# The kiwifruit (*Actinidia* sp.) vine root system: responses to vine manipulations

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# Abstract

The root system is a significant component of whole-plant biomass in kiwifruit vines and is likely to be a considerable carbohydrate sink. Seasonal trends in root growth were investigated to infer seasonal fluctuations in root carbohydrate demand. Root growth peaked in summer and autumn. Observations indicate that peak root demand for carbohydrates occurs when fruit demands are also high. This infers competition between fruit and root growth, disagreeing with observations in other species. Carbohydrate partitioning between fruit and roots was studied in small kiwifruit plants using the tracer $^{14}C$. Simultaneous tracer import was observed into fruit and roots. When half the root system was cut off, root system import was reduced. The fruit imported a larger proportion of the available tracer, whilst there was no change in import into the remaining root half. Simultaneous carbohydrate import into fruit and roots demonstrated competition between these sinks. However, increased carbohydrate import into fruit suggests that the fruit has higher priority. The findings of this research suggest that the root system is a more competitive sink in kiwifruit vines than in some other fruit crops.

Trunk girdling and root pruning are used to improve kiwifruit vine productivity. The effects of these practices on vine physiology, and root growth and function were investigated. Girdling reduced photosynthesis, with reductions attributed to stomatal limitation and feedback inhibition. Repeat annual girdling reduced root biomass by approximately 59%. Root growth rates were not affected, however, girdled vines produced less root tips. This suggests that new root production is more of a carbon expense than root extension. Root pruning reduced whole-vine hydraulic conductance. As treatment severity increased, reduced photosynthesis was evident, attributed to reduced sink demand. Roots in other species have demonstrated rapid changes in hydraulic conductance in response to environmental stresses. Hydraulic conductance in intact roots of kiwifruit vines did not respond when 80% of root biomass was removed. However, sap flow increased to a rate approximately 50% higher than pre-treatment. Kiwifruit roots exhibited hydraulic redundancy, which may be a stress tolerance mechanism.

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# Preface

This thesis reports the work of my PhD, carried out between August 2008 and September 2011. The work described has been designed and conducted by me at the Plant and Food Research facilities under the guidance of my supervisory panel; Dr Michael Clearwater (chief supervisor), Dr Peter Minchin, Dr Kevin Patterson and Professor Kevin Gould. My supervisors have helped me develop ideas, learn new techniques and provided revisions to manuscripts. All experimental work has been conducted by me, apart from: monitoring fruit and vegetative growth in the orchard grown vines (chapter 3), routine root image collection (chapter 4), determining X-ray detector efficiency and liquid scintillation spectrophotometry (chapter 5).

The thesis is laid out in six chapters, a general introduction, four experimental chapters and a general discussion. The four experimental chapters have been written as standalone scientific manuscripts for publication.

Chapter 2 has been published in the New Zealand Journal of Crop and Horticultural Science:

> Black MZ, Patterson KJ, Gould KS, Clearwater MJ 2011. Physiological responses of kiwifruit vines (*Actinidia chinensis* Planch. var. *chinensis*) to trunk girdling and root pruning. New Zealand Journal of Crop and Horticultural Science. DOI: 10.1080/01140671.2011.603343

Chapter 3 has been published in Tree Physiology:

> Black MZ, Patterson KJ, Minchin PEH, Gould KS, Clearwater MJ 2011. Hydraulic responses of whole-vines and individual roots of kiwifruit (*Actinidia chinensis*) following root severance. Tree Physiology 31: 508 - 518.

Chapter 4 is in preparation for submission to Plant and Soil.

Chapter 5 has been submitted as a techniques paper to Planta (date of submission 25th August 2011).

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# 1 Introduction

## 1.1 General introduction

Plant growth involves a complex suite of physiological processes and hormonal signals. In terms of resource acquisition there are two vital functions of land plants, firstly the ability to take $CO_2$ from the atmosphere and produce carbohydrates in a form that can be mobilised around the plant for use in tissue construction and the provision of energy. Secondly, the ability to acquire water from the soil and transport it around the plant to maintain tissue hydration and for use in physiological processes. Both of these processes are of interest to the study of plant physiology.

