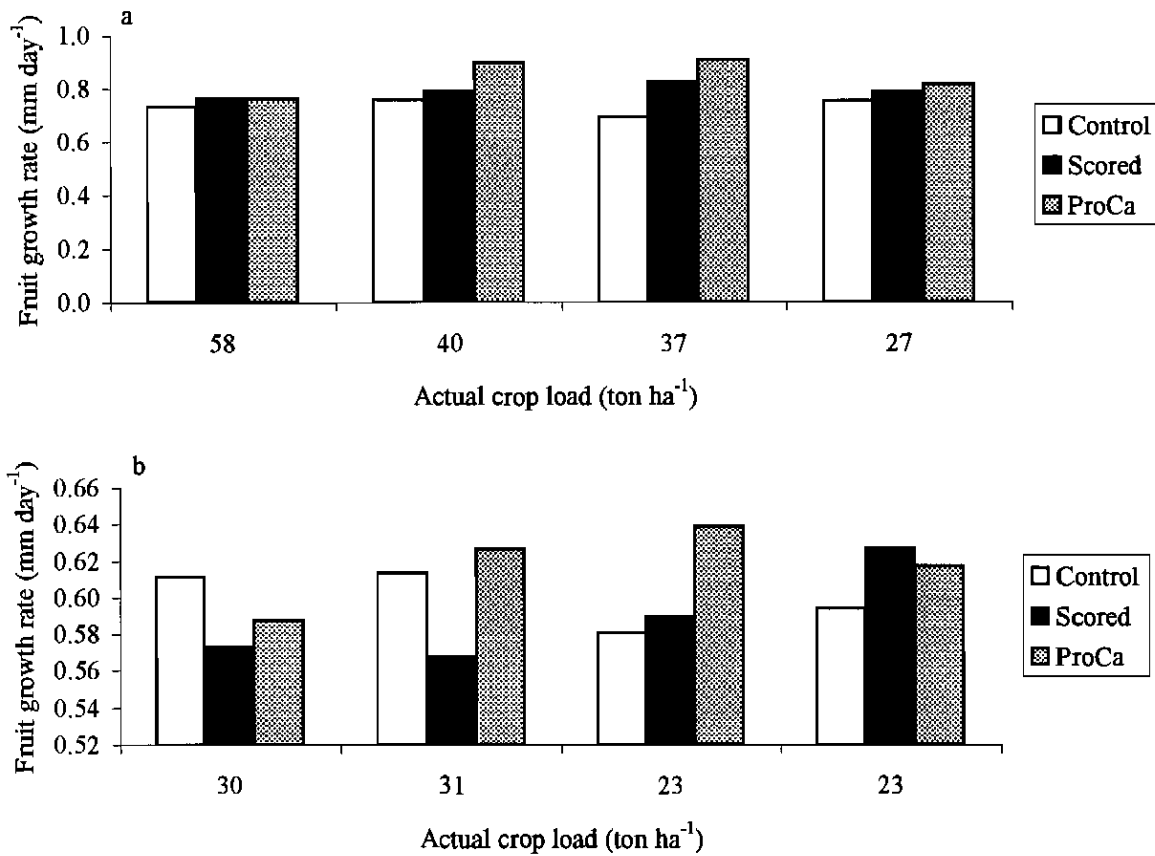


Fig. 4. Fruit growth rate of 'Royal Gala' for (a) 24-45 DAFB following scoring, spraying of prohexadione-calcium (CSP treatments) and thinning treatments during 2001/02 and (b) 45-100 DAFB for the CSP and thinning treatments during 2002/03. Means with the same letter within graphs are not significantly different (Tukey Pr < 0.05).

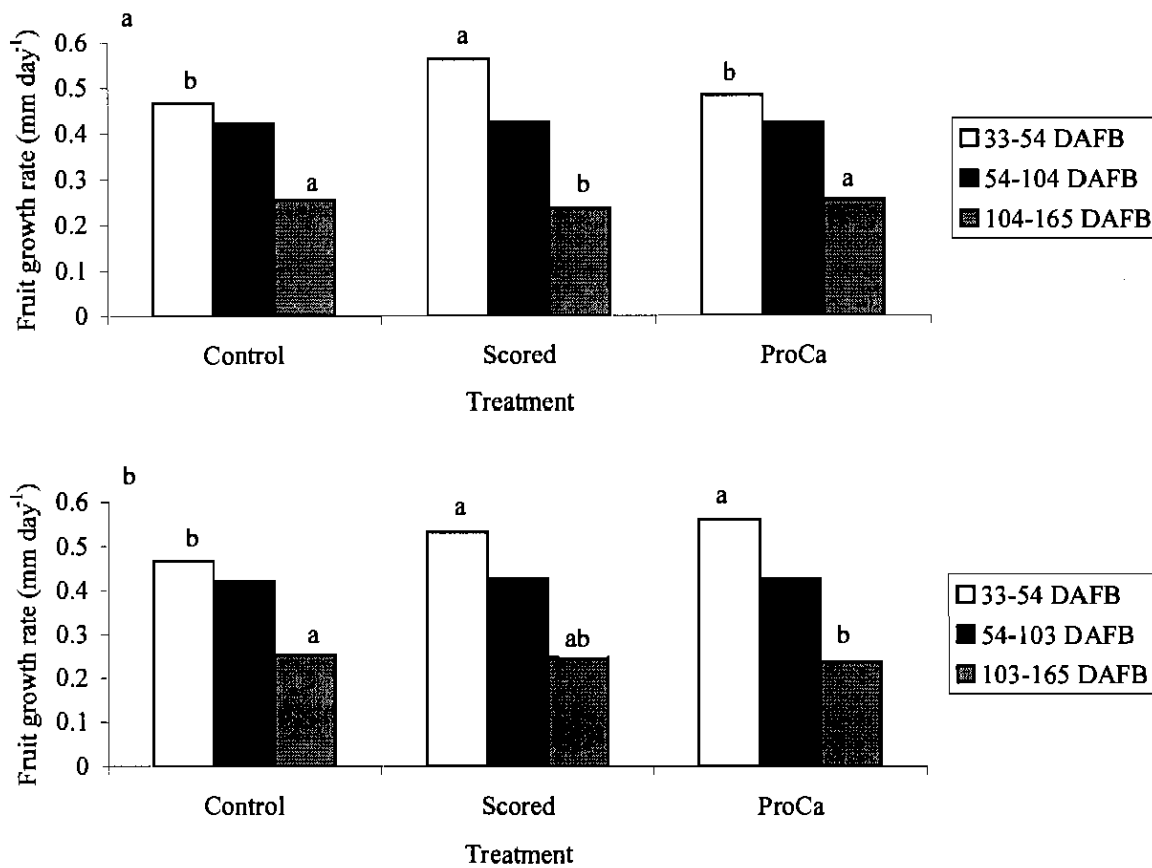


Fig. 5. Fruit growth rate of 'Fuji' for the periods 33-54 DAFB, 54-104 DAFB and 104-165 DAFB following scoring, spraying of prohexadione-calcium (CSP treatments) during 2001/02 and (b) fruit growth rate of 'Cripps' Pink' for the periods 33-54 DAFB, 54-103 DAFB and 103-165 DAFB for the CSP treatments during 2001/02. Means with the same letter within graphs are not significantly different (Tukey Pr < 0.05).

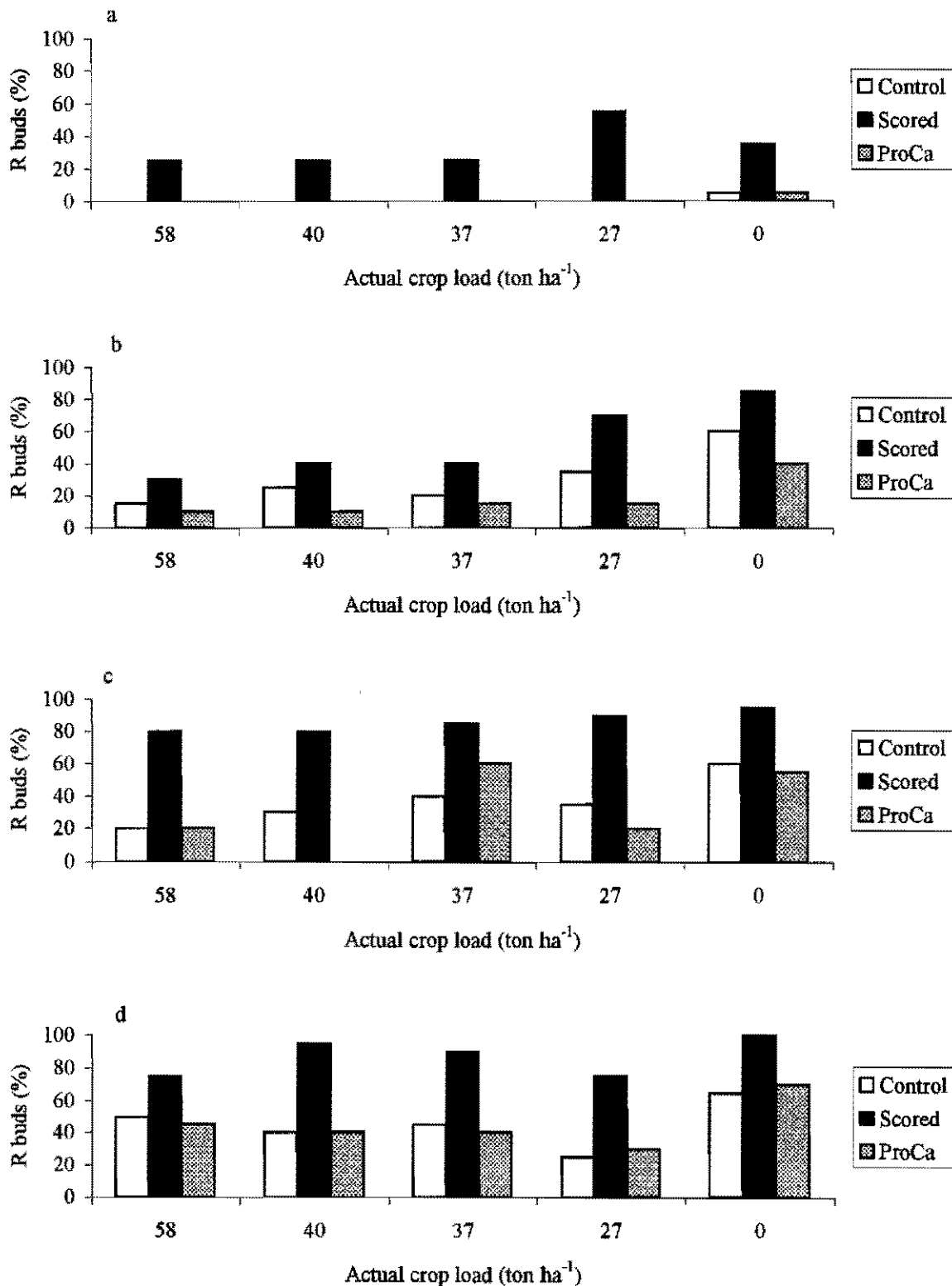


Fig. 6. Percentage reproductive (R) buds on (a) long shoots (>100 mm), (b) short shoots (30-100 mm), (c) new spurs and (d) old spurs on 'Royal Gala' after the 2001/02 season following scoring, spraying of prohexadione-calcium (CSP treatments) and thinning treatments.

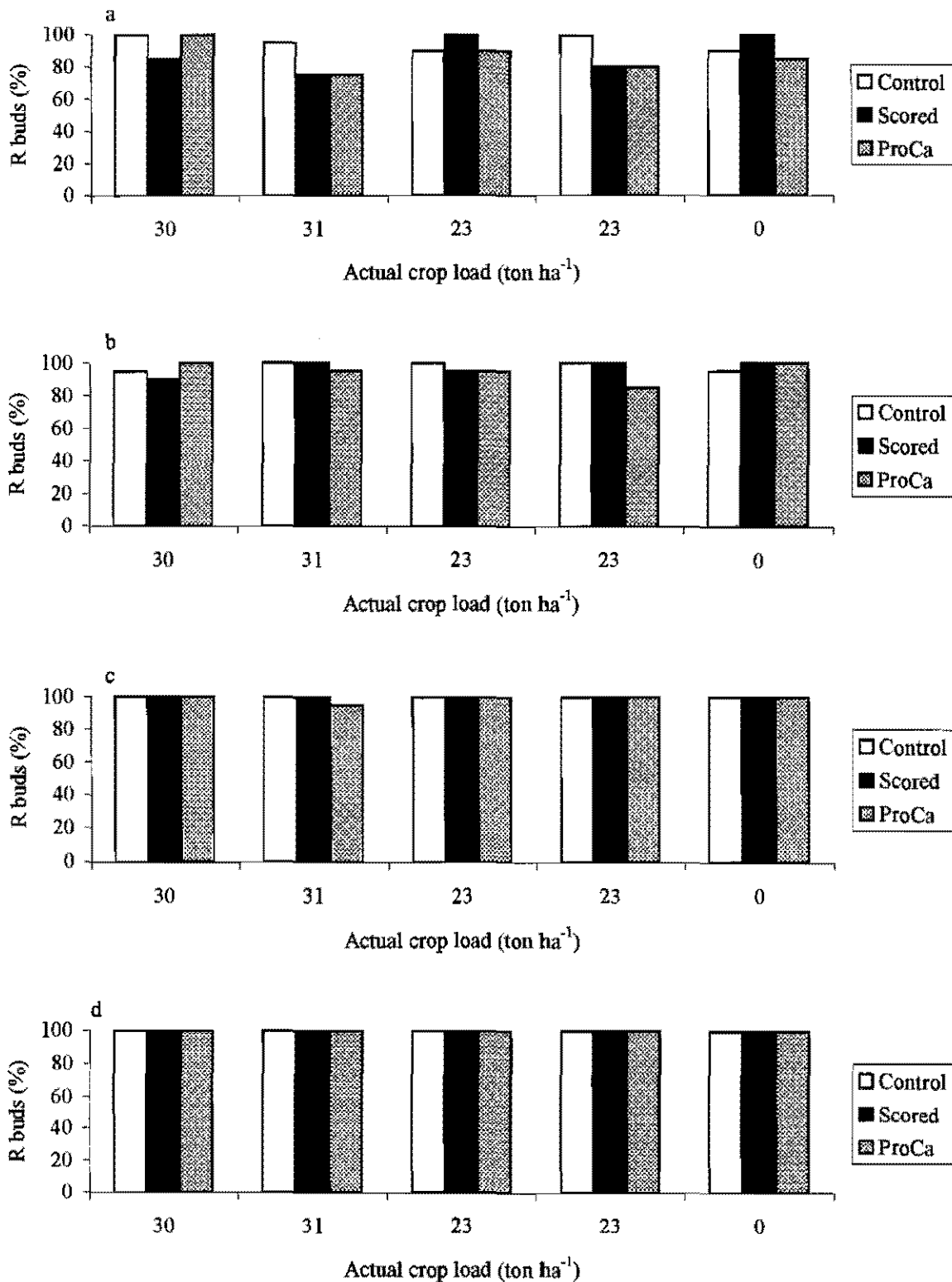


Fig. 7. Percentage reproductive buds on (a) long shoots (>100 mm), (b) short shoots (30-100 mm), (c) new spurs and (d) old spurs on 'Royal Gala' after the 2002/03 season following scoring, spraying of prohexadione-calcium (CSP treatments) and thinning treatments.

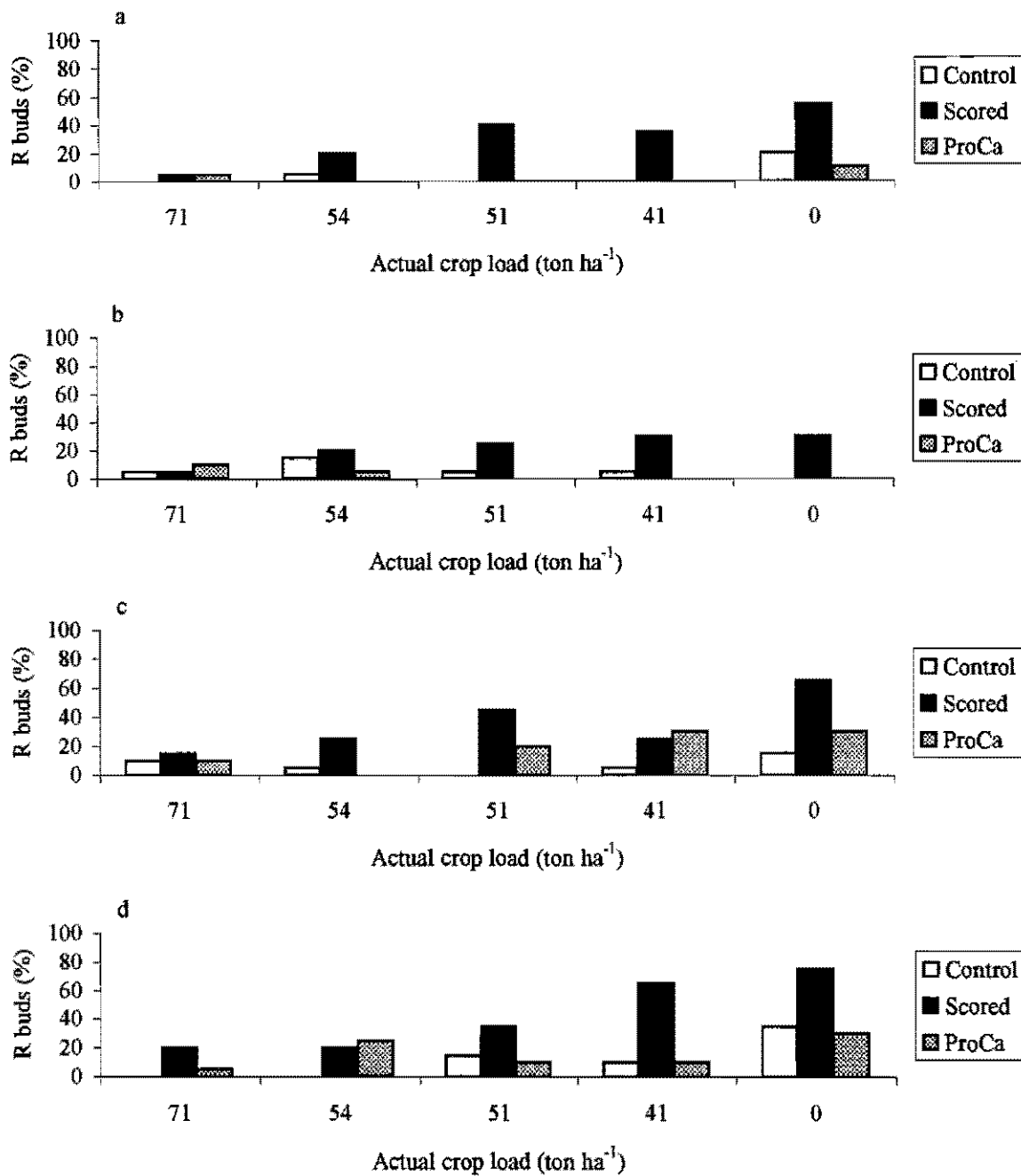


Fig. 8. Percentage reproductive buds on (a) long shoots (>100 mm), (b) short shoots (30-100 mm), (c) new spurs and (d) old spurs on 'Fuji' after the 2001/02 season following scoring, spraying of prohexadione-calcium (CSP treatments) and thinning treatments.

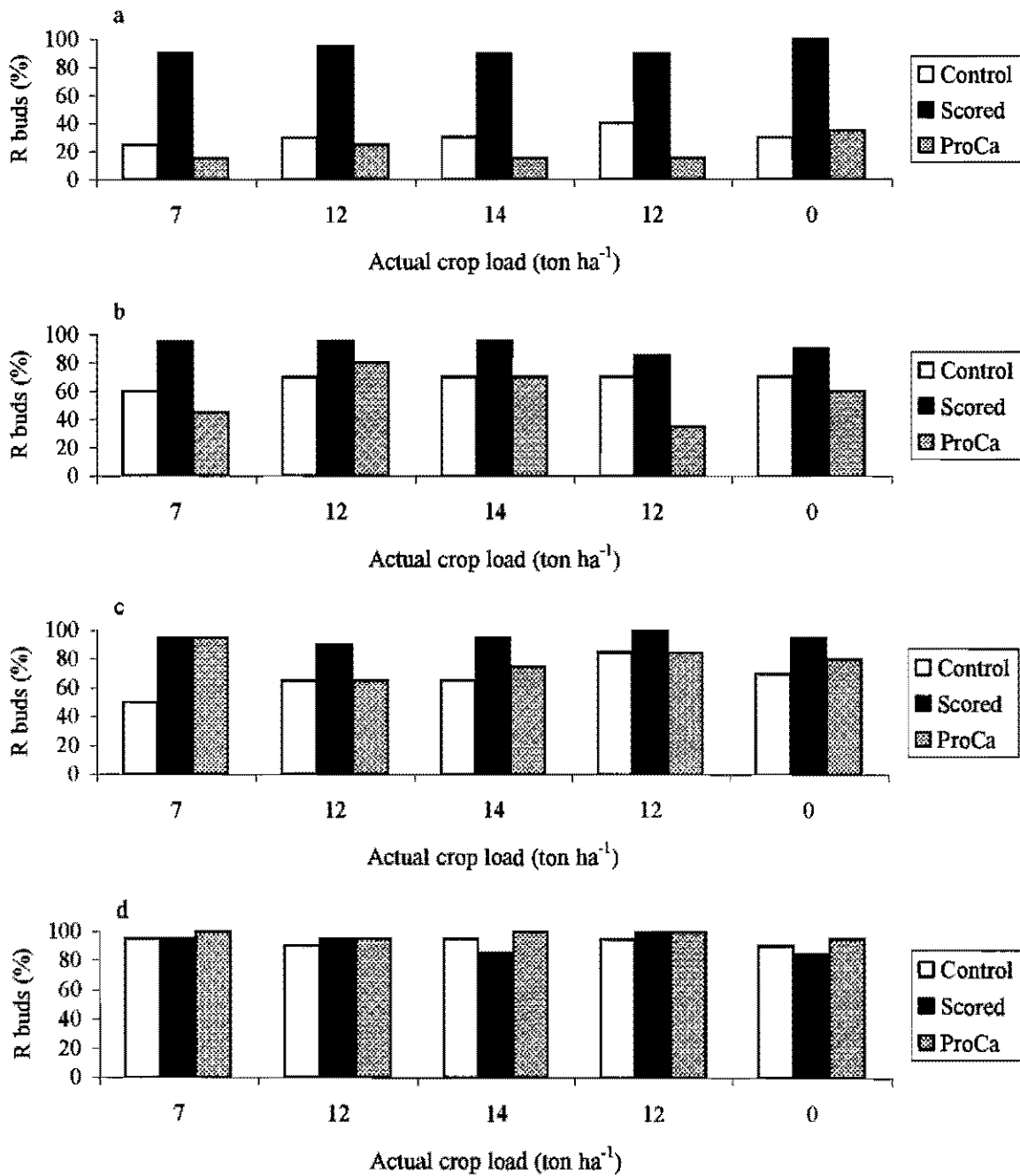


Fig. 9. Percentage reproductive buds on (a) long shoots (>100 mm), (b) short shoots (30-100 mm), (c) new spurs and (d) old spurs on 'Fuji' after the 2002/03 season following scoring, spraying of prohexadione-calcium (CSP treatments) and thinning treatments.