A plant is a composite of organs, connected via the vascular system. In regards to the partitioning of carbohydrates within a plant, organs can be divided into sources and sinks. Sources are organs that produce more carbohydrates than they require for growth, for example leaves. Sinks are organs that utilise more carbohydrates than they can produce, for example fruit and roots (Wareing & Patrick 1975). Carbohydrates are used in all plant organs for the production of amino acids and proteins for tissue construction and in respiration providing energy for physiological processes. Carbohydrates produced in the leaves are transported to the growing sinks by the phloem. Biomass accumulation is therefore a result of the photosynthetic capacity of sources, efficient carbohydrate distribution via the phloem vasculature and efficient utilisation by the sink organs (Minchin & Thorpe 2003).

Plants are considered to develop a functional equilibrium between roots and shoots (Richards & Rowe 1977; Brouwer 1983). The size of the root system will be sufficient to meet the water and nutrient requirements of the canopy. The canopy will be of sufficient size to meet the carbohydrate requirements of the root system. This highlights the importance of each part of the plant to the function and survival of the remainder and suggests that within the plant all organs are of equal importance. However, this is not the case with carbohydrate partitioning. The carbohydrate demand of organs is variable and is related to size and metabolic activity. Carbohydrate demand is referred to as sink strength and

indicates the amount of carbohydrates that may be utilised by an organ if supply is not limiting (Wareing & Patrick 1975). Sink strength does not determine the order in which sinks are provided with carbohydrates. A hierarchy has been suggested, describing the order in which the carbohydrate demands are met. This hierarchy is described as sink priority; fruit growth has a higher priority than shoot growth, which has a higher priority than root growth (Wardlaw 1990). An alternative theory is the 'optimality theory'. This suggests that carbohydrates are primarily partitioned to the organs that can obtain the resource that is in shortest supply. Therefore water deficiency would increase partitioning to the roots (Reich 2002). The ratio of root-shoot biomass and therefore the sink strength of organs will vary throughout the growing season and throughout the life of the plant as demands for substrates by these organs vary.

Kiwifruit (*Actinidia* sp.) production is a large horticultural industry in New Zealand. To achieve high yields and high quality fruit growers use techniques to manipulate carbohydrate partitioning in favour of fruit growth. Such manipulations are usually at the expense of carbohydrate partitioning to the roots. The importance of the root system as a carbohydrate sink remains to be determined. There is little knowledge of how manipulating carbohydrate partitioning in favour of the fruit impacts on the root system of the kiwifruit vine or how effects on the root system in turn affect vine physiology and productivity.

## 1.2 Carbohydrate partitioning in the kiwifruit vine: the significance of the root system

The roots require a continued carbohydrate supply to provide the raw materials required for growth and maintenance of root tissue and to provide energy for physiological processes, such as nutrient uptake (Fitter 2002). During respiration carbohydrates are metabolised to provide the energy required for growth and maintenance processes. Respiration accounts for a large proportion of root carbohydrate consumption, consuming up to two thirds of the carbohydrates transported to the roots. Daily root respiration can utilise up to half of a plants daily carbohydrate production. Respiratory consumption of carbohydrates changes with plant age, species vigour, nutrient availability, environmental conditions and

mycorrhizal associations (Lambers et al. 2002). Depending on root longevity, maintenance costs may exceed construction costs (Eissenstat & Yanai 1997).

The growth of the root system is usually considered to be a lower priority for carbohydrate supply than shoot and reproductive growth. However, root systems often comprise a significant component of whole-plant biomass and will therefore be a large consumer of carbohydrates. The root-shoot ratio of kiwifruit vines is high in comparison with other fruit trees. Biomass estimates in mature 'Hort16A' kiwifruit vines by Boyd et al (2010) indicated a perennial tissue root-shoot ratio of 1.1. This is considerably higher than root-shoot ratios of 0.4 in 2 year old *Betula pendula* (Yamaji et al. 2003), 0.3 in grape (*Vitis vinifera*) rooted cuttings (Grechi et al. 2007) and 0.2 in apple (*Malus domestica*) trees (Palmer 2007). Due to its large size it can be hypothesised that the root system of the kiwifruit vine will be a considerable sink for carbohydrate utilisation.

Numerous observations demonstrate the large size of the kiwifruit vine root system, suggesting that it will be a large carbohydrate sink. Kiwifruit vine root lengths have been estimated up to 34 km $vine^{-1}$ (Buwalda & Smith 1987). In mature vines, root dry weights have been estimated at 26.5 kg $vine^{-1}$ (Boyd et al. 2010). Uneven distribution of kiwifruit roots results in variable root length densities (RLD; Greaves 1985). Kiwifruit vines generally exhibit RLD that are higher than other fruit species (Xiloyannis et al. 1992; Hughes et al. 1995). Mean RLD have been estimated in the order of 0.2 – 1.6 cm $cm^{-3}$ (Greaves 1985; Gandar & Hughes 1988; Reid et al. 1992). These RLD can be compared to mean RLD of 0.93 cm $cm^{-3}$ for peach (*Prunus persica*), 0.15 cm $cm^{-3}$ for apple, and 0.09 cm $cm^{-3}$ for grape and Asian pear (*Pyrus pyrifolia*; Hughes et al. 1995).

Turnover is the continuous process of root death and re-growth. Turnover is thought to be a means where by the root system can lose roots that are inefficient or growing in less favourable conditions, in order to provide more energy to those in better soil conditions (Eissenstat & Yanai 2002). A considerable proportion of root carbohydrate consumption may be involved in root turnover. Reid et al (1993) calculated turnover indices for kiwifruit vines based on the average of relative growth and death rates of fine roots measured using a rhizotron. Over a two year period their estimates yielded an average turnover index of 1.2% per day,

ranging 0.5% – 3%. Different techniques have been used to estimate root turnover in kiwifruit and other species, complicating comparisons. The turnover rates estimated by Reid et al. (1993) are lower than those estimated by Cheng et al. (1990) for sorghum (*Sorghum bicolor;* 1% – 5.5% $day^{-1}$).

In perennial deciduous plants such as the kiwifruit vine the root system is an important carbohydrate reserve. Carbohydrates stored in the perennial tissues provide energy for spring canopy development. During spring reserves are mobilised and transported to the canopy. These reserves are especially important for kiwifruit vines as the period of carbohydrate mobilisation is longer than that in other fruit crops (Loescher et al. 1990). Approximately 30% of the starch reserves may be mobilised from perennial organs during spring, with largest losses from the roots (Smith et al. 1992). Root carbohydrate contents reach a minimum approximately 60 – 100 days post-bud break (Smith et al. 1992). Reserves in perennial tissues are replenished during the growing season when carbohydrate supply exceeds the demands of growing organs. In kiwifruit, root carbohydrate contents peaked between fruit harvest and leaf abscission (Smith et al. 1992).

The high root-shoot ratio in kiwifruit suggests that growth of the root system is not as low on the priority hierarchy as generally described (Wardlaw 1990). There have been observations of carbohydrate partitioning in kiwifruit vines that conflict with the accepted priority hierarchy. Minchin et al. (2010) found shoot growth to have a higher priority for carbohydrate supply than fruit growth. If the priority hierarchy does not stand true for the balance between fruit and shoot growth in kiwifruit then should we believe the low priority of the root system for carbohydrate supply? This question is also raised when we consider horticultural techniques, such as those described below, that are used in kiwifruit orchards to manipulate carbohydrate partitioning. These are used to reduce or temporarily remove the carbohydrate demands of the root system; this generally increases partitioning to the fruit and can result in increased fruit size and carbohydrate content. If the carbohydrate demands of the roots are not supplied until the carbohydrate requirements of fruit growth are fully supplied, why do these treatments enable increased fruit growth? The ability to increase fruit size and carbohydrate content by reducing carbohydrate supply to the roots indicates direct competition between fruit and root growth. Boyd et al (2010) suggested that in

comparison with other fruit crops, such as apple, kiwifruit vines partition a smaller fraction of carbohydrates to fruit growth. This may result from competition for available carbohydrates.