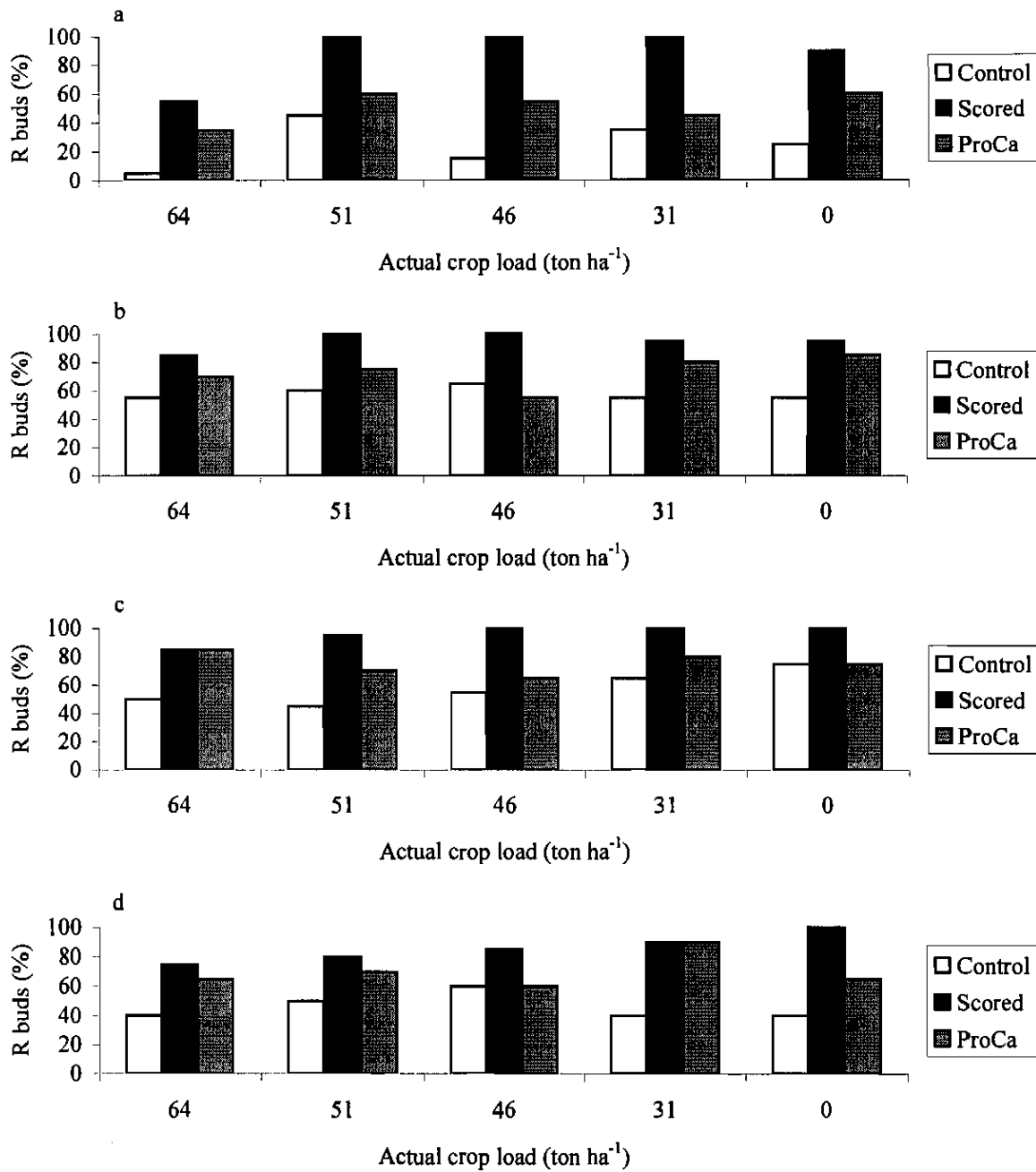


Fig. 10. Percentage reproductive buds on (a) long shoots (>100 mm), (b) short shoots (30-100 mm), (c) new spurs and (d) old spurs on 'Cripps' Pink' after the 2001/2002 season following scoring, spraying of prohexadione-calcium (CSP treatments) and thinning treatments.

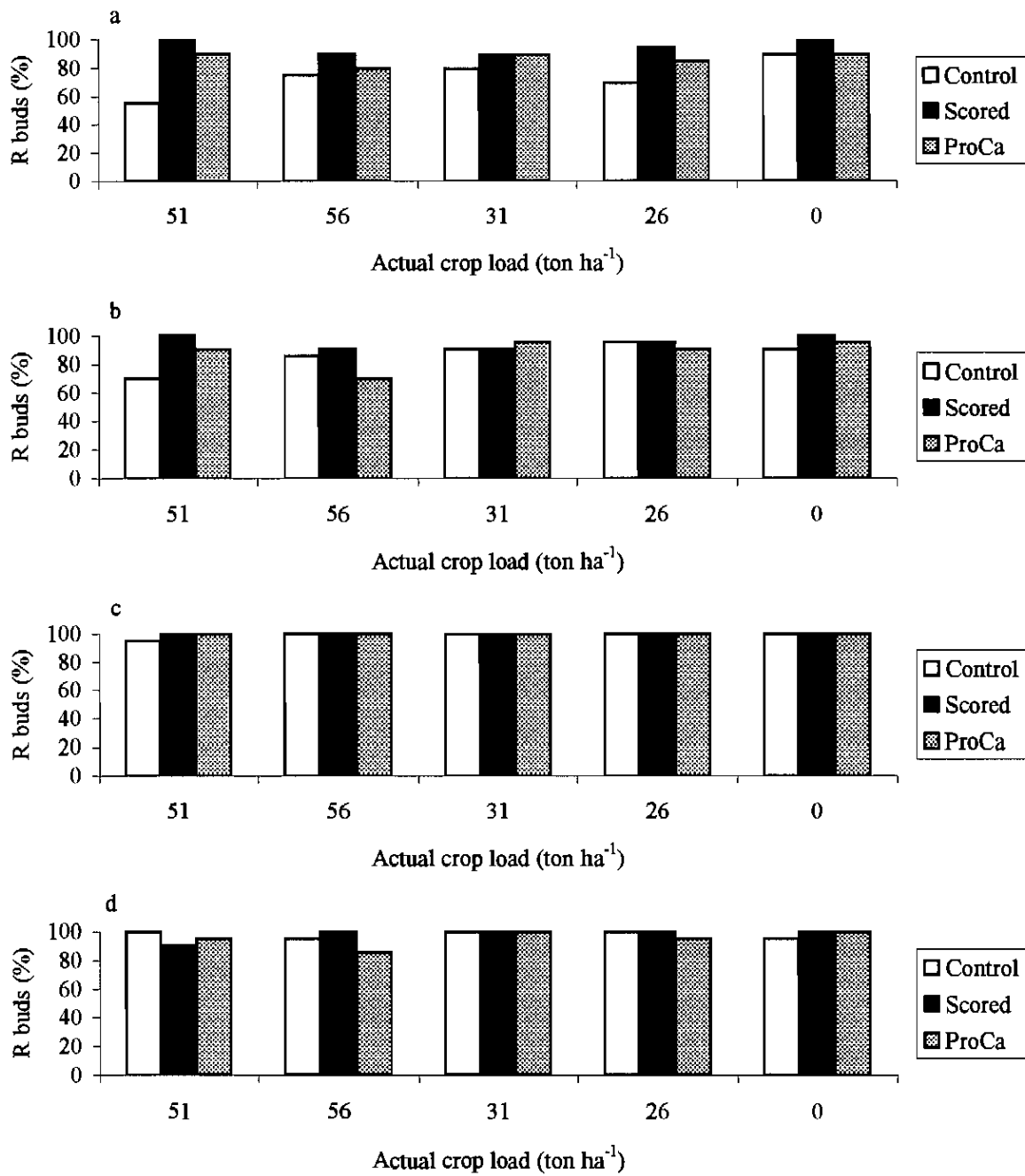


Fig. 11. Percentage reproductive buds on (a) long shoots (>100 mm), (b) short shoots (30-100 mm), (c) new spurs and (d) old spurs on 'Cripps' Pink after the 2002/2003 season following scoring, spraying of prohexadione-calcium (CSP treatments) and thinning treatments.

6. The combined use of scoring and prohexadione-calcium on 'Royal Gala', 'Fuji' and 'Cripps' Pink' apple trees to control vegetative growth and stimulate reproductive growth.

Abstract

Scoring and the spraying of prohexadione-calcium (ProCa) are practices that hold great potential for stimulating reproductive growth and controlling vegetative growth on apple trees. The objective of this study was to investigate the individual and combined effects of scoring (30-50 days after full bloom, DAFB) and ProCa (20-22 DAFB) on yield efficiency, fruit growth and quality, shoot growth and reproductive bud development of 'Royal Gala', 'Fuji' and 'Cripps' Pink' apple orchards. There were no treatment effects on yield efficiency. 'Royal Gala' fruit growth rate and final fruit mass were not affected by any treatment. 'Fuji' fruit growth rates were increased by the combined treatment following scoring, compared to control and ProCa treatments, and final fruit mass was higher, but not significantly so. The combined treatment also increased fruit growth rates in 'Cripps' Pink' following scoring, compared to the ProCa treatment, and final fruit mass was higher than in the ProCa and control treatments. Scoring alone or in combination with ProCa improved the total soluble solids concentration (TSS) of 'Royal Gala' fruit compared to control fruit, and improved TSS in 'Fuji' and 'Cripps' Pink' fruit compared to both the control and ProCa treatments. ProCa decreased extension shoot growth rates of 'Fuji' and 'Cripps' Pink' up to 81 DAFB compared to control and scored trees. This reduction in extension shoot growth rate contributed towards a faster fruit growth rate when combined with scoring. Final extension shoot length was significantly reduced by ProCa treatments only in 'Fuji'. Bourse shoot growth was not affected by any treatment. Scoring improved the reproductive bud development of 'Royal Gala' on long and short shoots and of 'Fuji' on all structures, but not of 'Cripps' Pink'.

Keywords. Apple, fruit growth, fruit quality, prohexadione-calcium, reproductive bud development, scoring, vegetative growth control, yield efficiency.

6.1 Introduction

Apple producers must manage fruit production in order to optimise quality and produce fruit with specific quality attributes such as increased fruit size and improved blush colour development in order to remain competitive on the international markets (Wünsche and Ferguson, 2005). In addition, modern high-density orchard planting systems must achieve high, early yields and high sustained yield of excellent fruit quality in order for the producer to survive financially (Robinson, 2003). This can be done by managing crop load and effectively restricting vegetative growth in vigorous apple cultivars in order to achieve a balance between vegetative and reproductive development (Forshey and Elfving, 1989; Wünsche and Ferguson, 2005).

Scoring (Goren et al., 2004) and in particular the spraying of prohexadione-calcium (ProCa; BAS-125, (3-oxido-4-propionyl-5-oxo-3-cyclohexene-carboxylate)) (Greene, 1999; Miller, 2002; Rademacher, 1995) are two practices that hold great potential to control vegetative growth on apple trees (Chapter 4) and stimulate allocation of substrates to reproductive growth. A reduction in vigour can have added benefits such as better fruit quality (blush colour development), improved yields, more open trees and improved light penetration leading to better pest control through better spray penetration and improved return bloom (Forshey and Elfving, 1989; Greene, 1999).

While ProCa may be more effective than scoring at inhibiting shoot growth, no evidence has been found for improved carbon allocation to fruits or reproductive buds (Greene, 1999, 2003; Miller, 2002; Chapter 5). On the other hand, scoring improves the carbohydrate supply to the fruits, which in turn can lead to increased fruit growth rates and better fruit size at harvest (Dennis, 1968; Goren et al., 2004). Scoring also increases reproductive bud formation, leading to high sustained yields (Greene and Lord, 1983; Li et al., 1996). It may be possible to utilise the individual benefits of scoring and ProCa by applying the treatments in combination, but little is known about the increased benefits of such a combined treatment approach.

The objective of this study was to investigate the effects of scoring and the use ProCa alone and in combination, with respect to fruit growth and quality, yield and reproductive bud development at a commercial crop load. We hypothesised that scoring has the potential to

increase carbohydrate allocation to fruit growth and reproductive bud development, but it would be even more effective when used in combination with a proven vegetative growth retardant (ProCa) for optimum benefit with respect to fruit size and bud development.

6.2 Materials and methods

This study was conducted in the same commercial 'Royal Gala', 'Fuji' and 'Cripps' Pink' apple orchards (Nooitgedacht Farm) in the Koue Bokkeveld (Ceres) region, (33°13'S, 19°02'E, 975 m above sea level) (Western Cape, South Africa) as described in Chapter 4, during 2002/03. The trial was laid out as a randomised complete block design with ten blocks and single tree replications (not the same trees as used in the parallel study described in Chapter 4). Treatments used were an un-manipulated control (C), trunk scoring (S), the spraying of the growth retardant prohexadione-calcium (100 mg kg^{-1}) (ProCa) and a combination of scoring and ProCa (S+ProCa). Scoring and chemical thinning were performed as described in Chapter 4. Scoring was performed at 30 and 51 days after full bloom (DAFB) in 'Royal Gala', at 49 DAFB in 'Fuji' and at 50 DAFB in 'Cripps' Pink'. ProCa was sprayed at 20 DAFB ('Royal Gala') and 22 DAFB ('Fuji' and 'Cripps' Pink'). All trees were hand thinned to a commercial crop load ('Royal Gala', 55 ton ha^{-1} , 'Fuji' and 'Cripps' Pink' 70 ton ha^{-1}).

For dates of full bloom, hand thinning, cell division stage, cell enlargement stages, first and second harvest refer to Chapters 4 and 5. The lengths of two (one on each side of the tree) tagged bourse and extension shoots were measured at different times during the season and the shoot growth rate calculated in mm day^{-1} . Fruit diameter of two (one on each side of the tree) tagged fruit was measured weekly and the relative fruit growth rate calculated in mm day^{-1} for the cell division stage and cell enlargement stages. These stages were retrospectively identified based on analysis of the seasonal fruit growth curves. At harvest, random sub-samples of 20 fruit per tree were analysed for fruit mass, number of seeds, and total soluble solids concentration (TSS). Slices cut from each side of the fruits were juiced together and a TSS reading taken using a refractometer (PR-100 9501, ATAGO CO. Ltd., Tokyo, Japan). Total yield per tree was recorded and expressed as yield cm^{-1} trunk circumference. During winter, microscopic bud analysis was performed on long shoots ($>100 \text{ mm}$), short shoots (30-100 mm) old spurs and new spurs to determine the percentage reproductive buds. Two buds per

structure were collected from every tree and the samples pooled, so that statistical analysis was not possible.

All data were analysed by ANOVA (SAS Enterprise Guide release 1.3) to test the significance ($Pr \leq 0.05$) of individual treatment effects, and crop load was used as co-variant. Multiple means comparison tests (Tukey, $Pr \leq 0.05$) were also performed.

6.3 Results

Total yield efficiency did not differ significantly between treatments for 'Royal Gala', 'Fuji' or 'Cripps' Pink' (Table 1). There were no significant differences in fruit mass at harvest for 'Royal Gala', 'Fuji' or first harvest for 'Cripps' Pink' (Table 2). Fruit harvested during the second harvest of 'Cripps' Pink' were significantly bigger on trees both scored and sprayed with ProCa (S+ProCa) compared to control trees and trees sprayed with ProCa (not scored) (Table 2).

Fruit harvested from 'Royal Gala' during the first harvest from trees both scored and sprayed with ProCa (S+ProCa) had a significantly higher total soluble solids concentration (TSS) compared to control trees (Table 2). During the second harvest fruit from all scored trees had a significantly higher TSS than fruit from control trees (Table 2). 'Fuji' fruit from all scored trees had a significantly higher TSS compared to fruit from control trees and those sprayed with ProCa (not scored) (Table 2). Fruit from scored (no ProCa) 'Cripps' Pink' trees during the first harvest had a significantly higher TSS compared to control trees (Table 2). During the second harvest fruit from all scored trees had a significantly higher TSS than fruit from control trees and those sprayed with ProCa (not scored) (Table 2).

There were no significant differences in fruit growth rate between treatments on 'Royal Gala' at any stage during the season (Table 3). Fruit growth rate of 'Fuji' during the period 46-81 DAFB was significantly higher on trees both scored and sprayed with ProCa (S+ProCa) compared to control trees and trees sprayed with ProCa (not scored) (Table 3). During the period 53-74 DAFB fruit growth rate of 'Cripps' Pink' was significantly higher on trees both scored and sprayed with ProCa (S+ProCa) compared to trees sprayed with ProCa (not scored) (Table 3).

During the period 44-72 DAFB scored 'Royal Gala' trees (no ProCa) had a significantly faster extension shoot growth rate than that of the other treatments (Table 4). During the period 32-46 DAFB, all 'Fuji' trees sprayed with ProCa has a significantly slower extension shoot growth rate than the control and scored (no ProCa) trees (Table 4). During the period 46-81 DAFB extension shoot growth rate of trees scored and sprayed with ProCa alone or in combination were significantly reduced compared to control trees. Extension shoot growth rate of trees sprayed with ProCa (not scored) was also significantly slower than that of scored trees during this period (Table 4). During the period 46-53 DAFB 'Cripps' Pink' trees scored and sprayed with ProCa and trees only sprayed with ProCa (not scored) had a significantly slower extension shoot growth rate than scored trees. During the period 53-81 DAFB trees sprayed with ProCa (not scored) had a significantly slower extension shoot growth rate than scored trees (Table 4).

Final extension shoot length was significantly longer on scored 'Royal Gala' trees compared to the other treatments (Table 4). Scored (no ProCa) and control 'Fuji' trees had significantly longer extension shoots compared to the S+ProCa and ProCa treatments (Table 4). There were no significant differences in final extension shoot length between treatments on 'Cripps' Pink' trees (Table 4). There were no significant differences in bourse shoot growth rates between the different treatments on any cultivar for any measurement period (data not shown), or in the final bourse shoot lengths (Table 4).

Scoring (no ProCa) favoured reproductive bud development on long and short shoots of 'Royal Gala' (Fig 1a). 'Fuji' trees which were scored and trees both scored and sprayed with ProCa showed improved reproductive bud development compared to control trees and trees sprayed with ProCa (not scored) on all bearing positions (Fig 1b). There was little difference in reproductive bud development between treatments in 'Cripps' Pink' (Fig 1c).

6.4 Discussion

Girdling/scoring can increase yield in various tree crops either due to increases in yield or fruit size (Goren et al., 2004). Scoring performed after initial fruit set would not be expected to have any effect on yield based on fruit numbers. In this study, scoring was done 30-50 DAFB, and no differences were observed in the yield efficiencies between different treatments on any

of the cultivars. Increases in yield are often observed in the second year after scoring due to better reproductive bud development on trees scored the previous year (Chapter 5; Greene and Lord, 1983; Li et al., 1996). In this study, scoring improved the reproductive bud development of 'Royal Gala' on long and short shoots, and on 'Fuji' on all structures, consistent with earlier results (Chapter 5), and one would expect yield increases only during the following season (Chapter 5). The promotion of flowering by scoring may be due to an increase of carbohydrates beyond a threshold level in the canopy of girdled trees (Goldschmidt et al., 1985). However, girdling also interferes with the transport of auxins and other plant growth regulators (Cutting and Lyne, 1993). The increase in flower bud formation can thus not solely be attributed to an increase in carbohydrate levels in the canopy of the girdled tree (Goren et al., 2004). The rich source of gibberellins in apple seeds and their translocation into the plant can inhibit reproductive bud development, especially in heavy cropping trees (Bangerth, 1993, 2000). ProCa acts as a GA biosynthesis inhibitor (Rademacher, 1995), thus reducing gibberellin levels in the plant. However, in spite of this, better reproductive bud development was not found with this treatment. Net CO₂ assimilation rates were reduced on 'Royal Gala' trees following scoring due to a feedback inhibition (Chapter 3, 4), indicating high levels of carbohydrates in the canopy. It thus remains unclear whether this increase in reproductive bud development on the scored treatments is due to higher carbohydrate levels or interference with plant growth regulator translocation.