## 1.3 Seasonal trends in root carbohydrate demand

As with fruit and shoot growth, root growth will show seasonal variability in its carbohydrate requirements. Seasonal trends in root carbohydrate demand can be identified by trends in root growth and respiration as both are heavily reliant on the supply of carbohydrates from the canopy. Root growth of many woody species is related to time of year or stage of plant development (Geisler & Ferree 1984a). Due to the suggestion that the root system is a low priority for carbohydrate supply, peaks in root growth might be expected to follow peaks in above-ground growth. In tree species that exhibit a semi-determinate growth habit shoot growth occurs in flushes throughout the growing season and flushes in root growth occur after shoot growth has slowed (Cardon & Herron 2005). In perennial deciduous fruiting species root growth often exhibits a seasonal trend with peaks in growth coinciding with slowed shoot or fruit growth, or fruit harvest. Historically root growth in apple and grape has been suggested to exhibit a bimodal trend with peaks in spring and autumn. When monitoring root growth in apple and grape Eissenstat et al. (2006) failed to find evidence to support this theory. In grape the main period of root production occurred between flowering and veraison, with little growth occurring before flowering, before harvest or during dormancy. Carbohydrate demand of the fruit would be expected to be high during early development so this observation suggests competition between fruit and root growth. In apple, Eissenstat et al. (2006) observed differences in the seasonal trend in root growth between scion cultivars and years. Peaks in root growth were observed either in spring or after harvest, but there was little evidence of bimodal growth (Eissenstat et al. 2006). Psarras et al. (2000) also suggested that root growth in apple trees was not bimodal. They observed peak root growth in summer with minimal growth evident before the onset of shoot growth. The timing of peak root growth occurred when fruit growth was high; at this time shoot growth was slowing. This suggests that the reduction in shoot growth reduced competition for carbohydrates and sufficient was available to meet the demands of fruit and root growth. In contrast to apple and grape, peach

trees exhibited a bimodal trend in root growth with peaks in spring and after fruit had been harvested (Basile et al. 2007). Basile et al. (2007) suggested that the differences in observations between species were attributable to the shorter fruit growth season in peach. Early fruit harvest would allow more time for the roots to obtain carbohydrates before leaf senescence.

Seasonal trends in root growth have been studied in Hayward kiwifruit (*A. deliciosa*), though discrepancies are evident in the results. Buwalda and Hutton (1988) observed peak root growth between February and March. They suggested that peaks in root growth followed peaks in shoot and fruit growth, and were related to competition for carbohydrate supply (Buwalda & Hutton 1988). The main period of shoot growth in Hayward kiwifruit ends around January (Buwalda & Hutton 1988; Davison 1990) and fruit growth is high between December and February (Richardson et al. 1997; Minchin et al. 2003). As with the observations in grape by Eissenstat et al. (2006), this suggests competition between fruit and root growth. Other observations in kiwifruit have shown less of a seasonal trend and combined with observations of white roots during winter months led to the suggestion that root growth occurred throughout the year (Lemon 1986; Reid et al. 1993). These discrepancies may result from different measuring techniques. If the seasonal carbohydrate demands of the root system are to be better understood in kiwifruit more research is required to clarify the occurrence of seasonal trends in root growth. Further, it remains to be clarified whether root growth competes with fruit growth for carbohydrate supply.

Root respiration is a considerable component of total soil respiration. Quantification of the contribution of root respiration to total soil respiration has estimated contributions between 10% and 60% (Wang et al. 2008; Ceccon et al. 2011). The large contribution of root respiration to total soil respiration enables trends in carbohydrate supply to the rhizosphere to be studied from trends in total soil respiration (Ryan & Law 2005). Root respiration is heavily reliant on continued supply of carbohydrates from the canopy, as has been demonstrated by trunk girdling of conifer trees (Högberg et al. 2001). Seasonal changes in root respiration will therefore also be related to the development of above-ground growth and the availability of carbohydrates (Cardon & Herron 2005). Wang et al (2008) observed considerable variation in the contribution of root respiration to

total soil respiration throughout the year. Variation was attributed to changing environmental conditions as well as changing physiological activity in the roots. Seasonal trends in root respiration could be used to identify seasonal trends in carbohydrate demand that are related to physiological processes such as nutrient uptake, that are not necessarily related to seasonal trends in root growth.