The growth rate of the developing fruit depends not only on whole-tree assimilate production but also on how successfully the fruit can compete with other sinks (Wünsche and Ferguson, 2005). Fruit size at harvest can be viewed as the result of a combination of cell number, determined during the early developmental stage of cell division, cell size and volume of intercellular space (Goffinet et al., 1995). Apple fruit growth rate may be determined in part by the ability to acquire sorbitol (Archbold, 1992). Of the three enzymes with important roles in the metabolism of imported sugars in apple fruit sorbitol dehydrogenase (SDH) might be the most important one (Archbold, 1999). Archbold (1999) found that SDH activity might be modulated by the availability of sorbitol and other carbohydrates. Reduced sorbitol availability arising from high levels of competition between sinks as well as from adverse environmental conditions reducing leaf photosynthetic rates, reduce SDH activity. As a result, sink strength and activity are regulated to fit the available resources, maintaining a balance between supply

and utilization of carbohydrates (Geiger et al., 1996). In this study, scoring was done towards the end of the cell division stage of fruit growth, since fruit growth rate increased for 'Fuji' and 'Cripps' Pink' following scoring only 50-80 DAFB. Scoring done during this developmental stage might be too late to establish the fruit as the dominant sink on the tree, and thus facilitate preferential carbon allocation to the fruit.

Excessive vegetative growth during the early growth period of fruit cell division may cause a deficit in carbohydrate availability to the developing fruit which can lead to decreased fruit growth rate and reduced final fruit size (Lakso, 1994). Non-chemical ways of restricting vegetative growth of apple trees, such as scoring, are only marginally effective when used alone (Greene, 2003). In this study ProCa only decreased final extension shoot length of 'Fuji', and scoring did not reduce final bourse or extension shoot length in any of the cultivars. There was an unexplained increase in final extension shoot length on scored 'Royal Gala' trees. Excessive vegetative growth is also a major concern for the producer because it competes with fruit growth (Forshey and Elfving, 1989). ProCa applied at 20 DAFB decreased extension shoot growth rate of 'Fuji' and 'Cripps' Pink' up to 81 DAFB compared to control and scored trees. This reduction in extension shoot growth rate might also have contributed towards a faster fruit growth rate on the S+ProCa treatments. Although 'Fuji' fruit harvested from the S+ProCa treatment were bigger than fruit from the control (267g vs. 242g) this difference was not significant ($P > F 0.1002$). In 'Cripps' Pink' the same trend was observed during harvest 1, but the significance level improved ($P > F 0.0517$), but during harvest 2 fruit from the S+ProCa treatment were significantly bigger than fruit from the control and ProCa treatments. No significant differences were observed in fruit growth rate or final fruit size of 'Royal Gala'.

Scoring causes the accumulation of carbohydrates throughout the canopy (Goren et al., 2004). Minchin et al. (1997) found that when assimilate supply to a fruit was increased by removing other nearby competing fruits, there was a three-fold increase in the amount of carbohydrates partitioned to the fruit within one day. They concluded that an increase in the ability of the fruit to utilize the available photosynthate through an increase in the expression of catabolic enzyme gave rise to their observation. All this supports the theory that carbohydrate metabolizing enzymes are induced by high levels of sorbitol and/or sucrose, providing a mechanism for carbohydrate supply to affect sink capacity. Scoring, which

increases carbohydrate levels in the canopy, would thus also increase the expression of catabolic enzymes and thus increase the sink strength of the fruit, leading to increased carbon allocation to the fruit and thus increased fruit growth rates. It would appear that although ProCa inhibited shoot growth effectively the strength of the shoot growth sink remained unaffected, therefore no positive effect on fruit growth was observed. In addition to the positive effects of increased carbohydrate supply to fruit on fruit size following girdling or scoring, this practice has also been found to improve other fruit quality characteristics such as firmness, TSS and skin blush colour (Greer et al., 2002). In this study, scoring alone or in combination with ProCa improved the TSS of 'Royal Gala', 'Fuji' and 'Cripps' Pink' fruit compared to control trees and trees sprayed with ProCa, indicating higher carbohydrate supply to the fruits. Elfving et al. (1991) have also found increased TSS on 'McIntosh' apples after girdling as late as three weeks before harvest. However, in this study, no evidence of increased fruit firmness or blush colour development was found in any of the cultivars (data not shown).

The combination of early-season scoring and application of ProCa seems to hold potential for increasing carbon allocation to the fruit and increasing fruit size and quality attributes. This manipulation would probably not be equally successful in all orchards, and vigorous orchards would benefit most from a combination of scoring and ProCa. In less vigorous orchards, manipulation of crop load and scoring alone may be sufficient to control vegetative growth and establish a balance between vegetative and reproductive growth. The exact timing of scoring for the achievement of certain goals is important and warrants further investigation.

6.5 References

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Table 1. Yield efficiency (kg cm^{-1} trunk circumference) of 'Royal Gala', 'Fuji' and 'Cripps' Pink' for harvests 1 and 2 in response to scoring, prohexadione-calcium (ProCa) and a combination of scoring and ProCa (S+ProCa) at an equal crop load. Means with the same letter within columns are not significantly different (Tukey Pr < 0.05).

	Yield 1	Yield 2	Total yield
'Royal Gala'			
Control	0.20 a	0.87 a	1.06 a
Scored	0.22 a	1.03 a	1.26 a
ProCa	0.20 a	0.99 a	1.19 a
S+ProCa	0.23 a	1.00 a	1.24 a
Pr > F	0.6746	0.7238	0.5544
'Fuji'			
Control			1.49 a
Scored			1.38 a
ProCa			1.45 a
S+ProCa			1.13 a
Pr > F			0.3837
'Cripps' Pink'			
Control	0.52 a	0.75 a	1.27 a
Scored	0.42 ab	0.59 a	1.00 a
ProCa	0.32 b	0.48 a	0.80 a
S+ProCa	0.40 ab	0.69 a	1.09 a
Pr > F	0.0528	0.2192	0.0926

Table 2. Fruit mass and total soluble solids (TSS) of 'Royal Gala', 'Fuji' and 'Cripps' Pink' for harvests 1 and 2 in response to scoring, prohexadione-calcium (ProCa) and a combination of scoring and ProCa (S+ProCa) at an equal crop load. Total yield efficiency was used as a co-variant. Means with the same letter within columns are not significantly different (Tukey Pr < 0.05).

	Harvest 1		Harvest 2	
	Fruit mass (g)	TSS (% Brix)	Fruit mass (g)	TSS (% Brix)
'Royal Gala'				
Control	151.6 a	12.5 b	150.7 a	11.7 b
Scored	157.3 a	13.1 ab	143.6 a	13.2 a
ProCa	158.6 a	13.0 ab	155.2 a	12.6 ab
S+ProCa	158.9 a	13.3 a	149.8 a	13.0 a
Pr > F	0.5769	0.0416	0.6028	0.0021
'Fuji'				
Control	241.8 a	13.6 b		
Scored	258.2 a	15.0 a		
ProCa	244.1 a	13.4 b		
S+ProCa	267.0 a	14.8 a		
Pr > F	0.1002	0.0007		
'Cripps' Pink'				
Control	175.2 a	13.7 b	186.4 b	13.2 b
Scored	186.5 a	14.5 a	197.0 ab	14.1 a
ProCa	189.5 a	13.8 ab	188.1 b	13.4 b
S+ProCa	192.1 a	14.3 ab	204.4 a	14.2 a
Pr > F	0.0517	0.0218	0.0037	<.0001

Table 3. Fruit growth rates (mm day⁻¹) for three different developmental periods for 'Royal Gala', 'Fuji' and 'Cripps' Pink' in response to scoring, prohexadione-calcium (ProCa) and a combination of scoring and ProCa (S+ProCa) at an equal crop load. Total yield efficiency was used as a co-variant. Means with the same letter within columns are not significantly different (Tukey Pr < 0.05). DAFB = days after full bloom.

'Royal Gala'			
	0-44 DAFB	44-72 DAFB	72-121 DAFB
Control	0.59 a	0.61 a	0.41 a
Scored	0.60 a	0.58 a	0.43 a
ProCa	0.58 a	0.63 a	0.45 a
S+ProCa	0.58 a	0.65 a	0.45 a
Pr > F	0.4319	0.2118	0.4415
'Fuji'			
	0-46 DAFB	46-81 DAFB	81-123 DAFB
Control	0.64 a	0.52 b	0.47 a
Scored	0.62 a	0.59 ab	0.47 a
ProCa	0.61 a	0.54 b	0.44 a
S+ProCa	0.63 a	0.70 a	0.48 a
Pr > F	0.5914	<i>0.0011</i>	0.6451
'Cripps' Pink'			
	0-53 DAFB	53-74 DAFB	74-123 DAFB
Control	0.54 a	0.44 ab	0.43 a
Scored	0.57 a	0.50 ab	0.44 a
ProCa	0.54 a	0.43 b	0.42 a
S+ProCa	0.55 a	0.52 a	0.42 a
Pr > F	0.4979	<i>0.0198</i>	0.9997

Table 4. Extension shoot growth rates (mm day⁻¹) for three different developmental periods, final extension shoot length and final bourse shoot length for 'Royal Gala', 'Fuji' and 'Cripps' Pink' in response to scoring, prohexadione-calcium (ProCa) and a combination of scoring and ProCa (S+ProCa) at an equal crop load. Total yield efficiency was used as a co-variant. Means with the same letter within columns are not significantly different (Tukey Pr < 0.05). DAFB = days after full bloom.

	'Royal Gala'				
	30-44 DAFB	44-72 DAFB	72-121 DAFB	Final extension shoot length (mm)	Final bourse shoot length (mm)
Control	4.93 a	0.69 b	0.12 a	349 b	140 a
Scored	6.00 a	3.38 a	0.10 a	464 a	134 a
ProCa	3.43 a	0.34 b	0.65 a	351 b	136 a
S+ProCa	3.36 a	0.54 b	0.05 a	320 b	141 a
Pr > F	0.2762	0.0029	0.4761	0.0467	0.8264
	'Fuji'				
	32-46 DAFB	46-81 DAFB	81-123 DAFB	Final extension shoot length (mm)	Final bourse shoot length (mm)
Control	7.64 a	3.31 a	1.26 a	521 a	209 a
Scored	7.43 a	1.83 b	0.12 a	414 a	195 a
ProCa	2.50 b	0.34 c	0.38 a	334 b	187 a
S+ProCa	2.86 b	0.43 bc	0.05 a	314 b	186 a
Pr > F	<.0001	<.0001	0.1175	0.0008	0.6676
	'Cripps' Pink'				
	46-53 DAFB	53-81 DAFB	81-123 DAFB	Final extension shoot length (mm)	Final bourse shoot length (mm)
Control	3.86 ab	0.79 ab	0.43 a	332 a	181 a
Scored	5.67 a	2.14 a	0.07 a	386 a	172 a
ProCa	2.86 b	0.07 b	0.29 a	324 a	172 a
S+ProCa	2.86 b	0.21 ab	0.24 a	305 a	182 a
Pr > F	0.0267	0.0292	0.8615	0.4259	0.6028

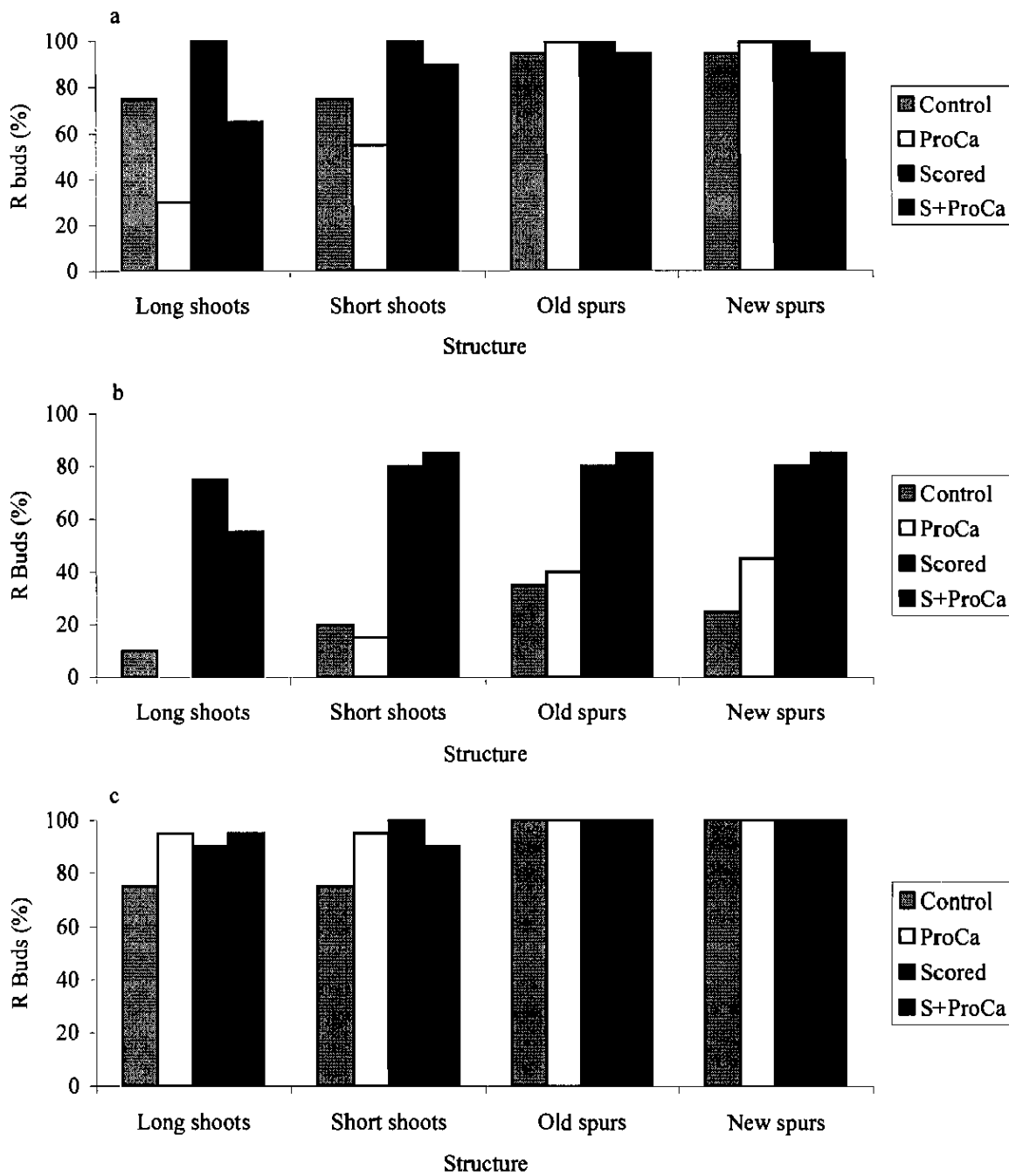


Fig. 1. Percentage reproductive buds (R Buds) on long shoots (>100 mm), short shoots (30-100 mm), new spurs and old spurs on (a) 'Royal Gala', (b) 'Fuji' and (c) 'Cripps' Pink' in response to scoring, prohexadione-calcium (ProCa) and a combination of scoring and ProCa (S+ProCa) at an equal crop load.

7. The effect of timing of scoring on yield, fruit and shoot growth and reproductive bud development on 'Royal Gala' Fuji' and 'Cripps' Pink' apple trees

Abstract

The effects of scoring of apple tree trunks at 1, 2, 4, 6 or 8 weeks after full bloom (WAFB) were investigated with respect to fruit and shoot growth, yield and return bloom. Scoring 1 and 2 WAFB appeared to improve fruit set (higher yield efficiency) on 'Royal Gala', but not on 'Fuji' or 'Cripps' Pink'. The high crop load on 'Royal Gala' trees scored 1 WAFB resulted in smaller fruit size during the second harvest (main crop) compared to the other treatments. Final extension shoot length was reduced only on scored 'Fuji' trees (all scoring times) compared to control trees. Final fruit size was improved on 'Fuji' (although not statistically significant) and 'Cripps' Pink' trees scored 2 or 4 WAFB compared to the control and other scoring treatments. Scoring improved reproductive bud development on all cultivars and on all bearing positions. On 'Royal Gala' and 'Cripps' Pink', the efficiency of scoring to stimulate reproductive bud development on old and new spurs declined after 4 WAFB. In 'Fuji', scoring later than 1 WAFB led to a decreasing positive response on old and new spurs. In contrast, reproductive bud development on long shoots increased with later scoring (6 and 8 WAFB) on all cultivars. It seems that the most beneficial time of scoring is 2-4 WAFB, as early as possible during the cell division stage of fruit growth, but not before natural drop has occurred, and as soon as possible after the cessation of bud elongation.

Keywords. Apple, fruit size, reproductive buds, shoot growth, timing of scoring, yield efficiency.

7.1 Introduction

Modern high-density orchard planting systems must achieve high early yields, high sustained yield and excellent fruit quality in order for the producer to survive financially (Robinson, 2003). This can be done by managing crop load and effectively restricting vegetative growth in vigorous apple cultivars in order to achieve a balance between vegetative and reproductive development (Goren et al., 2004; Wünsche and Ferguson, 2005).

Girdling or scoring are effective techniques to reduce vegetative growth, promote flowering, improve fruit set, increase fruit size and advance maturity in apples and pears (Dennis, 1968), peaches and nectarines (Dann et al., 1984), grapevines (Winkler et al., 1974), olives (Lavee et al., 1983) and several other species (Grierson et al., 1982; Goren et al., 2004). However, very little is known about the timing of this manipulation to achieve these goals.

The growth rate of the developing fruit depends not only on whole-tree assimilate production but also on how successfully the fruit can compete with other sinks (Wünsche and Ferguson, 2005). Fruit size at harvest can be viewed as the result of a combination of cell number, determined during the early developmental stage of cell division, cell size and volume of intercellular space (Goffinet et al., 1995). High crop densities and excessive vegetative growth during the early growth period of fruit cell division may cause a deficit in carbohydrate availability to the developing fruit which can lead to decreased fruit growth rate and reduced final fruit size (Lakso, 1994). Early scoring will thus increase fruit set due to better carbohydrate availability to the fruitlets, leading to decreased individual fruit growth rates and final fruit size due to increased competition between fruitlets before hand thinning. Late scoring (after cell division) would have very little effect on final fruit size as it would be too late to improve carbohydrate availability to the fruit during the cell division stage of fruit growth.

The promotion of flowering by scoring may be due to an increase in carbohydrates beyond a threshold level in the canopy of girdled trees (Goldschmidt et al., 1985). However, scoring also interferes with the transport of auxins and other plant growth regulators (Cutting and Lyne, 1993). The increase in flower bud formation can thus not solely be attributed to an increase in carbohydrate levels in the canopy of the scored tree (Goren et al., 2004).

The objective of this study was to investigate the effects of scoring at different times after full bloom, with respect to fruit growth and quality, yield and return bloom. We hypothesised that early scoring (1 week after full bloom) would enhance initial fruit set and late scoring (8 weeks after full bloom) would have little impact on final fruit size and that reproductive bud development would be enhanced by scoring.

7.2 Materials and methods

This study was conducted in the same commercial 'Royal Gala', 'Fuji' and 'Cripps' Pink' apple orchards (Nooitgedacht Farm) in the Koue Bokkeveld (Ceres) region, (33°13'S, 19°02'E, 975 m above sea level) (Western Cape, South Africa) described in Chapter 4, during 2001/02. The trial was laid out as a randomised complete block design with ten blocks and single tree replications. Treatments used were an un-scored control and a single trunk scoring done at 1, 2, 4, 6 and 8 weeks after full bloom (WAFB). Scoring was done with a girdling tool (Cape Agricultural Products, Somerset West, South Africa), cutting a complete circle around the trunk, through the phloem (no removal of the phloem), below the bottom scaffold branches. Chemical thinning was performed as described in Chapter 4. All trees were hand thinned to commercial crop load ('Royal Gala' 30 and 60 days after full bloom (DAFB), 'Fuji' and 'Cripps' Pink' 54 DAFB) ('Royal Gala', 55 ton ha⁻¹, 'Fuji' and 'Cripps' Pink' 70 ton ha⁻¹).

For dates of full bloom, cell division stage and cell enlargement stage of 'Royal Gala', 'Fuji' and 'Cripps' Pink' refer to Chapters 4 and 5. At harvest, random sub-samples of 20 fruit per tree were taken and the fruit individually weighed. Total yield per tree was recorded and expressed as yield cm⁻¹ trunk circumference. The lengths of two (one on each side of the tree) tagged bourse and extension shoots were measured at different times during the season and the shoot growth rate calculated in mm day⁻¹. During the following winter, microscopic bud analysis was performed on long shoots (>100 mm), short shoots (30-100 mm) old spurs and new spurs to determine the percentage reproductive buds. Two buds per structure were collected from every tree and the samples pooled, so that statistical analysis was not possible.

All fruit and shoot data were analysed by ANOVA (SAS Enterprise Guide release 1.3) to test the significance ($P > 0.05$) of treatment effects. Linear, quadratic and cubic orthogonal polynomial contrasts were used to analyse the response to timing of scoring.

7.3 Results

There was a linear decrease in total yield efficiency of 'Royal Gala' with time of scoring after full bloom, but no significant difference in yield efficiency between scored and control trees (Table 1). There were no significant differences in yield efficiency between scoring treatments or between scored and control trees in 'Fuji' and 'Cripps' Pink' (Table 1), although scored 'Cripps' Pink' trees generally had higher yield than control trees (non-significantly). Fruit fresh mass of scored 'Royal Gala' trees (harvest 2) was lower than controls after scoring at 1 WAFB, comparable with controls after scoring at 2, 4 and 8 WAFB, and slightly lower than controls after scoring at 6 WAFB, as indicated by the significant cubic response to timing of scoring (Table 2). There were no significant differences in fruit fresh mass between scored and control trees on both harvest dates (Table 2). Fruit fresh mass of 'Cripps' Pink' trees peaked after scoring at 2 WAFB after which there was a decline as indicated by the significant cubic response to timing of scoring (Table 2). Fruit fresh mass of scored 'Cripps' Pink' trees was significantly higher compared to control trees. There were no significant differences in fruit fresh mass between scoring treatments or between scored and control trees in 'Fuji' (Table 2), although trees scored 2 or 4 WAFB appeared to have the largest fruit (non-significant).

There were no significant differences between scoring treatments or between scored and control trees in final extension shoot lengths of 'Royal Gala' or 'Cripps' Pink' (Table 3). Final extension shoot lengths of scored 'Fuji' trees were significantly shorter compared to unscored control trees (Table 3). There were no significant differences between treatments in final bourse shoot lengths for any of the cultivars (data not shown).