## 1.4 Hydraulic capacity of the kiwifruit vine

Kiwifruit vines utilise large quantities of water (Chartzoulakis et al. 1993). Estimates of daily transpiration rates for kiwifruit vines in New Zealand include 31 L $day^{-1}$ in 7 year old *A. deliciosa* vines (Green & Clothier 1995) and 60 L $day^{-1}$ in *A. chinensis* vines (age unknown; Prendergast et al. 2007). These water use estimates can be compared with transpiration rates of up to 36 L $day^{-1}$ for 10 year old apple trees (Green et al. 1989) and up to 40 L $day^{-1}$ for 5 year old grapevines (Dragoni et al. 2006). Whole-plant hydraulic conductance is suggested to be high in kiwifruit in comparison with other species (Dichio et al. 1999), with conductance values of 0.44 – 0.69 $\times 10^{-4}$ kg $MPa^{-1}$ $m^{-2}$ $s^{-1}$ (Clearwater et al. 2004). High hydraulic conductance is typical of lianoid growth forms (Ewers 1985). In kiwifruit vines the vascular tissue is comprised of semi-ring porous wood. Large xylem vessels averaging 140 – 160 mm in length and up to 0.5 mm in diameter (Condon 1992) provide low resistance to water transport.

If soil water availability is not limiting, approximately 90% of daily transpiration will be obtained directly from the soil (Nuzzo et al. 1997). Therefore we can hypothesise that kiwifruit roots will be efficient in the uptake and transport of water and that mechanisms will exist in the roots to ensure water supply continues, even under stress conditions.

Under normal conditions when plants are transpiring, the flow of water through roots follows a water potential gradient (Steudle & Peterson 1998). Water can take one of three radial pathways into the root from the soil: apoplastic, moving through the cell walls; symplastic, moving from cell-to-cell or transcellular, moving across cell membranes (Javot & Maurel 2002). Cell-to-cell transport is facilitated by plasmodesmata (Steudle & Ranathunge 2007) and transcellular transport is facilitated by aquaporins, through gating or through changes in their abundance (Bramley et al. 2007; Steudle & Ranathunge 2007). Aquaporins are

proteins found in plant cell membranes that create channels for water movement (Javot & Maurel 2002). There is evidence of dynamic root responses to environmental stimuli that are considered to be a mechanism for minimising negative effects of environmental conditions on plant water status. These rapid responses have been attributed to aquaporin activity (Vandeleur et al. 2009). The contribution of aquaporins to root hydraulic conductance depends on the contributions of the different radial pathways to total radial flow, and this has been shown to vary between species (Bramley et al. 2009). Efficiency of water uptake and radial flow varies with age and development stage of the root. Anatomical development such as deposition of suberin and the development of Casparian bands increases resistance along the radial pathway, reducing apoplastic flow (Steudle & Ranathunge 2007). In onion (*Allium cepa*), older root segments showed higher hydraulic conductivity than younger segments. Higher conductivity in the mature root segments was attributed to the contribution of the cell-to-cell pathway to total radial flow and the involvement of aquaporins (Barrowclough et al. 2000). There is little knowledge as to the water uptake pathways involved in kiwifruit roots. If the transcellular pathway contributes significantly to water uptake in mature roots then with the large proportion of the kiwifruit vine root system having undergone secondary development it may be hypothesised that aquaporin activity will be important. High aquaporin involvement might enable tolerance to root manipulations and be a mechanism enabling this high water using species to tolerate reductions in water availability.

Root anatomy plays an important role in water transport. Kiwifruit root xylem vessel diameters have been measured in the range of 120 to 500 µm (McAneney & Judd 1983), which is large in comparison with other species. Avocado (*Persea americana*) roots for example, exhibit root xylem vessel diameters of approximately 27 µm (Fassio et al. 2009). These large diameter vessels in kiwifruit will provide a low resistance pathway for water transport and may result in high root hydraulic conductance. There have been few measurements of root hydraulic conductance in kiwifruit vines; however, rates of xylem sap flow have been measured. In a 7 year old vine, with mean transpiration rates of 0.3 – 0.4 ml $s^{-1}$, the rate of sap flow through roots of diameters 23 – 32 mm was between 0.003 – 0.02 ml $s^{-1}$ (Green & Clothier 1995). This is comparable to sap flow rates of

0.002 – 0.01 ml $s^{-1}$ for roots of diameter 19 – 21 mm, in a 14 year old apple tree (Green et al. 1997).

## 1.5 Horticultural manipulations in kiwifruit

In agricultural and horticultural crops growers continuously strive to manipulate carbohydrate partitioning in favour of the economically important components of the plant. These manipulations are often at the expense of the root system and may have negative impacts on water and nutrient uptake and carbohydrate reserves. In the kiwifruit industry there are two techniques that have raised concerns in regards to negative effects on the root system and the likely long-term impacts of repeated use. These techniques are trunk girdling and root pruning.

Girdling has been used in horticulture for many years to improve productivity in a range of crops, including grape, *Citrus* sp., apple and peach (Goren et al. 2003). Girdling involves the removal of a band of bark including the phloem tissue from around the circumference of the trunk, taking care to minimise damage to the cambium and xylem vessels (Goren et al. 2003). Trunk girdling disrupts phloem transport, temporarily preventing the downward transportation of carbohydrates to the roots. This temporarily removes the root system from the whole-plant carbon balance and makes more carbohydrates available to plant parts above the girdle. Girdles heal in kiwifruit, usually within 4 weeks. This is similar to the healing time observed in grape (Williams et al. 2000). Healing involves the formation of callus tissue starting at the edges of the wound and growing to bridge the entire girdle. Callus cells in contact with vascular cambium differentiate into cambial cells. New vascular cambium forms across the girdle, resulting in new phloem tissue that reconnects the undamaged phloem above and below the girdle (Goren et al. 2003). Phloem reconnection is assumed to occur within a few days of complete callus bridging (Sidlowski et al. 1971; Pang et al. 2008). The benefits of trunk girdling kiwifruit vines include; consistent increases in fruit dry matter content, increases in fruit size, increased flowering, reduced vegetative vigour and advanced maturity (Currie et al. 2005; Patterson et al. 2006).

Root pruning involves a tractor drawn blade being dragged through the soil along the row of plants, severing roots in its path. The severity of the treatment can be increased by using longer blades and cutting closer to the base of the trunk. In

kiwifruit vines root pruning has demonstrated potential to increase fruit size and dry matter content and advance fruit maturity (Patterson et al. 2009). The theory behind increased fruit growth following root pruning is that reducing the size of the root system results in reduced carbohydrate demand by the root system. This makes a larger proportion of the carbohydrate supply available to above-ground organs. An alternative theory is that reductions in vegetative vigour observed in a number of species following root pruning (Schupp & Ferree 1987; Khan et al. 1998) is a result of reallocation of carbohydrate reserves. When the size of the root system is reduced the root-shoot ratio is disrupted and carbohydrate reserves are re-directed to the root system to regain the balance between root and shoot area (Geisler & Ferree 1984a).

There has been little investigation into the physiological mechanisms involved in the responses of kiwifruit vines to these practices. The physiological response to trunk girdling has been studied in grape, citrus and conifer trees, amongst others. Following trunk girdling reductions in photosynthesis, stomatal conductance and transpiration have been observed and it has been suggested that the primary response is photosynthetic (Roper & Williams 1989; Williams et al. 2000; Iglesias et al. 2002; Domec & Pruyn 2008). Physiological responses to root pruning include reduced leaf xylem pressure potentials, stomatal conductance and photosynthesis in apple and grape (Geisler & Ferree 1984b; Smart et al. 2006), indicating that the response is primarily through a reduction in water supply to the canopy. The effects of these manipulations on the root system warrant investigation in kiwifruit. There is little evidence as to how trunk girdling affects the kiwifruit vine root system. Girdling has been shown to reduce root growth in Japanese persimmon (*Diospyros kaki*; Fumuro 1997), which may lead to reduced root biomass and result in reduced water and nutrient uptake. Further, root carbohydrate reserves have been reduced in grape and peach following trunk girdling (Roper & Williams 1989; Jordan & Habib 1996). In kiwifruit we need to determine the effects of annual girdling on the size of the root system and what this means for the long-term health and productivity of vines. Of particular interest with root pruning is the role of the root system in reducing negative effects on the whole-vine water status.

## 1.6 Objectives and hypotheses

The general aim of this research was to provide a better understanding of the function and structure of the kiwifruit vine root system.

Research objectives included:

- Identify physiological responses to vine manipulations.
- Investigate root hydraulic function in response to vine manipulations.
- Clarify seasonal trends in root carbohydrate demand.
- Determine factors influencing root growth.
- Investigate the priority of the root system for carbohydrate supply.

The objectives and hypotheses of each of the experiments are outlined below.