Scoring favoured reproductive bud development in all the cultivars (Fig. 1). Scoring 1, 2 or 4 WAFB had the best overall effect on reproductive bud development in 'Royal Gala' (Fig. 1a). Reproductive bud development on long and short shoots improved equally well on trees scored later than 4 WAFB as on trees scored before 4 WAFB, but the response was less positive after 4 WAFB on old and new spurs (Fig. 1a). In 'Fuji', scoring at 1 WAFB led to the best reproductive bud development on short shoots, new and old spurs (Fig. 1b). Reproductive bud development on long shoots was the best on trees scored 8 WAFB (Fig. 1b). In 'Cripps' Pink', trees scored 1, 2 or 4 WAFB showed the best overall reproductive bud development on

short shoots, new and old spurs (Fig 1c). Reproductive bud development on long shoots was again the best on trees scored 8 WAFB (Fig. 1c).

7.4 Discussion

The use of girdling/scoring as a horticultural technique requires consideration of tree age, health and vigour, as well as growing conditions (Goren et al., 2004). Under South African conditions, scoring could be used to improve fruit set, increase fruit size and quality, ensure good return bloom and reduce vigour. Fruit size can be improved through cultural manipulations which shift the allocation of available carbohydrates from vegetative to reproductive growth (Corelli Grappadelli et al., 1994), provided that this increase in carbon availability to the fruitlets does not improve set to the extent that between-fruit competition reduces individual fruit growth rates.

Simulated carbohydrate supply and demand curves for apple trees through the season show that carbon supply for fruit development can be potentially limiting two to four weeks after full bloom, when carbohydrate demand of the rapidly growing crop is high (Lakso et al., 1999). At this stage, the vegetative development of extension and bourse shoots appears to have priority over reproductive development, thus negatively affecting fruit set and fruit growth by cell division (Bepete and Lakso, 1998). Scoring leads to the accumulation of carbohydrates in the canopy and thus provides a rich source of energy for all stages of reproductive development (Goren et al., 2004). Scoring 1 and 2 WAFB seems to have improved set (higher yield efficiency) on 'Royal Gala', but not on 'Fuji' or 'Cripps' Pink'. The higher crop load on the 'Royal Gala' trees scored 1 WAFB resulted in reduced fruit size during harvest 2 compared to the control trees and trees scored 2 and 4 WAFB. On trees that normally set well, an early scoring will reduce the natural drop by temporarily increasing carbohydrate supply in the canopy. This higher crop load, although adjusted later in the season via hand thinning, will affect fruit growth rates negatively since this depends not only on whole-tree assimilate production but also on how successfully the fruit can compete with other sinks (Wünsche and Ferguson, 2005).

Final extension shoot length was reduced only on scored 'Fuji' trees compared to control trees. A reduction in vigour can have added benefits such as better fruit quality (blush

colour development), improved yields, better pest control through better spray penetration and improved light penetration leading to better return bloom (Greene, 1999). Excessive vegetative growth is also a major concern for the producer because it competes with fruit growth (Forshey and Elfving, 1989).

Fruit size at harvest can be viewed as the result of a combination of cell number, determined during the early developmental stage of cell division, cell size and volume of intercellular space (Goffinet et al., 1995). Minchin et al. (1997) found that when assimilate supply to a fruit was increased by removing other nearby competing fruits, there was a three-fold increase in the amount of carbohydrates partitioned to the fruit within one day. Scoring, which increases carbohydrate levels in the canopy (Goren et al., 2004), would thus also increase the expression of catabolic enzymes and thus increase the sink strength of the fruit, leading to increased carbon allocation to the fruit and increased fruit growth rates. In this study, final fruit size was improved on 'Fuji' (non-significantly) and 'Cripps' Pink' trees scored 2 or 4 WAFB compared to the control and other scoring treatments. It seems that the strengthening of the fruit growth sink due to scoring is most beneficial if scoring is performed as early as possible during the cell division stage of fruit growth. Care should, however, be taken that the accumulation of assimilates due to scoring does not increase fruit set and therefore increase competition between individual fruits.

Scoring improved reproductive bud development on all cultivars, probably due to an increase in assimilate supply. For this response to occur effectively, scoring should be performed as soon as possible after the elongation of the structure has stopped. On 'Royal Gala' and 'Cripps' Pink', the efficiency of scoring to stimulate reproductive bud development on old and new spurs declined after 4 WAFB. In 'Fuji' scoring later than 1 WAFB led to a decreasing positive response in reproductive bud development on old and new spurs. In contrast, the reproductive bud development on long shoots increased with later scoring (6 or 8 WAFB) on all cultivars. It has been shown in Chapters 5 and 6 that prohexadione-calcium which acts as a GA biosynthesis inhibitor (Rademacher, 1995), thus reducing gibberellin levels in the plant, did not have a positive effect on reproductive bud development, whereas scoring, which improves assimilates in the canopy, had a positive effect. It would seem as though it is not so much the high levels of gibberellins in the tree due to a high crop load that

inhibits reproductive bud development, but rather a limited supply of carbohydrates in the canopy following cessation of elongation of the structure.

Scoring temporarily increases the availability of assimilates in the canopy, which increases the strength of the fruit growth sink. This increase can be positive (improved set, fruit size and quality) or negative (too many fruit) if done very early. Recommendations for optimum timing of scoring would thus have to be orchard- and cultivar-specific depending on the goals that are to be achieved. The interaction between the responses of plant growth regulators and carbohydrate supply levels in the tree warrants further investigation.

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Table 1. Yield efficiency (kg cm^{-1} trunk circumference) of 'Royal Gala', 'Fuji' and 'Cripps' Pink'. Trees were scored 1, 2, 4, 6 or 8 weeks after full bloom (WAFB).

	'Royal Gala'	'Fuji'	'Cripps' Pink'
1 WAFB	0.84	1.26	1.43
2 WAFB	0.82	1.46	1.22
4 WAFB	0.77	1.38	1.32
6 WAFB	0.57	1.18	1.35
8 WAFB	0.60	1.40	1.60
Control	0.67	1.44	1.16
	Pr > F		
Scoring vs. Control	0.5300	0.4606	0.1097
Linear	0.0033	0.9272	0.1750
Quadratic	0.8101	0.8427	0.1207
Cubic	0.3229	0.0975	0.7434

Table 2. Individual fruit mass (g) of 'Royal Gala', 'Fuji' and 'Cripps' Pink'. Trees were scored 1, 2, 4, 6 or 8 weeks after full bloom (WAFB).

	'Royal Gala'		'Fuji'	'Cripps' Pink'
	Harvest 1	Harvest 2		
1 WAFB	125.0	112.9	165.5	178.8
2 WAFB	124.4	123.0	175.4	200.6
4 WAFB	128.5	123.9	175.0	191.5
6 WAFB	120.6	116.3	170.1	185.9
8 WAFB	127.6	120.3	168.9	177.9
Control	124.2	122.6	167.5	178.7
	Pr > F			
Scoring vs. Control	0.8308	0.3494	0.4392	<i>0.0095</i>
Linear	0.9060	0.5977	0.9198	<i>0.0440</i>
Quadratic	0.7202	0.2038	0.1977	<i>0.0029</i>
Cubic	0.2955	<i>0.0129</i>	0.2899	<i>0.0119</i>

Table 3. Final extension shoot length (mm) of 'Royal Gala', 'Fuji' and 'Cripps' Pink'. Trees were scored 1, 2, 4, 6 or 8 weeks after full bloom (WAFB).

	'Royal Gala'	'Fuji'	'Cripps Pink'
1 WAFB	438	396	379
2 WAFB	415	491	429
4 WAFB	510	600	335
6 WAFB	363	466	387
8 WAFB	428	572	408
Control	398	727	378
	Pr > F		
Scoring vs. Control	0.5366	0.0018	0.8512
Linear	0.6160	0.1267	0.9342
Quadratic	0.7379	0.3626	0.4594
Cubic	0.2654	0.0847	0.7385

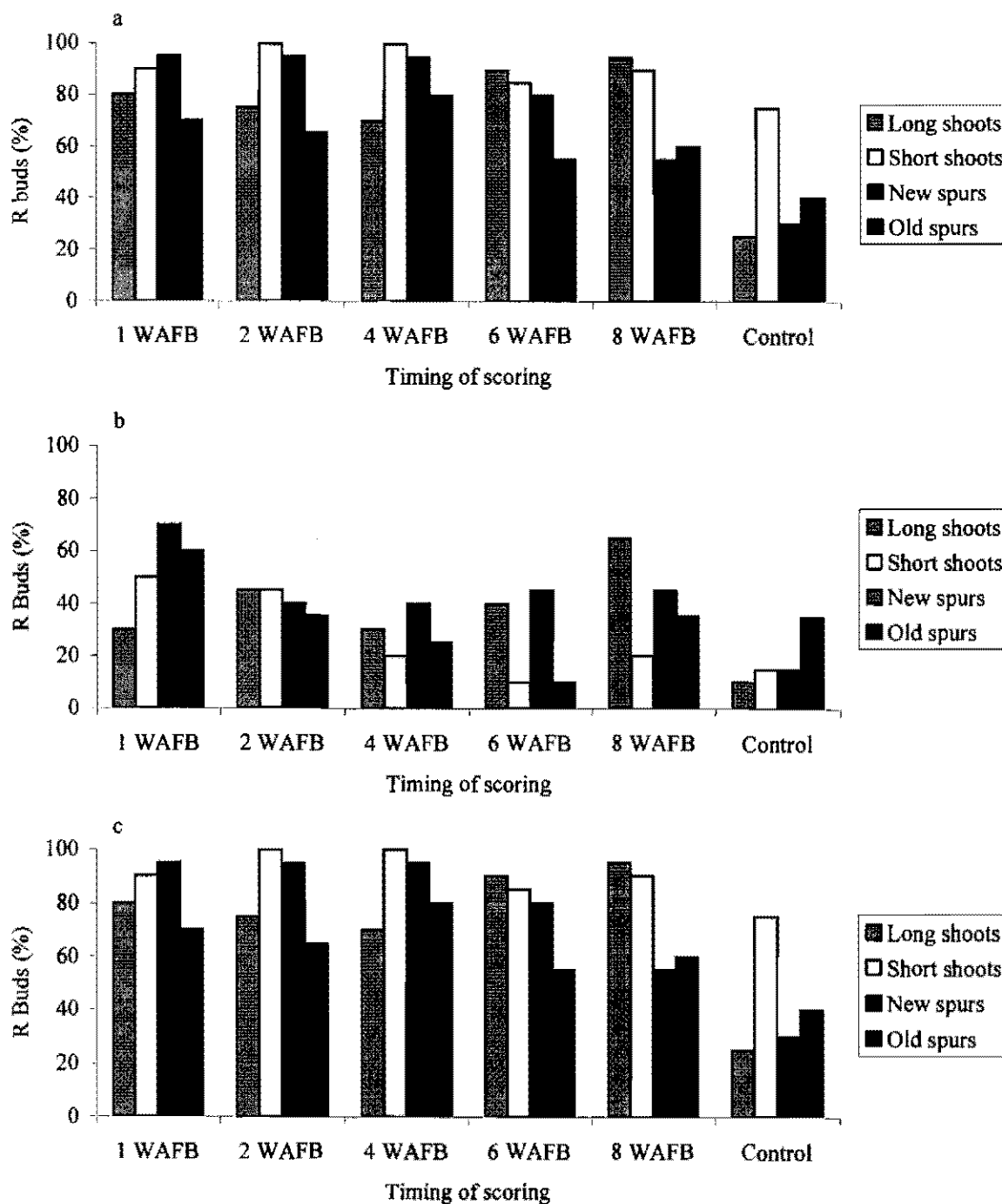


Fig. 1. Percentage reproductive buds (R Buds) on the different structures (long shoots >100 mm; short shoots 30-100 mm; new spurs and old spurs) on a) 'Royal Gala', b) 'Fuji' and c) 'Cripps' Pink' after the 2001/02 season. Trees were scored 1, 2, 4, 6 or 8 weeks after full bloom (WAFB) or not scored (control).

8. The effect of temperature and developmental stage on carbon dioxide exchange of attached 'Royal Gala', 'Fuji' and 'Cripps' Pink' apple fruits

Abstract

In the Western Cape region of South Africa post-bloom spring temperatures can be high, resulting in high respiration rates during a period when carbohydrate supply can be potentially limiting. Chlorophyllous apple flesh tissues exposed to light are capable of assimilating CO₂ diffusing through stomata or lenticels, or re-fixing CO₂ lost through respiration. This could represent a significant contribution to the carbon balance of growing fruits. In this study, light-saturated net CO₂ assimilation rate (A_{max}), dark respiration rate (R_d), and light-saturated photosynthetic rate (P_{max} , the difference between A_{max} and R_d), were measured at different fruit surface temperatures on attached 'Royal Gala', 'Fuji' and 'Cripps' Pink' apple fruits in the Ceres and Elgin regions (Western Cape, South Africa). Measurements were performed during the cell division stage (20-30 days after full bloom, DAFB) and during the cell enlargement stage (50-60 DAFB) of fruit growth. In all cultivars, P_{max} and R_d increased, and A_{max} decreased with increasing fruit surface temperature. After the cell division stage, P_{max} and R_d decreased and A_{max} increased with increasing fruit fresh weight. 'Fuji' fruit reached compensation point (A_{max} = positive due to P_{max} exceeding losses by R_d) during the mid-season (55 DAFB) at temperatures of up to 30°C, but A_{max} remained negative at 35°C. 'Royal Gala' almost reached compensation at 20 and 25°C (53 DAFB), but A_{max} remained negative at 30 and 35°C. The rapid increase in dark respiration rate with increasing temperatures during the cell division stage of fruit growth creates a high demand for assimilates and could have serious implications for fruit growth and final size in warm climates such as the Western Cape.

Keywords: Apple, carbon balance, cell division stage, compensation point, fruit growth, fruit photosynthesis, heat stress.

8.1 Introduction

Every year 10-50 tons per hectare of apple fruit are harvested from orchards worldwide. This corresponds to 400-2000 kg of carbon per hectare originating from photosynthesis, both of the leaves and fruit. This does not mean that high net photosynthetic rates will necessarily result in high yields or large fruit size, since in deciduous fruit trees the acquired carbon must also be partitioned efficiently to growing fruit and developing flower buds which compete with growing shoots (Forshey and Elfving, 1989; Lakso, 1994). Source-sink relationships and the regulation of carbon allocation to reproductive processes therefore strongly influence yields and fruit size. Fruit exposed to light are capable of photosynthesis and contribute a portion of their carbohydrate requirements (Jones, 1981), thus potentially alleviating sub-optimal allocation from leaves during critical developmental periods. An increased understanding of the role of fruit photosynthesis in the carbon balance, and the factors which influence it, is essential when trying to improve yield and fruit size (Pavel and DeJong, 1993b).

The presence of chloroplasts and mitochondria within the apple fruit has been demonstrated by microscopic observations (Rhodes and Wooltorton, 1967). The chloroplasts are only found in the green tissue of the hypodermal and inner perivascular tissue. The latter comprises the four to five layers of cells surrounding the vascular bundle. In young apple fruit, stomata are the preferred sites of CO₂ exchange due to their low diffusive resistance to CO₂, and they largely regulate the rate of CO₂ exchange. During fruit ontogenesis, stomatal density decreases with expanding surface area, and during mid-season the stomata are transformed into lenticels which have a higher diffusive resistance (Blanke, 1987). Gas exchange may also take place through the cuticle, especially in young fruit. During fruit growth, wax is secreted at a faster rate than the expansion of the surface area (Blanke and Lenz, 1989) thus increasing the amount of wax per unit area. These wax constituents obstruct gas diffusion, therefore the overall permeability of the fruit cuticle decreases with age. Apple fruit are also capable of re-fixing CO₂ lost internally through respiration (Jones, 1981).

The seasonal pattern of apple fruit growth is defined by an initial 35-50 day period of exponential growth following fertilisation, coinciding with rapidly increasing fruit cell numbers. This phase is followed by a more or less linear growth phase, during which fruit size increases predominately through cell expansion (Palmer et al., 2003). Fruit size is determined by the combination of cell number, cell size and percentage intercellular space (Goffinet et al.,

1995). Pearson and Robertson (1953) stated that the variation in fruit size is determined early in development by the number of cell divisions (cell number), and that within one season cell volume in individual fruit of the same age is fairly uniform. Jones (1981) found that fruit dark respiration rate per unit fresh weight is highest during the cell division stage of fruit growth. The respiration rate, comprising maintenance and growth respiration, shows an exponential increase with increasing temperature (Lakso, 1994). Fruit growth during the cell division stage is very temperature sensitive due to a higher growth respiration component (Warrington et al., 1999).

Fruit respiration utilises much of the carbohydrate produced during fruit photosynthesis. The compensation point refers to the situation where there is no net exchange of CO₂ with the atmosphere, since photosynthetic CO₂ exchange rate equals respiration CO₂ exchange rate (Willmer and Johnson, 1976). In physiological experiments under standard laboratory conditions, apple fruits were not capable of reaching compensation point (Jones, 1981). However, peach fruits have been reported to reach compensation point under specific conditions *in situ* (Pavel and DeJong, 1993a).

The CO₂ exchange of developing apple fruit thus appears to be largely affected by developmental stage and temperature, assuming that light conditions are not limiting. In the Western Cape region, post-bloom spring temperatures can be high (Fig. 1). This could result in potentially high respiration rates during a period when carbohydrate supply can be limiting due to competition between a large number of actively growing fruitlets and shoots (Lakso et al., 1999), reserve depletion, and incomplete canopy development and leaf photosynthetic machinery. In Chapter 3 it was shown that at 20 DAFB, the maximum rate of electron transport (J_{max}) and light-saturated net CO₂ assimilation rate (A_{max}) of leaves were substantially lower, and dark respiration rate higher than at 50 DAFB. Supply of carbohydrates from leaves is thus not yet optimal during this early period and the contribution by fruit potentially important. There are few published reports of *in situ* apple fruit CO₂ exchange and the conditions which influence it, and none under South African conditions. We suspect that this factor may play a significant role in the small fruit problem experienced for some cultivars.

The aim of this experiment was to study the effects of temperature and developmental stage on the CO₂ exchange rate of attached 'Royal Gala', 'Fuji' and 'Cripps' Pink' fruit under South African conditions.

8.2 Materials and methods

During 2000/01, the study was conducted in the Elgin region (34°10'S, 19°05'E, Western Cape, South Africa), on a commercial 'Royal Gala' orchard. Trees on seedling rootstock were planted in 1992, spaced 4.5 x 1.5 m and trained to a central leader (see Chapter 3). During 2001/02 and 2002/03, commercial 'Royal Gala', 'Fuji' and 'Cripps' Pink' apple orchards were used in the Koue Bokkeveld (Ceres) region (33°13'S, 19°02'E). Trees on M793 rootstock were planted in 1996, spaced 4.5 x 1.25 m and trained to a central leader (see Chapter 4). Commercial orchard management practices were followed. Spring weather data for the past five seasons (1-8 weeks after full bloom) were obtained from the Two-a-Day meteorological station in Elgin and the De Keur station in the Koue Bokkeveld (Fig. 1).

In the Elgin region during 2000/01, measurements were done on trees that were part of a larger scoring and crop load trial (Chapter 3). This trial was laid out as a randomised complete block design with ten single tree replications. Measurements were taken on well-exposed spur fruit on scored trees thinned to one fruit per cluster or one fruit every second cluster (five trees for each thinning treatment). Since there were no differences in results between fruit on trees thinned to different crop loads, these data were pooled. Measurements of net CO₂ assimilation rate (*A*) of attached fruits were made 30 days after full bloom (DAFB) using a LI-6400 portable photosynthesis system (Li-Cor, Lincoln, Nebraska, USA) with the conifer chamber attachment (LI-6400-05, Li-Cor, Lincoln, Nebraska, USA). The system uses the equations derived by Von Caemmerer and Farquhar (1981) to calculate net CO₂ assimilation rate (*A*), transpiration rate (*E*) and stomatal conductance (*g_s*). Energy balance equations were used to calculate fruit surface temperature and this was controlled electronically at 20, 25, 30 or 35°C. Ten replicates were measured at each temperature, using different fruit at each temperature. Since the conifer chamber does not have its own light source, incident light levels (full sunlight) were used and PPF (photosynthetic photon flux density) measured using a quantum sensor (LI-190SA, Li-Cor, Lincoln, Nebraska, USA) attached to the conifer chamber. Layers of shade cloth were used to block out progressively more light and finally a black photographic cloth was used to block out all light in the chamber to determine dark respiration rate (*R_d*) of the fruit. Cuvette CO₂ concentration was controlled at 380 μmol mol⁻¹ using the CO₂ injection system (LI-6400-01, Li-Cor, Lincoln, Nebraska, USA) and compressed CO₂-cylinders.

For the 2001/02 trials in the Ceres region, measurements of attached 'Royal Gala' 'Fuji' and 'Cripps' Pink' fruit were made 25-35 DAFB and again 53-55 DAFB. During 2002/03, measurements were made 33-35 DAFB. Measurements were made on trees which were part of a trial testing scoring and prohexadione-calcium, combined with different crop load levels, and laid out as a randomised complete block design with ten blocks (see Chapter 4). Well-exposed spur fruit from scored trees thinned to a crop load of 50 ton ha⁻¹ were measured. During the measurements made 25-35 DAFB, fruits were small enough to fit into the 2 x 3 cm opaque leaf cuvette fitted with an internal red/blue LED light source (LI-6400-02B, Li-Cor, Lincoln, Nebraska, USA). Irradiances were controlled at 1500, 1000, 800, 600, 400, 200, 100 and 0 $\mu\text{mol m}^{-2} \text{s}^{-1}$ photosynthetic photon flux density (PPFD). Cuvette CO₂ concentration was controlled at 380 $\mu\text{mol mol}^{-1}$. During measurements made at 53-55 DAFB the conifer chamber attachment was used as describe above. Five replicates were measured at each temperature (different fruit).