**Chapter 2**

***Physiological responses of kiwifruit vines to trunk girdling and root pruning***

This experiment investigated whole-vine hydraulic and photosynthetic responses to trunk girdling and root pruning. The objective of the experiment was to identify and compare the physiological response of kiwifruit vines to these two manipulations. It was hypothesised that temporary removal of the root system as a carbohydrate sink by trunk girdling would reduce sink demand leading to reductions in photosynthesis. Further, it was hypothesised that root pruning would moderate photosynthesis through stomatal closure, following a reduction in whole-vine hydraulic conductance.

**Chapter 3**

***Hydraulic responses of whole-vines and individual roots of kiwifruit following root severance***

In this experiment the response of whole-vine and individual-root hydraulic conductance to root pruning was monitored. The objective of this experiment was to investigate the physiological response to root pruning and to identify physiological changes in the root that may negate the negative effects of reducing water uptake capacity. It was hypothesised that following root pruning, these

mature, woody vines would acclimate to the reduction in root surface area through rapid increases in the hydraulic conductance of the remaining intact roots.

**Chapter 4**

***Seasonal trends in root growth and soil respiration of kiwifruit vines: considerations for carbohydrate partitioning***

This experiment used an adaptation of the mini-rhizotron to investigate the seasonality of root growth in kiwifruit vines and the effects of trunk girdling. The objective was to identify seasonal trends in root growth and soil respiration that could be used to determine the seasonality of root carbohydrate demand. A second objective was to determine the impact of annual trunk girdling on root growth, biomass and function. It was hypothesised that seasonal trends in root growth would be related to plant carbohydrate availability, with peaks in root growth occurring after peaks in fruit and shoot growth had slowed. Further, it was hypothesised that disrupting carbohydrate supply to the roots by trunk girdling would impose a transitory reduction in root respiration and root growth rates. The repeated annual use of trunk girdling was hypothesised to reduce root biomass.

**Chapter 5**

***Carbohydrate partitioning in kiwifruit: the effects of root manipulation***

A series of experiments were conducted using a novel system for *in vivo* monitoring of the distribution of recently fixed tracer-labelled photosynthates. The objective of these experiments was to investigate competition between fruit and roots to determine the effect of root pruning on carbohydrate partitioning to the fruit and the remaining roots. The experiments investigated the hypothesis that when part of the root system is cut-off, root system demand for carbohydrates is reduced and partitioning of tracer to the fruit is increased.

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# 2 Physiological responses of kiwifruit vines to trunk girdling and root pruning

## 2.1 Introduction

In order to meet quality standards set by kiwifruit (*Actinidia* sp.) marketers, New Zealand kiwifruit growers have adopted a range of innovative orchard practices. Two such practices, trunk girdling and root pruning, are quite invasive to the plant and raise questions regarding how plant function is affected. Trunk girdling and root pruning were introduced to New Zealand kiwifruit orchards with the aim of increasing fruit size and percentage dry matter (DM), with DM being associated with fruit sweetness (Harker et al. 2009).

Trunk girdling involves the removal of a band of bark and phloem tissue, approximately 5 mm in width, from around the circumference of the trunk, avoiding damage to the xylem. Trunk girdling temporarily increases the availability of carbohydrates to the developing fruit through disruption of the source:sink ratio of the vine, by temporarily removing the root system as a carbohydrate sink (Noel 1970). The technique was adopted by New Zealand kiwifruit growers in the early 2000s, following demonstration of its success in a number of other fruit crops (Goren et al. 2003). In grapes, for example, berries are larger and mature earlier (Weaver 1976; Williams et al. 2000). Consistent positive effects on fruit size and DM content have been achieved as a consequence of trunk girdling kiwifruit vines at particular times of the year (Currie et al. 2005) and the technique is now widely used.

Root pruning entails a tractor-drawn blade, approximately 300 – 400 mm in length (Schupp & Ferree 1987; Kurtural & Taylor 2004), being dragged through the soil, along the row, within 450 – 600 mm of the trunk (Ferree et al. 2000; Kurtural & Taylor 2004). Detling et al (1980) suggested that root pruning had a temporary effect on carbohydrate partitioning, with the reduced size of root system requiring a smaller fraction of carbohydrates. Root pruning is a more recent introduction to the kiwifruit industry; trials have shown consistent increases

in fruit DM content and the potential to increase fruit size (Patterson et al. 2009), promoting interest in the technique. In addition, root pruning has the potential to reduce vegetative vigour of the kiwifruit vine, as has been observed in other fruit crops (Ferree 1990; Maas 2008).