Sixty fruit were randomly harvested on a weekly basis from trees in a row adjacent to the experimental rows in the same orchards during all three seasons, their equatorial diameters measured and the fresh weight determined. The fresh weight-diameter relationship was fitted to a power function, which was then used to determine the fresh weight of the measured fruit from the fruit diameter. CO₂ exchange rates were calculated on a fresh weight basis.

Although light response curves were performed, only light-saturated net CO₂ assimilation rate (A_{max}), dark respiration rate (R_d), and light-saturated photosynthetic rate (P_{max} , the difference between A_{max} and R_d), are reported. Data were analysed by one-way ANOVA (SAS v. 6.12, SAS Institute, Cary, NC, USA) to test the significance ($\text{Pr} \leq 0.05$) of temperature effects. Fruit weight were used as covariate. Multiple comparison tests (Tukey, $\text{Pr} \leq 0.05$) were also performed to separate means.

8.3 Results

Overall, higher temperatures resulted in lower A_{max} and higher P_{max} and R_d , expressed on a fruit fresh weight basis, for all cultivars and developmental stages (Tables 1, 2, 3, Fig. 2). With an increase in fruit fresh weight there is an increase in A_{max} and a decrease in P_{max} and R_d for all cultivars at all temperatures (Tables 1, 2, 3, Fig. 3).

'Royal Gala' fruit with an average fruit weight of 2.0 g had a significantly higher A_{\max} at 20 and 25°C than at 30 and 35°C during 2001/02. At this stage there were no significant differences in P_{\max} at different temperatures, while at 20°C R_d was significantly lower than at 30 and 35°C (Table 1). During 2002/03, fruit with an average weight of 3.2 g had significantly lower A_{\max} at 35°C compared to 20 and 25°C, while P_{\max} and R_d were significantly higher at 30 and 35°C compared to 20 and 25°C (Table 1). During 2000/01, P_{\max} and R_d of fruit with an average weight of 8.8 g were significantly lower at 20 and 25°C compared to 30°C, which was in turn significantly lower than at 35°C. There were no significant difference in A_{\max} between different temperatures (Table 1). During 2001/02, A_{\max} of fruit with an average weight of 20.1 g were significantly higher at 20 and 25°C than at 35°C, while P_{\max} and R_d were significantly higher at 35°C compared to lower temperatures (Table 1).

During 2001/02, P_{\max} and R_d of 'Fuji' fruit with an average weight of 2.4 g were significantly lower at 20°C compared to 25 and 30°C, while at 30°C A_{\max} was significantly lower than at 20°C (Table 2). During 2002/03, P_{\max} and R_d of fruit with an average weight of 3.0 g were significantly lower at 20 and 25°C compared to 30 and 35°C, while at 35°C A_{\max} was significantly lower than at 20 and 25°C (Table 2). During 2001/02, A_{\max} of fruit with an average weight of 17.5 g was significantly lower at 35°C compared to 20°C. R_d and P_{\max} were significantly higher at 35°C compared to 30°C, and significantly higher at 30°C than at 20°C (Table 2).

Temperatures ranging from 20-30°C did not have a significant effect on A_{\max} , P_{\max} or R_d of 'Cripps' Pink' fruit with an average weight of 2.9 g during 2001/02 (Table 3). During 2002/03, A_{\max} of fruit with an average weight of 3.5 g was significantly lower at 35°C compared to 20°C, while P_{\max} was significantly lower at 20°C than at 25°C, which was in turn significantly lower than at 30°C. At this stage, R_d at 20 and 25°C was significantly lower than at 35°C (Table 3). During 2001/02, P_{\max} and R_d of fruit with an average weight of 16.5 g were significantly lower at 30°C compared to 35°C, while there was no significant difference in A_{\max} between these two temperatures (Table 3).

Since temperature and developmental stage were the most important determinants of A_{\max} , P_{\max} and R_d , results from all cultivars, regions and seasons were plotted against temperature (Fig. 2) and fresh fruit weight (Fig. 3) to illustrate trends. R_d and P_{\max} increased with an increase in temperature across all cultivars and regions (Fig. 2 a,b). The gradient of this increase was apparently larger for smaller fruit (< 5 g, during the first 40 DAFB)

compared to larger fruit (Fig. 2 a,b). The opposite trend was observed for A_{\max} , and only larger fruit (> 15 g) were capable of approaching compensation point ($A_{\max} = 0$) (Fig. 2c).

There was a weight-specific decrease in R_d and P_{\max} with an increase in fruit fresh weight across all cultivars and regions (Fig. 3 a,b). Fruit larger than 15 grams came close to compensation point at temperatures lower than 30°C. In smaller fruit (< 5 g) an increase in temperature resulted in a sharp decrease in A_{\max} (Fig. 3c). R_d , P_{\max} and A_{\max} of larger fruit (> 10 g) were less temperature sensitive than smaller fruit (Fig. 3 a,b,c).

8.4 Discussion

'Royal Gala', 'Fuji' and 'Cripps' Pink' apple fruit responded to increasing temperatures with increasing light-saturated photosynthetic and dark respiration rates, but decreasing net CO_2 exchange rates. This was similar to what Jones (1981) found for 'Golden Delicious' apple fruit and what Pavel and DeJong (1993a) found for developing peach fruit. The continued increase in light-saturated photosynthetic rate of the fruit with an increase in temperatures up to 35°C could be attributed to various factors. The generally low conductance of CO_2 through stomata, lenticels and the epidermis in apple fruit surfaces (in comparison with thin leaves) probably leads to a build-up of internal CO_2 concentration with an increase in temperature, generated by the temperature-sensitive increase in dark respiration rate (Blanke and Lenz, 1989). This is similar to what Pavel and DeJong (1993a) found for developing peach fruits. Higher internal CO_2 concentrations would favour the carboxylating reaction of rubisco. In addition, the enzymatic reactions associated with the dark reactions of photosynthesis only become negatively affected above about 40°C in most chlorophyllous tissues (Björkman et al., 1980).

There appeared to be differences between cultivars in the efficiency of photosynthesis compared to respiration. 'Fuji' fruit reached compensation point during the mid-season period (55 DAFB) at temperatures of up to 30°C (Table 2), whereas 'Royal Gala' did not reach compensation point even though the A_{\max} values were close to zero (Table 1). We could not compare 'Cripps' Pink' due to the absence of data at milder temperatures (20 and 25°C, Table 3) when A_{\max} is at its highest. 'Golden Delicious' fruit used by Jones (1981) also did not reach compensation point. However, Pavel and DeJong (1993a) reported that during mid-season under optimal conditions, peach fruit were able to reach compensation point. A shaded peach fruit could contribute 5% of its total seasonal carbohydrate requirement and an exposed fruit

up to 9% (Pavel and DeJong, 1993b). This contribution is, however, clearly temperature dependent, and our data suggest that at temperatures above 25°C high respiratory losses could reduce the fruit contribution substantially.

During the cell division stage of fruit growth (first \pm 40 DAFB), R_d was very high on a fresh weight basis but declined rapidly as the fruit grew. According to Warrington et al. (1999) this is the stage of fruit growth during which temperature has the largest effect on fruit growth and final fruit size. It is the period during the season when carbohydrate supply to the fruit can be potentially limiting due to competition between a large number of actively growing fruitlets and shoots (Lakso et al., 1999), reserve depletion, and incomplete canopy development and leaf photosynthetic machinery. High temperatures during this period, particularly at night, would place additional strain on the limited carbohydrate supply in the trees, which could severely limit final yield and fruit size.

In both the Elgin and Ceres production areas, average minimum temperatures lie between 10 and 17°C, with the Elgin area being warmer than the Ceres area (Fig. 1). Average maximum air temperatures are between 20 and 27°C during the cell division stages of fruit growth, rarely being limiting to fruit set and initial growth in the lower range (Warrington et al., 1999). However, temperatures of more than 30°C are encountered (Fig. 1). Exposed fruit could easily experience fruit surface temperatures of 35°C or higher under these conditions (Thorpe, 1974). This means that exposed fruit in warm spring climates such as experienced in the Western Cape would have a high respiration rate, especially during the cell division stage (Lakso, 1994), with resulting negative impacts on whole-tree net carbohydrate availability (Francesconi et al., 1997) and carbon supply to the fruit. This could place severe restrictions on fruit growth rates during this period and account for the small fruit problem of some apple cultivars in this region. In a study by Warrington et al. (1999), increases in daytime temperature of up to 25°C proved beneficial for fruit growth. Unfortunately, higher temperatures were not tested and it seems that temperatures higher than 25°C could affect fruit growth negatively.

The rapid increase in dark respiration rate with increasing temperatures during the cell division stage of fruit growth creates a high demand for assimilates and could have serious implications for fruit growth and final size in warm climates such as the Western Cape. Heat-reducing technologies such as reflective particle film (Surround[®] WP) and evaporative cooling

could reduce fruit temperatures and therefore have a positive effect on whole-tree carbon balance, which might influence fruit size positively.

8.5 References

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Table 1. Light-saturated net CO₂ exchange rate (A_{max}), maximum rate of photosynthesis (P_{max}) and dark respiration rate (R_d) expressed per unit fruit fresh weight (ng CO₂ g fruit fresh weight⁻¹ s⁻¹) for 'Royal Gala' apples at different fruit surface temperatures.

Fruit Temperature	A_{max}	SE	P_{max}	SE	R_d	SE
2001/02 (25 DAFB) average fruit weight 2.0g						
20°C	-13.01 a	7.80	47.16 a	4.51	60.17 b	8.64
25°C	-11.02 a	19.93	68.92 a	22.01	79.94 ab	41.76
30°C	-115.01 b	8.26	136.29 a	7.77	251.29 a	14.25
35°C	-101.23 b	7.84	141.90 a	8.87	243.13 a	5.49
Pr>F	0.0005		0.0561		0.0057	
2002/03 (33 DAFB) average fruit weight 3.2g						
20°C	-23.62 a	11.89	16.34 b	3.81	39.96 b	15.51
25°C	-58.59 a	8.51	44.17 b	2.75	102.75 b	10.77
30°C	-86.95 ab	26.06	60.73 a	15.74	147.69 a	41.33
35°C	-91.77 b	15.65	81.05 a	13.77	172.82 a	28.33
Pr>F	0.0125		0.0002		0.0020	
2000/01 (30 DAFB) average fruit weight 8.8g						
20°C	-8.20 a	1.05	21.18 c	2.68	29.39 c	2.91
25°C	-8.21 a	2.34	27.10 c	2.79	35.31 c	3.17
30°C	-15.96 a	2.14	45.10 b	2.96	61.06 b	3.80
35°C	-15.83 a	3.05	76.96 a	7.09	92.79 a	8.11
Pr>F	0.0237		<.0001		<.0001	
2001/02 (53 DAFB) average fruit size 20.1g						
20°C	-0.20 a	0.34	14.97 b	3.44	15.17 b	3.72
25°C	-0.38 a	0.49	19.69 b	3.04	20.07 b	3.39
30°C	-2.09 ab	0.79	24.88 b	3.16	26.97 b	3.92
35°C	-5.79 b	2.10	37.20 a	4.41	43.00 a	4.79
Pr>F	0.0085		0.0002		<.0001	

Values are means (n=10 (2000/01) or n=5 (2001/02 and 2002/03)) with standard errors (SE). Means within columns followed by the same letter are not significantly different according to Tukey's test ($Pr \leq 0.05$).

Table 2. Light-saturated net CO₂ exchange rate (A_{max}), maximum rate of photosynthesis (P_{max}) and dark respiration rate (R_d) expressed per unit fruit fresh weight (ng CO₂ g fruit fresh weight⁻¹ s⁻¹) for 'Fuji' apples at different fruit surface temperatures.

Fruit Temperature	A_{max}	SE	P_{max}	SE	R_d	SE
2001/02 (25 DAFB) average fruit weight 2.4g						
20°C	-27.12 a	7.41	64.18 b	8.32	91.30 b	4.95
25°C	-35.49 ab	6.00	93.08 a	11.12	128.57 a	13.94
30°C	-45.70 b	7.39	113.10 a	9.98	158.80 a	17.26
Pr>F	0.0575		0.0002		<.0001	
2002/03 (35 DAFB) average fruit weight 3.0g						
20°C	-11.55 a	9.45	25.65 b	5.06	37.19 b	14.10
25°C	-29.25 ab	8.75	42.26 b	5.00	71.50 b	10.92
30°C	-64.77 bc	12.82	74.93 a	8.86	139.70 a	20.77
35°C	-90.29 c	10.56	101.03 a	6.48	191.31 a	12.01
Pr>F	0.0004		<.0001		<.0001	
2001/02 (55 DAFB) average fruit weight 17.5g						
20°C	0.93 a	0.34	12.95 c	0.88	12.02 c	0.59
25°C	0.12 ab	0.45	19.63 bc	2.08	19.51 bc	1.71
30°C	0.40 ab	0.20	24.76 b	1.40	24.36 b	1.41
35°C	-1.13 b	0.63	36.20 a	5.09	37.34 a	5.29
Pr>F	0.0401		<.0001		<.0001	

Values are means (n=5) with standard errors (SE). Means within columns followed by the same letter are not significantly different according to Tukey's test ($Pr \leq 0.05$).

Table 3. Light-saturated net CO₂ exchange rate (A_{\max}), maximum rate of photosynthesis (P_{\max}) and dark respiration rate (R_d) expressed per unit fruit fresh weight (ng CO₂ g fruit fresh weight⁻¹ s⁻¹) for ‘Cripps’ Pink’ apples at different fruit surface temperatures.

Fruit Temperature	A_{\max}	SE	P_{\max}	SE	R_d	SE
2001/02 (33 DAFB) average fruit weight 2.9g						
20°C	-9.22 a	3.95	17.35 a	1.22	26.57 a	3.83
25°C	-12.76 a	4.90	34.40 a	7.59	47.16 a	12.40
30°C	-17.21 a	3.37	42.83 a	8.94	60.04 a	11.55
Pr>F	0.6246		0.0683		0.1421	
2002/03 (35 DAFB) average fruit weight 3.5g						
20°C	-27.96 a	6.71	33.47 c	4.77	61.44 b	8.37
25°C	-44.06 ab	3.89	45.84 b	5.13	89.90 b	7.32
30°C	-76.95 ab	20.70	43.35 bc	1.55	120.30 ab	20.93
35°C	-87.21 b	15.84	92.23 a	10.65	179.44 a	22.32
Pr>F	0.0294		<.0001		0.0005	
2001/02 (55 DAFB) average fruit weight 16.5g						
30°C	-3.19 a	0.93	33.40 b	1.13	36.59 b	1.62
35°C	-3.59 a	1.19	47.55 a	6.44	51.14 a	6.87
Pr>F	0.9935		0.0235		0.0277	

Values are means (n=5) with standard errors (SE). Means within columns followed by the same letter are not significantly different according to Tukey’s test ($Pr \leq 0.05$).

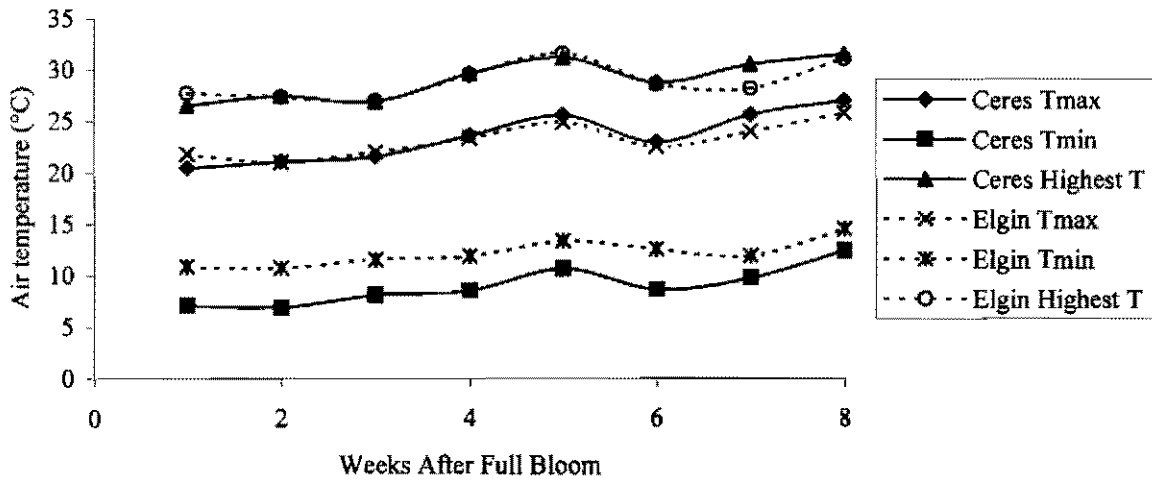


Figure 1. Mean daily minimum (Tmin) and maximum (Tmax) air temperature, and highest air temperature (Highest T) during one to eight weeks after full bloom in the Ceres and Elgin regions for the past five years.

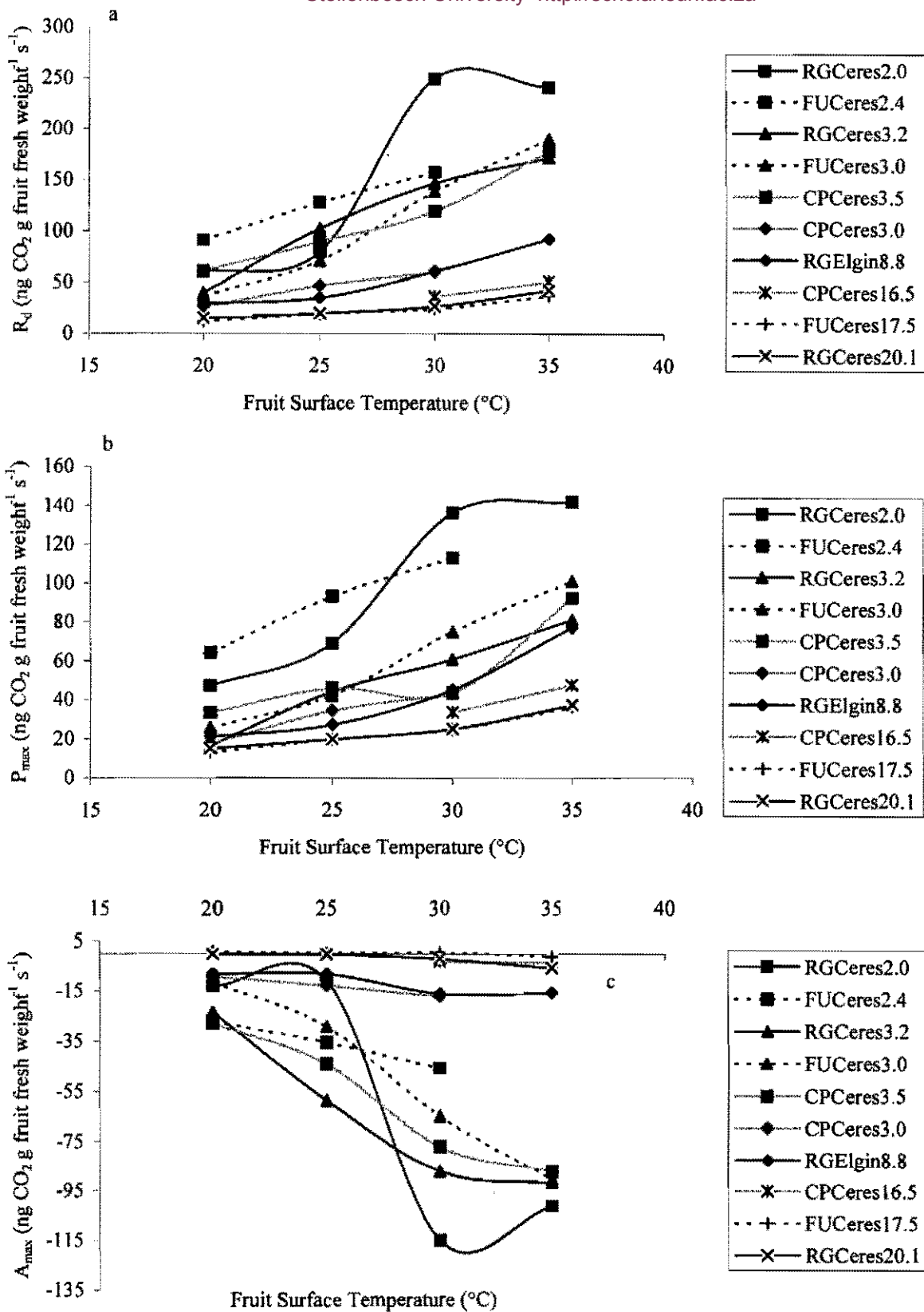


Figure 2. (a) Dark respiration rate (R_d), (b) light-saturated photosynthetic rate (P_{max} , the difference between A_{max} and R_d), and (c) light-saturated net CO₂ assimilation rate (A_{max}), for all cultivars and regions at different fruit surface temperatures. Cultivar, region and mean fruit weight are indicated in the legend. RG = 'Royal Gala'; FU = 'Fuji'; CP = 'Cripps' Pink'.

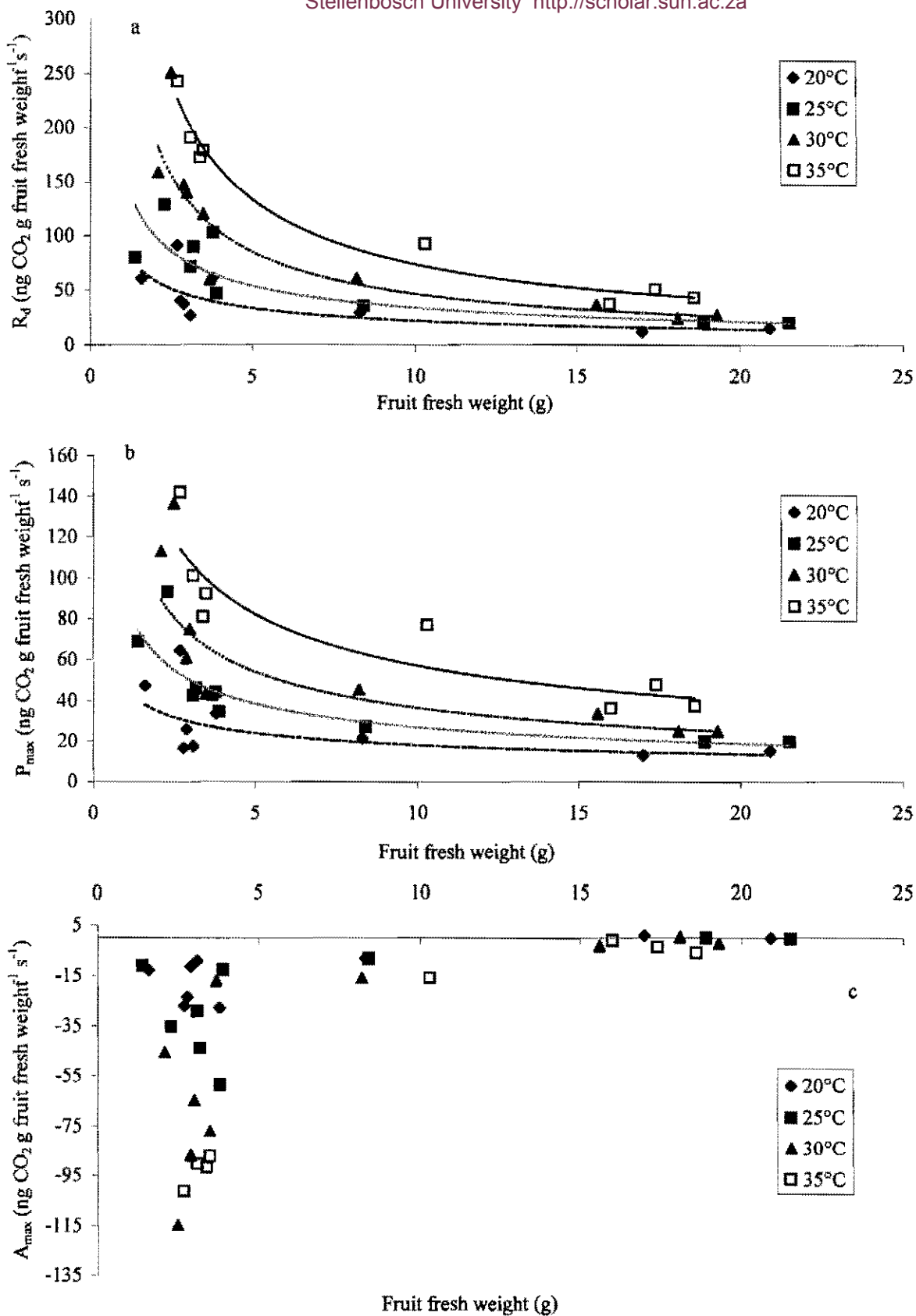


Figure 3. (a) Dark respiration rate (R_d), (b) light-saturated photosynthetic rate (P_{max} , the difference between A_{max} and R_d), and (c) light-saturated net CO₂ assimilation rate (A_{max}), for all cultivars and regions at different fruit surface temperatures, plotted against fruit fresh weight.

9. Late-season stomatal sensitivity to microclimate is influenced by sink strength and soil moisture stress in 'Braestar' apple trees in South Africa

Abstract

In the Western Cape region of South Africa, mild weather during the late season is conducive to continued high rates of photosynthesis in apple trees. During the post-harvest period, both stressful environmental conditions and altered tree carbohydrate demand (sink strength) may influence stomatal function and thus gas exchange and tree water requirements. In this study we investigated the interactive effects of the main atmospheric determinants of stomatal conductance (light, leaf temperature, leaf-to-air vapour pressure deficit [VPD]) in response to fruit removal at harvest and soil moisture stress after harvest (cessation of irrigation). An eight-year-old 'Braestar' orchard was used. Pre- and post-harvest (pre-senescent) photosynthetic light response curves were generated at leaf temperatures of 20-40°C. Stomatal conductance showed increased sensitivity to increasing leaf temperature after harvest, resulting in a reduction in the optimum temperature for photosynthesis from 25-30°C to 20-25°C. This ensured the maintenance of high instantaneous leaf water use efficiency (WUE) post-harvest at all temperatures, compared to reductions in WUE but higher photosynthetic rates at high leaf temperature pre-harvest. We suggest that this reflects a change from a carbohydrate-demanding strategy (high sink demand) pre-harvest, to a water-conserving strategy post-harvest when sink demand is reduced. Following harvest, daily irrigation was either shut off or continued for 11 days, and light-saturated gas exchange measured regularly at varying levels of VPD in droughted trees. Photosynthetic rates, stomatal conductances and intercellular CO₂ concentration (c_i) remained relatively constant for the first 6 days, followed by reductions linked to a period of high ambient atmospheric evaporative demand and subsequent partial recovery. Droughted and non-droughted trees showed similar gas exchange values at the end of the droughting period when measured at 25°C, but gas exchange of droughted trees remained repressed when measured at 30°C due to increased non-stomatal limitation. Throughout the study, only cuvette VPD levels above 3.5 kPa (measured at 30°C) significantly reduced gas exchange, with no interaction with duration of droughting. This study showed that post-harvest stomatal control was more attuned to

ambient atmospheric evaporative demand in the orchard than to slowly developing soil moisture stress. We conclude that both atmospheric factors and changing sink demand influence stomatal control of apple leaves post-harvest, and that this should be taken into account when determining irrigation strategies.

Keywords: Apple, soil moisture stress, gas exchange, sink strength, stomatal conductance, temperature.

9.1. Introduction

In the Western Cape region of South Africa, the late season (roughly between March and May, from harvest until leaf senescence) is long and conducive to continued high rates of photosynthesis until shortly before leaf drop (S.J.E. Wand, unpublished), as well as good carbohydrate reserve accumulation and bud development, provided that trees are not stressed. Climatic conditions and soil water status can, however, still be stressful, and in the last decade autumn temperatures have been unusually high (S.J.E. Wand, unpublished) and the rains have come later than usual. In a region where winter rainfall begins around April/May and summer rainfall is low, some dams dry up during the late season, forcing growers to stop irrigating.

We are interested in the effects of post-harvest environmental conditions and within-tree demand on carbohydrate assimilation and water use in apple trees, and whether the stomata adjust to reductions in sink strength and altered climatic conditions. For efficient functioning of the tree, stomatal conductance to gas exchange must be tuned to the environment and photosynthetic metabolism of the leaf, as well as the hydraulic characteristics of the soil and tree. Stomatal conductance, and thus photosynthesis, are regulated in the short-term primarily by light, but also by temperature and leaf-to-air vapour pressure deficit (VPD), soil and plant water status, and mineral nutrition (Burrows and Milthorpe, 1976; Farquhar and Sharkey, 1982; Schulze, 1986). The effect of these variables also depends on the source:sink balance of the tree (Flore and Lakso, 1989; Lakso, 1994). Apple stomata are known to be regulated over the longer-term primarily by photosynthetic demand for CO₂, as governed by current sink strength of growing vegetative and reproductive organs.

Environmental factors influence photosynthesis through both stomatal and non-stomatal responses. The stomatal behavior of apple leaves appears to be better correlated with photosynthetic rate (as governed by light) than with leaf temperature, VPD or leaf water potential (Lakso, 1994; Jones et al., 1985) under normal non-droughted conditions. Decreases in stomatal conductance with increasing leaf temperature can often be attributed to increased VPD, therefore VPD should be held constant if the effects of increasing temperature are studied. Berry and Björkman (1980) found that in the absence of water stress and high VPD gradients, stomata tend to respond to temperature in concert with the changing photosynthetic demand for CO₂. VPD leads to stomatal closure only at fairly high levels in mature apple leaves (>2.5 kPa) (Faust, 1989; Jones et al., 1985).

However, both rapid and prolonged soil moisture deficit will lead to stomatal closure, albeit at a more advanced stage of stress the older the leaf is (Faust, 1989), since mature apple leaves are capable of osmotic adjustment in order to maintain leaf turgor (Lakso, 1979). An indirect indicator of stomatal limitation to photosynthesis is the intercellular CO₂ concentration (c_i) which is calculated from CO₂ and water vapour fluxes (Jones, 1992). This parameter allows one to determine whether a reduction in net CO₂ assimilation rate was due to stomatal closure (c_i decreasing) or non-stomatal reduction such as reduced light use efficiency (c_i increasing). The relative roles of stomatal versus non-stomatal limitation in apple leaves appear to depend on how rapidly water deficit develops.

Little is known about how short-term stomatal sensitivity of apple leaves to atmospheric factors may change through the season in response to changes in environmental conditions and sink demand. Declining temperatures during the post-harvest period may lead to downward shifts in the optimal temperature range for photosynthesis, since many plants adjust to seasonal changes in their thermal environment (Berry and Björkman, 1980; Lambers et al., 1998). This was demonstrated by Lange et al. (1974) in apricot trees. In addition, absolute rates of photosynthesis generally decline after harvest, due firstly to the reduction in sink strength leading to feedback inhibition of photosynthesis, and at a later stage the physiological aging of the leaves (Palmer et al., 1997). Stomata may be regulated so as to favour critical processes, such as rapid fruit growth during early summer and the pre-harvest period (high carbon requirement and high water loss), or carbohydrate reserve accumulation and bud development post-harvest (lower carbon requirement but sensitive to water status).

Apple stomata generally maintain a high leaf water use efficiency (WUE, defined as photosynthesis per unit of transpiration) throughout the season depending on the demand for carbohydrates by the plant. Achievement of high WUE could be a particular priority later in the season (Cheng and Luo, 1997). This may be seen in an increasing sensitivity to high temperature during autumn, as well as to soil moisture stress, leading to increases in both stomatal and non-stomatal limitation of photosynthesis.

In this study we investigated the interactive effects of the main atmospheric determinants of stomatal conductance (light, leaf temperature, leaf-to-air VPD) in response to fruit removal at harvest and soil moisture stress after harvest (cessation of irrigation). Firstly, we hypothesise that strongly reduced sink demand after harvest will increase stomatal sensitivity to atmospheric stress, and thus reduce carbon assimilation in favour of water use efficiency. Secondly, we hypothesise that post-harvest soil moisture stress will increase stomatal sensitivity to atmospheric drought (VPD) at two levels of constant leaf temperature. This information is important in the development of management strategies, particularly concerning the continued use or reduction of over-tree and under-tree irrigation regimes following harvest in the Western Cape.

9.2. Materials and Methods

9.2.1. Plant material and orchard management

A five-year-old orchard of 'Braestar' apple trees on M25 rootstock was used in the Villiersdorp district, Western Cape, South Africa, during the 1999/2000 season. The region experiences hot dry summers and mild wet winters, with an extended post-harvest period prior to leaf senescence. The planting system was a double row, with trees spaced 4.5 m between double rows and 0.75 m within double rows. Trees were spaced 1.5 m within each row and staggered within the double row. Trees were trained to a V-shape with the 0.75 m wide row forming the basis. Row orientation was north-south.

Trees were irrigated daily using drip fertigation, refilling the soil profile to field capacity. Irrigation requirements were calculated using tensiometers. Daily irrigation requirements were split into six equal portions and delivered during the daylight hours, approximately every 90 minutes. The irrigation system was converted from a micro-system to

a daily daylight fertigation system two years prior to the study. Fertilizer as required was added to the irrigation water on a daily basis early in the growth season, but not during the course of this study. No rainfall was recorded during the period of the droughting treatment.

9.2.2. Pre- and post-harvest gas exchange

During late summer, gas exchange measurements were made on the western side of the row using a portable photosynthesis system (LI-6400, Li-Cor, Lincoln, Nebraska, USA). Measurements were made one week before harvest (second week of March 2000) and three weeks after harvest. Leaves only started to senesce about 6-8 weeks after harvest, therefore it is unlikely that senescence influenced the results. It is known that apple leaf photosynthetic ageing is remarkably slow under high light exposure later in the season (Porpiglia and Barden, 1980; S.J.E. Wand, unpublished data)

Photosynthetic light response curves were generated at leaf temperatures of 20°, 25°, 30°, 35° and 40°C, controlled electronically by the LI-6400. Irradiance levels of 2000, 1500, 1000, 800, 600, 400, 200, 100 and 50 $\mu\text{mol m}^{-2} \text{s}^{-1}$ photosynthetic photon flux (PPF) were provided by an internal red/blue LED light source (LI-6400-02B Li-Cor, Lincoln, Nebraska, USA). Cuvette carbon dioxide (CO_2) concentration was controlled at 380 $\mu\text{mol mol}^{-1}$ using the LI-6400 CO_2 injection system and compressed CO_2 -cylinders. Leaf-to-air vapour pressure deficit (VPD) was controlled at between 2.0 and 2.7 kPa, depending on leaf temperature, by manipulating the flow rate.

Only exposed sun leaves on a fruiting spur at a height of about 1.2m were selected for measurements. Five replicates of photosynthetic light response curves were obtained at each leaf temperature. Five rows were used, and one tree randomly selected in each row for measurements. All data were analysed using SAS (v. 6.12). A two-way ANOVA was used to test the significance ($P \leq 0.05$) of temperature and harvest (time) effects.

Linear models were fitted individually to the light response of transpiration rate (E) and stomatal conductance (g_s). Response curves of net CO_2 assimilation rate (A) were fitted individually using non-linear regression (Statistica 5.5) and the monomolecular function $y = a(1 - e^{-bx})$ given by Causston and Dale (1990). In this function, the coefficient "a" represents the light-saturated rate of net CO_2 assimilation (A_{max}), and the apparent quantum

efficiency (α) (gradient at $x=0$) is given by “ace^b”. R^2 values for the fits obtained by the model were in the range 0.90-0.99.

9.2.3. Post-harvest soil moisture stress

After harvest, but well before the onset of leaf senescence, irrigation of one row of trees was cut off (on 4 May 2000) for 12 days. All other rows received normal daily irrigation. Net CO_2 assimilation rates of droughted trees were measured at increasing levels of leaf-to-air VPD on 4, 5, 6, 8, 9, 10, 13 and 15 May 2000 using the LI-6400. Leaf temperatures were electronically controlled at either 25°C or 30°C, using different leaves. At 25°C VPD in the leaf cuvette was controlled at 1.5, 2.0, 2.5 and 2.7 kPa, and at 30°C VPD was controlled at 2.0, 2.5, 3.0, 3.5 and 3.7 kPa. This was achieved by a combination of coarse control (regulation of flow through the dessicant) and electronically-driven fine control by means of flow rate adjustment. Cuvette CO_2 concentration was controlled at 380 $\mu\text{mol mol}^{-1}$ and PPF was controlled at 1200 $\mu\text{mol m}^{-2}\text{s}^{-1}$. One leaf from each of three randomly selected trees in the droughted row was used on each date. On 15 May measurements were also performed on three non-droughted trees 2 rows away.

Gas exchange data were analysed by two-way ANOVA (SAS v. 6.12) with duration of droughting and VPD level and factors, separately for the two leaf temperatures. Significance levels were set at $\text{Pr} \leq 0.05$. Final differences on 15 May between treatments (droughted and control) and VPD level were also analysed by two-way ANOVA. Multiple comparison tests (Tukey, $\text{Pr} \leq 0.05$) were used to establish trends.

Leaf water potential was measured using a Scholander pressure chamber (PMS Instrument Co., Corvallis, USA) on 10 May 2000. Three leaves from each of three trees in the irrigated and a nearby non-irrigated row were measured between 11h45 and 12h20, and again between 13h00 and 13h40. Irrigation came on at 12h40 during this period. Data were statistically analysed for each period using one-way ANOVA (SAS v. 6.12).

Soil matrix potential was measured with electronic tensiometers at depths of 30 and 60 cm, and connected to a datalogger (custom built by S. Lorentz, University of Natal, South Africa). Tensiometers were installed between two drippers in the row forming the basis of the V-shape. Readings were taken every 16 minutes. Leaf temperature of a representative outer

canopy leaf was measured with an infrared thermocouple sensor (Apogee IRTS-P, Logan, Utah, USA) connected to a datalogger (CR10X, Campbell Scientific, Inc., Logan, Utah, USA). Readings were taken every 10 seconds and the mean stored every 5 minutes.

Weather data (air temperature, relative humidity and potential evapotranspiration [ET₀]) were obtained from the Villiersdorp weather station approximately 5 km from the orchard.

9.3. Results

9.3.1. Pre- and post-harvest gas exchange

The response of light-saturated net CO₂ assimilation rate (A_{\max}) to increasing leaf temperature shows that the optimum temperature was reduced from about 25-30°C pre-harvest to 20-25°C post-harvest (Fig. 1a). A_{\max} was lower at leaf temperatures of 30°C or higher after harvest compared to before harvest. There was a significant temperature*time interaction (Table 1), indicating the faster reduction in A_{\max} with increasing temperature post-harvest.

Light saturated stomatal conductance (g_s) was strongly reduced post-harvest at high temperatures (>30°C, Fig. 1b), leading to a significant temperature*time interaction (Table 1). This was accompanied by a similar strong reduction in intercellular CO₂ concentration (c_i) above 30°C during the post-harvest period (Fig. 1c). In contrast, g_s and c_i remained relatively constant at all leaf temperatures pre-harvest. Transpiration rate (E) followed the same pattern as g_s (Table 2). Instantaneous leaf water use efficiency (WUE) fell at 40°C pre-harvest, but remained relatively constant at all leaf temperatures post-harvest (Fig. 1d, Table 1).

Analysis of photosynthetic light responses (Fig. 2a,b) showed a reduction in apparent quantum efficiency (α) at 35-40°C, both pre- and post-harvest (Table 2). C_i was not influenced by changing irradiance, and showed a similar trend at different leaf temperatures pre-harvest, but was consistently lower at 35-40°C post-harvest (Fig. 2c,d). Increasing irradiance caused increases in g_s and E at all temperatures, both pre- and post-harvest, but the slopes of the responses did not differ between different temperatures (data not shown).

Thus at leaf temperatures of 35-40°C, non-stomatal limitation occurred during both periods, but stomatal limitation also played a large role after harvest, accounting for the steeper decline in A with increasing leaf temperature (Fig. 1a).

9.3.2. Post-harvest soil moisture stress

At 25°C leaf temperature, A_{\max} remained relatively constant during the first week of soil drying, followed by a significant decrease on 13 May and a partial recovery on 15 May (Fig. 3a, Table 3). There was no effect of cuvette VPD levels (1.5 to 2.7 kPa) throughout the drying period. At this temperature, photosynthetic rates were similar in droughted and control trees at the end of the trial on 15 May (Table 4). A similar time trend was observed at 30°C until 13 May (Fig. 3b), but A_{\max} continued to decline in droughted trees, and was significantly lower than in control trees on 15 May (Table 4). At this temperature (30°C), the highest level of VPD (3.7 kPa) reduced net CO₂ assimilation rate throughout the droughting period.

Stomatal conductance (g_s) followed a similar trend as A_{\max} (Fig. 3c,d, Tables 3,4) indicating that changes in A_{\max} could be primarily attributed to stomatal responses. In particular, the effects of high VPD and droughting at 30°C on A_{\max} were mediated via changes in stomatal conductance. Although c_i remained relatively constant during the first week of droughting (Fig. 4a,b), stressful climatic conditions on 13 May reduced c_i , particularly at the lower levels of VPD. There was a partial recovery of c_i on 15 May, but c_i of droughted trees remained lower at 30°C than that of control trees (Table 4), in tandem with the decreases in g_s and A_{\max} .

Leaf water potential of droughted and irrigated trees on 10 May (after 6 days of droughting) did not differ significantly during late morning or early afternoon (Fig. 5; $P=0.8593$ and $P=0.2554$ respectively), and remained relatively high (-1 to -1.5 MPa). This day had moderate maximum air temperature and relative humidity (Fig. 6a), giving low values for potential evapotranspiration (Fig. 6b).

Air temperatures were cool to moderate during the first week of the droughting treatment and then increased, accompanied by a decrease in relative humidity. Together this resulted in increased evaporative demand from the atmosphere (Fig. 6b). Leaf temperature

increased with air temperature during the second half of the droughting treatment (Fig. 6c). The topsoil (upper 30 cm) was already dry at the start of the droughting treatment, and the deeper soil layer (60 cm) dried out (matrix potential increased) with treatment duration (Fig. 6d).

9.4. Discussion

9.4.1 Pre- and post-harvest gas exchange

Several factors are known to control stomatal conductance, such as light, VPD, soil water potential, internal CO₂ concentration as well as sink strength. However, there are likely to be several feedback and feed forward relationships that can also regulate stomatal conductance (Jones, 1998). The feedbacks have been separated into CO₂ feedback, possibly operating through either c_i or through assimilation rate (Wong et al., 1985), and hydraulic feedbacks dependent on aspects of stomatal or plant water relations (Jarvis and Davies, 1998). Evidence from this study would suggest that it is mainly CO₂ feedback following a strong reduction in sink strength that increases stomatal sensitivity of mature apple leaves to environmental variables e.g. high leaf temperatures.

The optimum temperature range for photosynthesis was between 25 and 30°C pre-harvest and between 20 and 25°C post-harvest (Fig. 1a). This was likely due to differential stomatal responses to temperature (Fig. 1b), but may also have been related to the reduction in average maximum air temperature from 28°C (pre-harvest) to 22°C (post-harvest) during this period. Watson et al. (1978) also reported a similar late-season decrease in the optimum temperature range in 'Golden Delicious' apple trees. Photosynthetic acclimation to seasonal temperature variation has also been observed in other temperate fruit species e.g. *Prunus armeniaca* (Lange et al., 1974). Slatyer and Ferrar (1977) found that acclimation could occur in two weeks or less.

At temperatures higher than the optimum the ratio CO₂/O₂ decreases and this decreases the carboxylation efficiency of rubisco, with a resulting increase in photorespiration and thus a decrease in carbon fixation (Berry and Björkman, 1980). The increase in respiratory production of CO₂ with increasing temperature also decreases A_{max} at higher temperatures.

The pre-harvest reduction in A_{\max} with an increase in temperature from 35-40°C (Fig. 1a) can be attributed to non-stomatal limitations since g_s and c_i remain constant with an increase in temperature from 35-40°C (Fig. 1b, c). Amongst other potential non-stomatal factors, we show that apparent quantum efficiency (α , Table 2) was significantly reduced at 40°C. This decrease in A_{\max} resulted in a decreased WUE (Fig. 1d) since transpiration rate (E) increased in response to high temperature (Table 2) at a constant g_s .

After harvest, the decrease in A_{\max} at temperatures higher than 35°C (Fig. 1a) was the result of both stomatal (Fig. 1b, c) and non-stomatal (Table 2) limitations. Stomatal limitation already influenced A_{\max} at 35°C, as shown by decreased g_s and c_i . This resulted in the maintenance of high WUE (Fig. 1d).

The post-harvest change in stomatal sensitivity to increases in temperature can only be explained by the reduction in sink strength. Wünsche and Palmer (1997) found that after harvest A_{\max} of 'Braeburn' apple trees declined to the level of non-cropping trees. Due to a reduced demand for carbohydrates by the plant, A_{\max} is reduced post-harvest. Following this reduction in sink strength and A_{\max} stomata became more sensitive to high leaf temperatures, thus restricting water loss. Pre-harvest there is a strong demand for carbohydrates, therefore stomata must be kept open to ensure a high rate of CO₂ incorporation, even if this comes at the cost of a reduced WUE. Wibbe and Blanke (1995) found a decrease in WUE of 'Golden Delicious' apple trees after harvest, apparently related to the onset of leaf senescence. However after fruit removal earlier in the season, WUE increased. In this study, leaves only senesced 6-8 weeks after harvest, therefore it is unlikely that senescence influenced WUE. Cheng and Luo (1997) also reported increased WUE of apple leaves in autumn, due to declining transpiration rates.

From Fig. 2a it appears as if g_s increases with an increase in light intensity, however this increase is rather due to a decrease in c_i with an increase in light intensity (Fig. 2c). As net CO₂ exchange rate increase with an increase in light intensity there is an decrease in c_i until the light saturation point is reach between 800 and 1200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PFD, after which c_i remains relatively constant. These saturation values are consistent with those found by Francesconi et al. (1997) for potted 'Gala' trees. Net CO₂ exchange rate with increased light intensity post-harvest follows the same trend as in the pre-harvest measurements, however the

reduction in net CO₂ exchange rate at 40°C is much larger than with pre-harvest measurements (Fig. 2b and d).

Our hypothesis that stomatal sensitivity to environmental stresses, such as high temperature, increases with a decrease in the demand for carbohydrates by sinks, thereby improving water use efficiency during the late season, is thus supported by this study.

9.4.2 Post-harvest soil moisture stress

Coupling of stomata with the atmosphere is particularly strong in fruit trees, due to the poor hydraulic conductivity of their root systems (Jones et al., 1985). Transpirational water loss is not always replenished at a sufficient rate when evaporative demand is high. Even under conditions of optimal soil water supply, strong water deficits may develop and result in low water potentials in the above-ground tree parts. The latter is dealt with by means of highly effective osmotic adjustments in mature leaves (Faust, 1989), which ensure maintenance of cell turgor for photosynthesis and other metabolic processes. Osmotic adjustment of mature leaves is a striking characteristic of apple trees compared to many other crops. The amount of active osmotic adjustment has been found to be up to 2.5 MPa over a season (Fanjul and Rosher, 1984). This allows the apple tree to tolerate mild to moderate droughts while maintaining canopy photosynthesis. It appears that water stress induces the preferential role of sorbitol as the osmoticum. In our study, leaf water potential of droughted and irrigated trees on 10 May (after 6 days of droughting) did not differ (Fig. 4) and was fairly high throughout the morning and early afternoon (-1 to -1.5 MPa). However, this day was characterised by moderate maximum temperature and relative humidity (Fig. 6a), giving low values for potential evapotranspiration (Fig. 6b). Another explanation for similar leaf water potentials may be that the capacity for osmotic adjustment in apple leaves is highest late in the season (Lakso, 1979). Moderate water stress which develops gradually therefore has no apparent detrimental effects on gas exchange after harvest (although it may directly affect other processes such as cell division and differentiation, for example in developing reproductive buds).

Massacci and Jones (1990) found that the relative roles of stomatal versus non-stomatal limitation in apple trees appear to depend on how rapidly the water stress develops.

When stress development is rapid, only gas exchange is affected (stomatal limitation only), but after long term stress both stomatal and non-stomatal components limit gas exchange.

In our study, moderate temperatures and high relative humidity resulted in a low potential evapotranspiration during the first week of the droughting treatment (Fig. 6a, b) so that water stress developed over a relatively long time period. Due to the fact that this orchard was only converted to drip irrigation two years previously it is likely that it still had an extensive root system, and greater water foraging capacity than would be expected for newly established orchards using drip irrigation. Leaf temperatures followed the same trend as air temperature, reaching peak values above 25°C on the last three days of the droughting treatment (Fig. 6c). Increasing soil moisture deficit (Fig. 6d) was thus accompanied by increasing atmospheric moisture deficit.

During the droughting treatment stomatal conductance followed a similar trend as A_{\max} throughout (Fig. 3c,d), indicating that the variability in A_{\max} could largely be attributed to stomatal responses, which were regulated so as to maintain c_i at relatively constant levels (except on 13 May under stressful atmospheric conditions). However, on the last day (15 May) when measured at 30°C (Fig. 3b,d), g_s and c_i values were similar compared to 13 May and could not account for the further decrease in A_{\max} . This indicates increased non-stomatal limitation of photosynthesis in droughted trees at high temperature. At 25°C there were no significant differences in A_{\max} and g_s between control trees receiving regular irrigation and droughted trees after 12 days. However, at 30°C, A_{\max} and g_s were significantly reduced (Table 4). Throughout the droughting treatment A_{\max} was only significantly reduced at VPD levels above 3.5 kPa (Table 3). Stomatal sensitivity to increased VPD levels was thus unaffected by the increasing soil moisture deficit.

Only one leaf on the tree was subjected to high temperatures and VPD levels in the cuvette during measurements. It is unlikely to have caused a limitation in the supply of water from the roots. However, if the whole tree were subjected to these conditions it is likely that a supply limitation would have occurred. This supply limitation might affect the sensitivity of stomata to increased VPD levels. This statement is supported by the fact that during days when there was a high atmospheric evaporative demand A_{\max} and g_s were significantly reduced at all VPD levels (Table 3).

This study showed that post-harvest stomatal control was finely attuned to atmospheric evaporative demand as determined by air and leaf temperatures and VPD of the atmosphere around the whole tree, rather than by slowly developing soil moisture stress. Stomata did not become more sensitive to increased VPD levels with an increase in soil moisture deficit late in the season.

Apple leaf adjustments which allow the maintenance of high leaf water potential during the late season should be taken into account by producers when deciding on an irrigation strategy. Greater soil water depletion levels after harvest than currently employed may thus be tolerable and perhaps even beneficial, especially in Mediterranean type climates where winter rainfall will replenish the soil profile. This could help to reduce irrigation costs and save dwindling water supplies. An added benefit of controlled deficit irrigation at this post-harvest stage could be to help control late vegetative growth (Jerie et al., 1989). Care should however be taken not to negatively affect the development of reproductive buds.

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Table 1. Probability values for light-saturated net CO₂ assimilation rate (A_{\max}), apparent quantum efficiency (α), stomatal conductance (g_s), intercellular CO₂ concentration (c_i), transpiration rate (E) and instantaneous leaf water use efficiency (WUE) at differing leaf temperatures pre- and post-harvest. Data were analysed by two-way ANOVA.

	Pr>F (Temperature)	Pr>F (Time)	Pr>F (Temperature*Time)
A_{\max} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	0.0001	0.0305	0.0001
α ($\mu\text{mol CO}_2 \mu\text{mol}^{-1}$ quanta)	0.0450	0.2121	0.3214
g_s ($\text{mol m}^{-2} \text{s}^{-1}$)	0.0001	0.7134	0.0004
c_i ($\mu\text{mol mol}^{-1}$)	0.0001	0.0001	0.0001
E ($\text{mmol m}^{-2} \text{s}^{-1}$)	0.0214	0.3227	0.0004
WUE ($\mu\text{mol CO}_2 \text{mmol}^{-1} \text{H}_2\text{O}$)	0.0570	0.1504	0.0016

Table 2. Apparent quantum efficiency (α) and light-saturated transpiration rate (E) at differing leaf temperatures pre- and post-harvest. Values are means with standard deviations (n = 5).

Leaf temperature °C	Time	α ($\mu\text{mol CO}_2 \mu\text{mol}^{-1}$ quanta)	E ($\text{mmol m}^{-2} \text{s}^{-1}$)
20	Pre-harvest	0.039 \pm 0.010	3.86 \pm 0.93
	Post-harvest	0.045 \pm 0.005	4.95 \pm 0.63
25	Pre-harvest	0.047 \pm 0.006	5.04 \pm 0.76
	Post-harvest	0.045 \pm 0.007	5.96 \pm 0.52
30	Pre-harvest	0.045 \pm 0.005	4.59 \pm 1.31
	Post-harvest	0.054 \pm 0.015	3.77 \pm 0.29
35	Pre-harvest	0.036 \pm 0.011	4.12 \pm 1.75
	Post-harvest	0.057 \pm 0.008	4.08 \pm 1.23
40	Pre-harvest	0.030 \pm 0.011	5.63 \pm 1.46
	Post-harvest	0.025 \pm 0.013	1.91 \pm 0.51

Table 3. Probability values for light-saturated net CO₂ assimilation rate (A_{max}), stomatal conductance (g_s) and intercellular CO₂ concentration (c_i) at differing leaf temperatures for the duration of the soil water stress treatment. Data were analysed by two-way ANOVA.

	A_{max}	g_s	c_i
25°C leaf temp.:			
Pr>F	<i>0.0001</i>	<i>0.0001</i>	<i>0.0001</i>
(Duration of drought)			
Pr>F (VPD level)	0.4232	0.4147	<i>0.0002</i>
Pr>F (interaction)	1.0000	0.9996	<i>0.0001</i>
30°C leaf temp.:			
Pr>F	<i>0.0001</i>	<i>0.0001</i>	<i>0.0001</i>
(Duration of drought)			
Pr>F (VPD level)	<i>0.0003</i>	<i>0.0012</i>	<i>0.0002</i>
Pr>F (interaction)	0.9911	0.3908	<i>0.0001</i>

Table 4. Probability values for light-saturated net CO₂ assimilation rate (A_{max}), stomatal conductance (g_s) and intercellular CO₂ concentration (c_i) at differing leaf temperatures of droughted trees compared to irrigated control trees on 15 May (last day of water stress treatment). Data were analysed by two-way ANOVA.

	A_{max}	g_s	c_i
25°C leaf temp.:			
Pr>F (Treatment)	0.1052	0.7091	0.9193
Pr>F (VPD level)	0.1073	0.6108	<i>0.0390</i>
Pr>F (interaction)	0.9861	0.6928	0.8951
30°C leaf temp.:			
Pr>F (Treatment)	<i>0.0008</i>	<i>0.0002</i>	<i>0.0067</i>
Pr>F (VPD level)	0.9110	0.9450	0.1207
Pr>F (interaction)	0.9803	0.9926	0.8948

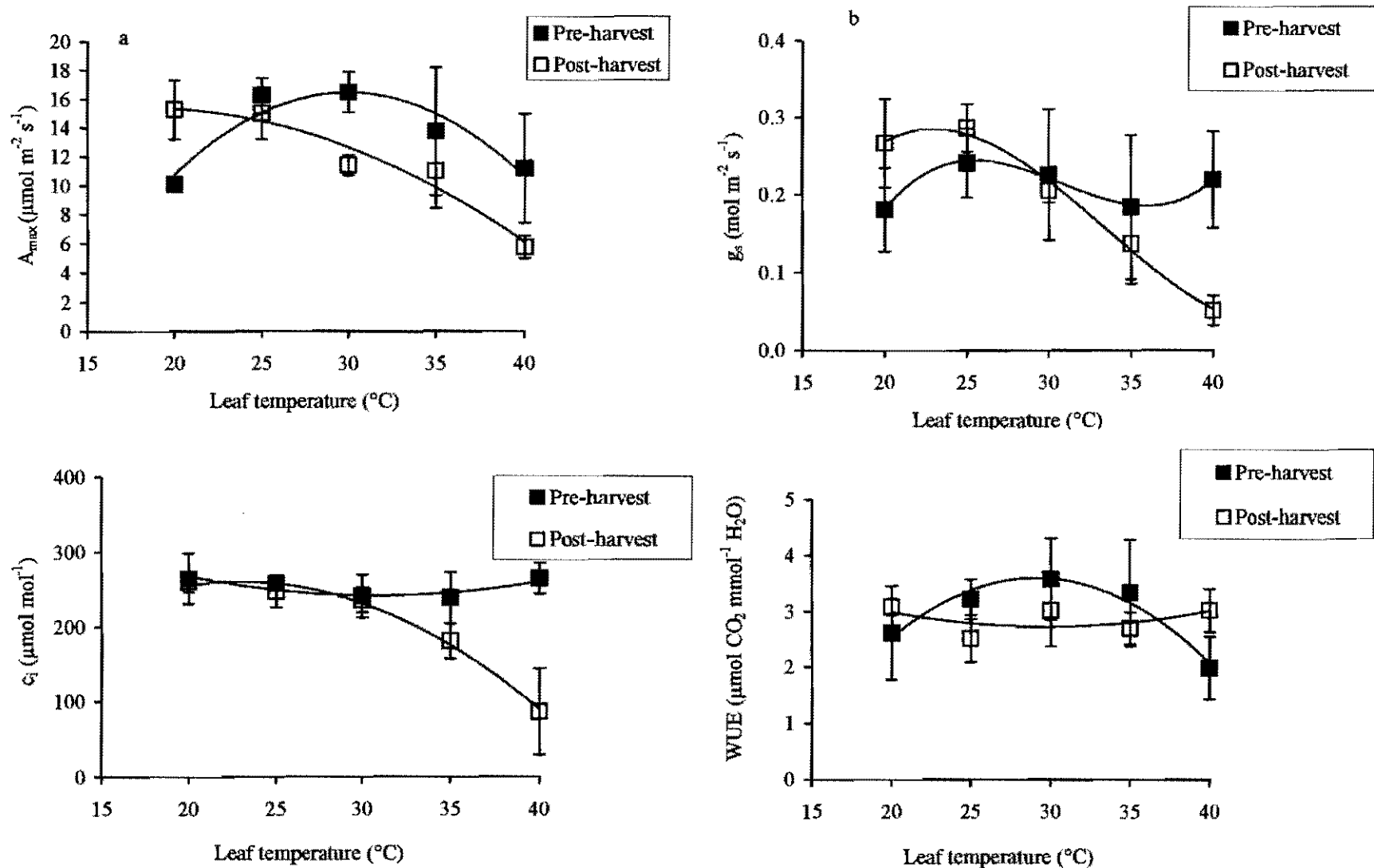


Fig. 1. Responses to increased leaf temperature under light saturation, before and after harvest, of (a) net CO₂ assimilation rate (A_{max}), (b) stomatal conductance to water vapour (g_s), (c) intercellular CO₂ concentration (c_i), and (d) instantaneous leaf water use efficiency (WUE). Values are means ($n = 5$) and error bars represent the standard deviation.

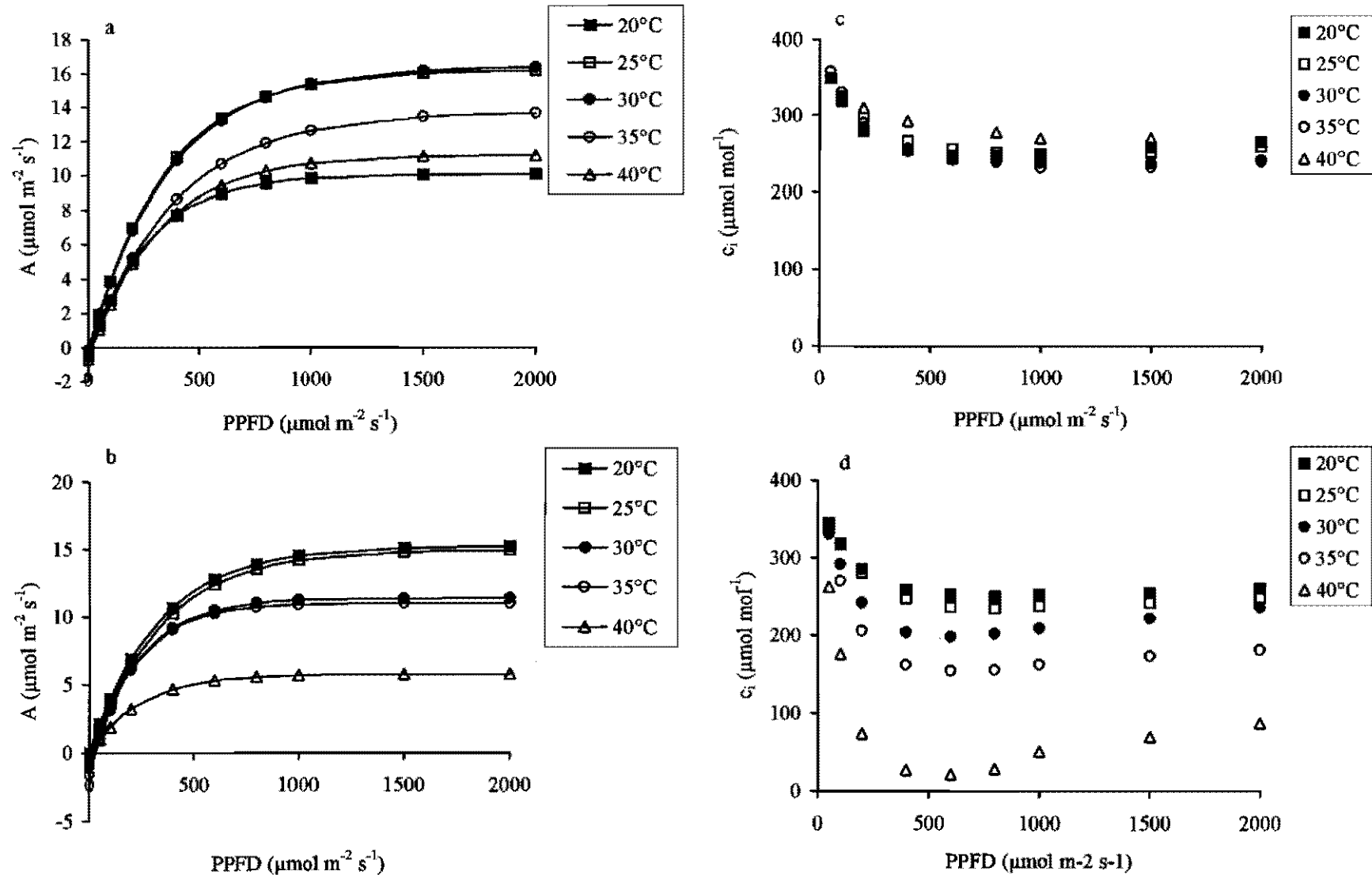


Fig. 2. Response of net CO₂ assimilation rate (A) to increasing levels of photosynthetic photon flux densities (PPFD) at different leaf temperatures, (a) before harvest, (b) after harvest. Response of intercellular CO₂ concentration (c_i) to increasing levels of photosynthetic photon flux densities (PPFD) at different leaf temperatures, (c) before harvest and (d) after harvest.

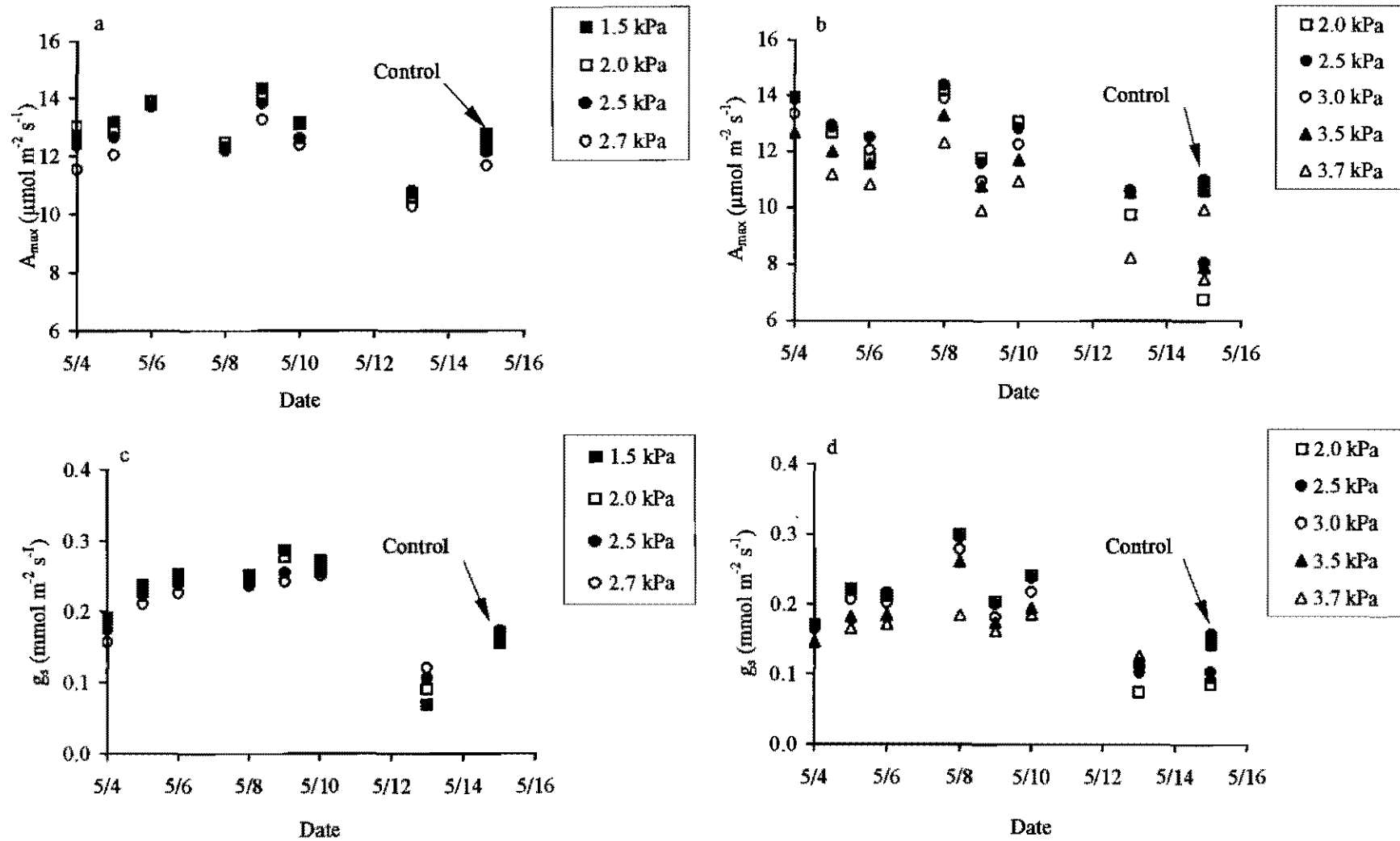


Fig. 3. Response of net CO₂ assimilation rate at saturated light intensity (A_{max}) to different levels of leaf to air vapour pressure deficit (VPD) at a leaf temperature of (a) 25°C, and (b) 30°C. Response of stomatal conductance to water vapour at a saturated light intensity (g_s) to different levels of VPD at a leaf temperature of (c) 25°C and (d) 30°C at the different dates during the droughting period.

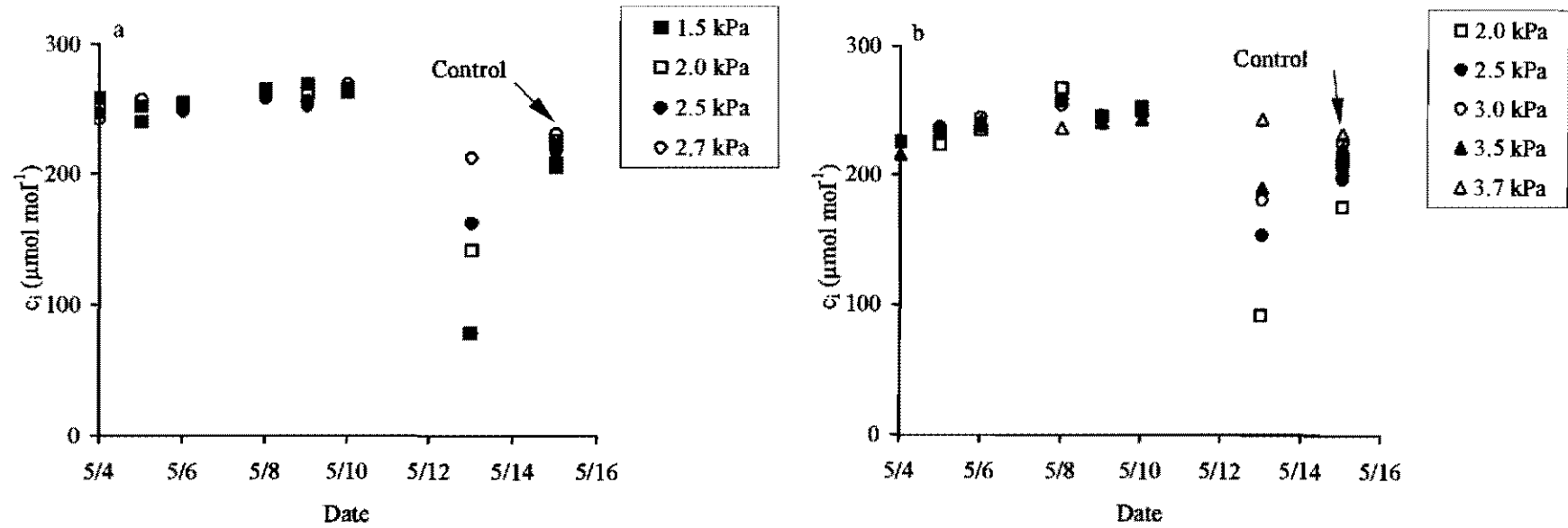


Fig. 4. Response of intercellular CO₂ concentration (c_i) to different levels of leaf to air vapour pressure deficit (VPD) at a leaf temperature of (a) 25°C, and (b) 30°C at the different dates during the droughting period.

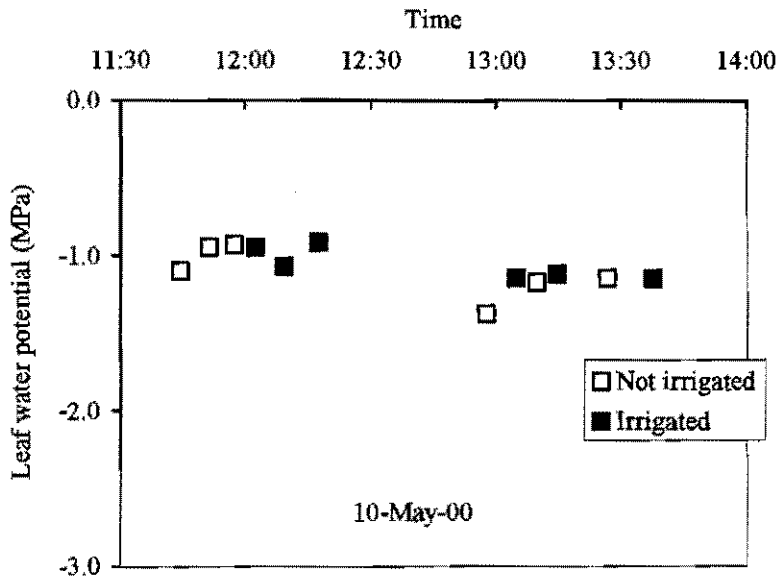


Fig. 5. Leaf water potential (Y) of irrigated and droughted trees on 10 May 2000, six days after the cessation of irrigation.

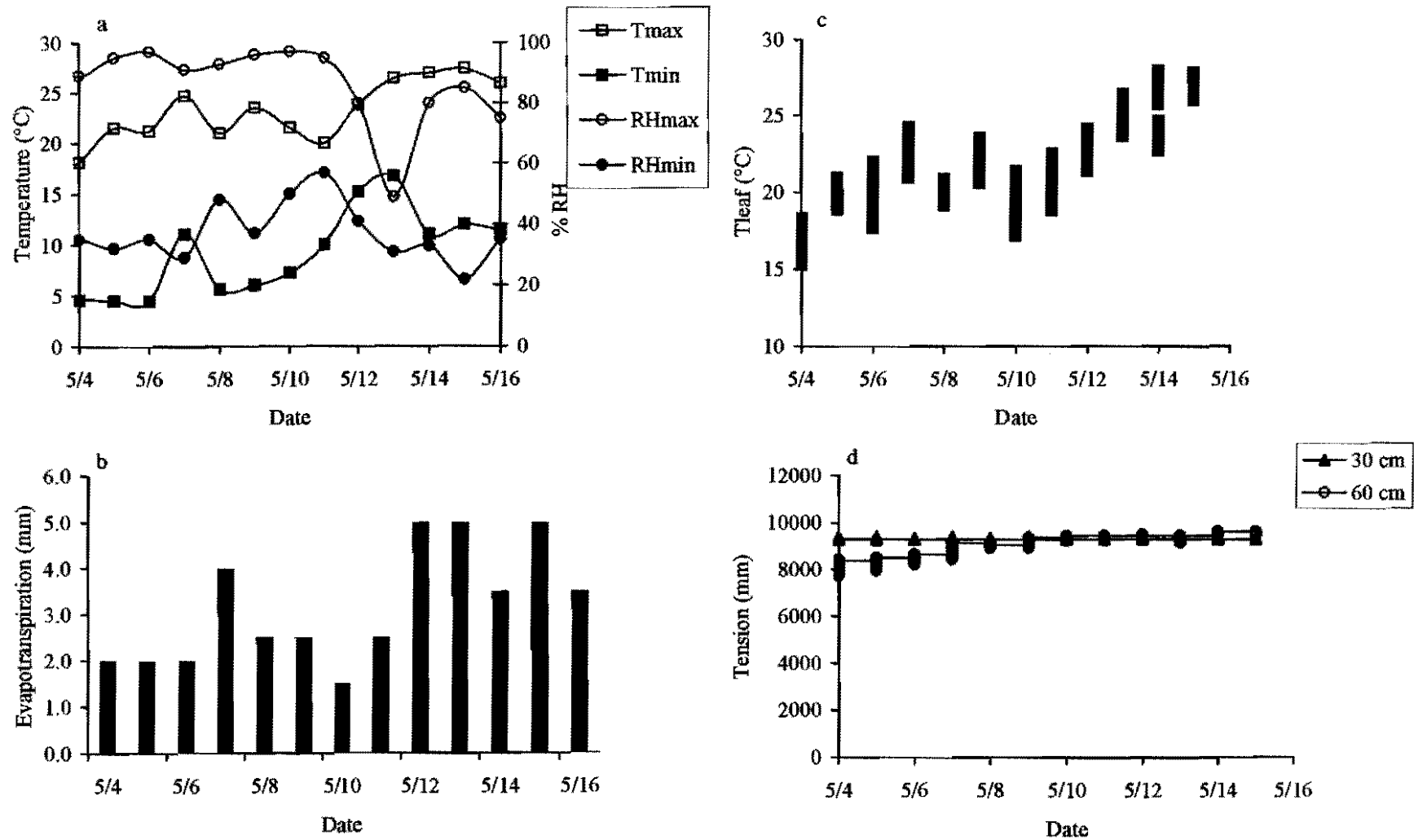


Fig. 6. (a) Maximum (Tmax) and minimum (Tmin) air temperatures and maximum (RHmax) and minimum (RHmin) relative humidity; (b) potential evapotranspiration; (c) leaf temperatures between 12h00 and 15h00; and (d) soil matrix potential for the soil droughting period.

10. General conclusions

Market preferences for larger fruit have forced producers to adopt cultural practices that will ensure bigger fruit even if this comes at the expense of reduced total yields. In order to obtain acceptable fruit size there must be an adequate supply of photosynthetic carbon products especially during the cell division stage of fruit growth. Competition between fruits and between fruit and vegetative growth may limit the carbon supply to the fruits at this critical period and thus limit the final fruit size. Adverse environmental conditions such as low light levels as well as high temperatures can limit carbon assimilation rates, thus negatively influencing carbon supply to the developing fruit. Thinning reduces the number of competing fruit and should ensure that the remaining fruit have an adequate supply of carbon from the source leaves.

In deciduous fruit trees, carbohydrates must not only be produced but also allocated efficiently to fruit and new flower buds. Source-sink relationships and the regulation of carbon allocation therefore determine growth of individual organs. This may be restricted by assimilate availability (source limitation) or by the organ's ability to utilise assimilates (sink limitation). The partitioning pattern of photosynthate between vegetative development and reproductive development early in the season, when fruit growth is mainly due to cell division, has a pronounced influence on fruit development and size. Cell division takes place primarily during the first 40 days after full bloom (DAFB), followed by cell enlargement. In order to maximise fruit size, it is imperative to optimise carbon allocation to the fruit during this period. In the hierarchy of sinks fruits and shoots are equal, but since shoots develop prior to fruits they compete more efficiently for carbohydrates during the early part of the season. Excessive vegetative growth is thus a major concern to the producer because it competes with fruit growth.

In the Western Cape region of South Africa, dwarfing rootstocks offer no solution to excessive tree vigour due to inadequate winter chilling, causing poor bud break and canopy development, and generally poor soil conditions, all of which restrict the growth of dwarfing rootstocks severely. Currently, semi-vigorous rootstocks such as MM 106 or MM 793 are used for high-density orchards (1000-1666 trees ha⁻¹). Their lack of precocity is a serious limitation and this makes management of mature high density orchards difficult. These orchards must

achieve high early yields, high sustained yield and excellent fruit quality in order for the producer to survive financially. In addition, growth on these rootstocks is often too vigorous and an effective restriction of vegetative growth is required. There is an international trend away from harsh chemical treatments and growth retardants and towards a more environmentally acceptable approach.

Numerous non-chemical techniques are available to control excessive vigour, including summer and root pruning, limb spreading, restricted fertiliser application and the control of irrigation. However, these techniques may be only marginally effective. There is now increasing interest in trunk or limb girdling or scoring as an effective and environmentally friendly cultural practice to control tree vigour and increase fruit size, and it has been widely used in South African orchards in recent years. However, recommendations for the timing and severity of girdling are usually based on an empirical formula giving rise to mixed results. A new and milder chemical growth retardant, prohexadione-calcium (ProCa), is now also available for vegetative growth control of apple trees. At the same time, fruit thinning is usually required to regulate the crop load and optimise yields and fruit quality. Although much is known about thinning, the simultaneous use of thinning, scoring and ProCa during the early season is poorly understood on a physiological basis and was thus investigated over three seasons.

In this study, both scoring and ProCa inhibited shoot growth, but the mechanisms through which they acted were apparently quite different (Chapters 3, 4, 6 and 7). Extension shoot growth was generally more sensitive than bourse shoot growth to both scoring and ProCa. ProCa was a more effective way of controlling shoot growth than scoring, with 'Fuji' and 'Cripps' Pink' being more sensitive to the application of ProCa than 'Royal Gala'. The apparent inhibiting effect of scoring on shoot growth was more likely an effect of a bigger crop load on scored trees than a direct effect of scoring on shoot growth. This was especially true in the second year of scoring.

Scoring reduces the sink strength of competing vegetative growth by altering the internal plant growth regulator balance of the tree, thus ensuring that more carbon is available for fruit growth. Sink strength of developing fruits is determined in part by the ability of the fruit to metabolize the carbon supplied by the source leaves. Evidence from other published studies suggests that the imported carbon compounds regulate the activities of the enzymes

responsible for the metabolism of imported carbon. Thus an increase in the supply of carbon early in the season will lead to an increase in the expression and activity of the enzymes, thus ensuring that the fruits are established as a strong sink early in the season.

This increase in the carbohydrate supply to the fruit during the cell division stage of fruit growth generally culminates in increased fruit growth rates and ultimately bigger fruit on scored trees. Increased carbohydrate supply to fruit thus results in bigger fruit with favourable characteristics such as higher firmness, higher total soluble solids (TSS) and improved skin colour. This was confirmed in this study (Chapters 3, 5, 6 and 7), as scoring often had a beneficial effect on fruit size and also improved the TSS of 'Fuji' and 'Cripps' Pink' fruit compared to control and trees sprayed with ProCa, indicating higher carbohydrate supply to the fruits. In 'Cripps' Pink' fruit firmness and colour were also improved in scored trees compared to control trees. ProCa did not improve fruit firmness compared to control trees in 'Cripps' Pink', but fruit from the ProCa treatment showed better colour development than fruit from the control treatment. As there was no evidence of improved carbohydrate supply to the fruit in the ProCa treatment, this improvement in fruit colour was probably due to less shoot growth and better light penetration into the canopy of these trees.

Scoring disrupts the basipetal transport of indoleacetic acid (IAA) to the roots, as well as reducing the carbohydrate flow to the roots, which in turn leads to a reduction in the synthesis of cytokinins and gibberellins by the roots. Gibberellins and cytokinins are transported in the xylem to the shoots (scoring does not disrupt their transport) where they increase the sink strength of growing shoots and also enhance shoot elongation. It is likely that in this study too, reduced concentrations of cytokinins and gibberellins in shoots due to scoring led to a reduction in shoot sink strength. Therefore, more of the carbohydrates produced by photosynthesis were allocated to the fruit, which in turn led to increased fruit growth. ProCa reduces shoot growth by acting as a GA biosynthesis inhibitor, thus reducing gibberellin levels in the plant. However, no evidence of increased carbon allocation to the fruit was found in this study, suggesting that the strength of the shoot growth sink was not affected. It would appear as if the strength of the shoot growth sink is determined primarily by IAA and cytokinins and thus remains un-affected by the application of ProCa.

Source leaves react to the demand from the sink organs and regulate photosynthesis accordingly. Maximum rate of photosynthesis is determined by prevailing environmental

conditions. Once the light environment inside the trees is optimized there is very little the grower can do to increase the maximum rate of photosynthesis. Therefore, cultural practices to increase fruit size should aim to establish the fruits as dominant sinks very early in the season. This ensures an adequate supply of carbon to the fruit at all times. Early thinning and scoring can achieve this by eliminating competition between fruitlets and between fruits and vegetative sinks early in the season when the adequate supply of carbon to the fruits is critical.

Scoring and severe thinning cause the accumulation of carbohydrates throughout the canopy, which can lead to a reduction in photosynthesis. Excess carbohydrates may interfere with photosynthesis through several mechanisms: (1) enlarged starch granules damaging the chloroplasts; (2) closure of stomata; (3) accumulation of phosphorylated intermediates and depletion of inorganic phosphate (P_i); and (4) indirect action by repressing the expression of genes that encode proteins needed for photosynthesis. In order to achieve optimum yields and fruit size, photosynthesis should be slightly demand limited throughout the season, and care must be taken to control the vegetative sinks in order to partition as much as possible of the photosynthate to the fruits.

In this study, the reductions in light saturated net CO_2 assimilation rate (A_{max}) due to scoring and severe thinning (Chapters 3 and 4) were attributable to both reductions in stomatal conductance, and reductions in maximum rate of carboxylation by rubisco ($V_{c,max}$) and maximum rate of electron transport (J_{max}) (Chapter 3). $V_{c,max}$ was significantly lower in the scored treatments later in the season. J_{max} increased substantially from 20 to 50 DAFB and was decreased significantly by scoring later in the season, and by thinning early in the season but not thereafter. Therefore, slight feedback inhibition was evident only during the early season in thinned trees but did not influence A_{max} , whereas small reductions in A_{max} thereafter were due to lower stomatal conductances. In contrast, scoring resulted in season-long reductions in stomatal conductance and gradual down-regulation of photosynthetic capacity during the mid- and late-season probably due to a reduction in the shoot growth sink. No feedback inhibition during the middle part of the season (50 DAFB) might indicate source-limitations, even on trees with a reduced fruit sink strength, due to excessive vegetative growth.

The sharp increase in dark respiration (R_d) in leaves and fruit (Chapter 8) with an increase in temperature would mean that significant carbohydrate shortages could occur in trees during source limited periods early in the season, especially under warm weather

conditions commonly experienced in the Western Cape region. This situation can be aggravated by excessive vegetative growth, during the early cell division period.

The optimum temperature range for photosynthesis was found to adjust according to seasonal temperature variations. The harvest-induced reduction in sink strength changed stomatal sensitivity to higher temperature (Chapter 9). Due to a reduced demand for carbohydrates by the plant, A_{max} was reduced post-harvest, in a similar manner to the effects measured after complete fruit removal earlier in the season (Chapters 3 and 4). Following this reduction in sink strength and A_{max} , stomata became more sensitive to high leaf temperatures, thus restricting water loss. Pre-harvest there was a strong demand for carbohydrates, therefore stomata were kept open at higher temperatures to ensure a high rate of CO_2 incorporation, but at a cost with regard to water use efficiency.

South African producers are being forced to alter their cultural practices to increase fruit size and improve quality attributes such as blush colour development in order to stay competitive on the international markets. Modern high-density orchard planting systems must achieve high early yields, high sustained yield and excellent fruit quality in order for the producer to survive financially. This can be done by managing crop load and effectively restricting vegetative growth in vigorous apple cultivars in order to achieve a balance between vegetative and reproductive development. Increases in yield were observed in the second year after scoring (Chapter 5) due to better reproductive bud development on trees scored the previous year (Chapters 5, 6 and 7), leading to high sustained yields. The promotion of flowering by scoring may be due to an increase in carbohydrates beyond a threshold level in the canopy of girdled trees. However, girdling also interferes with the transport of auxins and other plant growth regulators. The increase in flower bud formation can thus not solely be attributed to an increase in carbohydrate levels in the canopy of the girdled tree. The rich source of gibberellins in apple seeds and their translocation into the plant can inhibit reproductive bud development, especially in heavy cropping trees. ProCa acts as a GA biosynthesis inhibitor, thus reducing gibberellin levels in the plant. However, in spite of this and better light penetration due to reduced shoot growth, better reproductive bud development was not found with this treatment in this study. Net CO_2 assimilation rates were reduced on 'Royal Gala' trees following scoring due to a feedback inhibition, indicating high levels of carbohydrates in the canopy. It thus remains unclear whether this increase in reproductive bud

development on the scored treatments is due to higher carbohydrate levels or interference with plant growth regulator translocation. For this response to occur effectively, scoring should be performed as soon as possible after growth cessation. On 'Royal Gala' and 'Cripps' Pink', the efficiency of scoring to stimulate reproductive bud development on old and new spurs declined after 4 WAFB. In 'Fuji' scoring later than 1 WAFB led to a decreasing positive response in reproductive bud development on old and new spurs. In contrast, the reproductive bud development on long shoots increased with later scoring (6 or 8 WAFB) on all cultivars. It would seem as though it is not primarily the high levels of gibberellins in the tree due to a high crop load that inhibits reproductive bud development, but also a limited supply of carbohydrates in the canopy following reproductive bud initiation.

Scoring temporarily increases the availability of assimilates in the canopy, which increases the strength of the fruit growth sink. This increase can be positive (improved set, fruit size and quality) or negative (too many fruit) if done very early. Recommendations for optimum use of scoring would thus have to be orchard- and cultivar-specific depending on the goals that are to be achieved. ProCa decreases shoot growth very effectively in all cultivars studied. This reduction in shoot growth can have added benefits such as improved light penetration leading to better fruit quality (blush colour development) and improved yields, and better pest control through better spray penetration. However, no evidence of increased carbon allocation to the fruit was found. The combination of early-season scoring and application of ProCa seems to hold potential for increasing carbon allocation to the fruit and improving fruit size and quality attributes to a greater degree than when used individually (Chapter 6). The interaction between the responses of plant growth regulators and carbohydrate supply levels in the tree warrants further investigation.