Previous research into the use of these techniques in kiwifruit has studied the effects on fruit quality and growth (Currie et al. 2008; Patterson et al. 2009); however, little is known regarding the effects on physiological processes within kiwifruit vines. Trunk girdling and root pruning both have an impact on the root system. Following trunk girdling, reductions in root carbohydrate reserves have been observed in peach and kiwifruit (Jordan & Habib 1996; Currie et al. 2005), along with reductions in fine root production in Japanese persimmon (Fumuro 1997). Annual girdling and the application of multiple girdles in a single season may magnify these effects in the long term. The nature of the physiological response to trunk girdling is not clear. Correctly applied girdles should not cause substantial damage to the xylem and should therefore not directly affect hydraulic conductance. In grape, reductions in stomatal conductance have been observed following trunk girdling (Roper & Williams 1989; Williams et al. 2000), although this was not associated with reductions in leaf water potential (Williams et al. 2000), which suggests a photosynthetic rather than hydraulic response. In oak, however, trunk girdling reduced xylem sap flux within 2 days of treatment (De Schepper et al. 2010). The observed reduction was too rapid to have resulted from effects of limited carbohydrate supply on root system size. Reduced water use following trunk girdling has been attributed to an indirect response, with reduced transpiration related to stomatal limitation of photosynthesis (Williams & Ayars 2005; De Schepper et al. 2010).

Root pruning has an immediate, long-term impact on the size of the root system, the severity of which is determined by the proportion of root system removed. Reducing root surface area will disrupt the plant's carbohydrate balance, as well as affecting water uptake capacity. The effects of root pruning on plant hydraulics and carbon assimilation are not clearly understood. The reduction in root area is thought to reduce root system demand for carbohydrates, making more available to other organs. Consistent with that hypothesis are observations of larger fruit and increases in fruit carbohydrate content (Patterson et al. 2009). In contrast,

vegetative growth is often reduced; this reduction in vegetative growth is unlikely to occur through carbohydrate limitation; therefore, it may be suggested that a hydraulic effect is limiting vegetative growth. Reductions in stomatal conductance, transpiration and leaf water potentials have been found in apple, grape, pear (*Pyrus communis*) and peach (Geisler & Ferree 1984; Poni et al. 1992; Smart et al. 2006), confirming a hydraulic response following root pruning.

The objective of this experiment was to compare the physiological responses of kiwifruit vines to trunk girdling and root pruning. We hypothesised that in kiwifruit, trunk girdling manipulates carbohydrate partitioning, thereby reducing sink demand and resulting in reduced photosynthesis. Photosynthetic reductions could involve both biochemical and stomatal limitations. We further hypothesised that root pruning moderates photosynthesis through reduced stomatal conductance. It was hypothesised that a reduction in root area would result in rapid and significant reductions in hydraulic conductance, stomatal conductance and transpiration.

## 2.2 Materials and methods

### 2.2.1 Plant material

An experiment was conducted in a plastic greenhouse at the Plant & Food Research Te Puke research orchard, New Zealand. Vines were 9 year-old yellow kiwifruit (*Actinidia chinensis* Planch. var. *chinensis* 'Hort16A') grafted onto open pollinated green kiwifruit (*Actinidia deliciosa* (A.Chev.) C.F. Liang et A.R. Ferguson var. *deliciosa*) rootstocks. Grafted seedlings were transferred to 100 L fabric root control bags (Smart Pot – In Ground™), before being planted out in the orchard. The root control bags were buried and vines were maintained for approximately 7 years, managed according to standard practice for the cultivar, adapted from recommendations for *A. deliciosa* (Sale & Lyford 1990). The annual fertiliser regime for the vines included a base application of potassium, phosphate, sulphur and magnesium in October and applications of nitrogen in November, January and July. Vines were irrigated with individual sprinklers in close proximity to the trunk. The vines were trained to a T-bar support system.

### 2.2.2 Experimental conditions

In November 2008, approximately 1 month post-anthesis, plants were transferred from the ground, remaining in the root control bags, to custom-made 150 L pots. Pots were fitted with a drainage tube and a metal frame work for supporting the trunk and leader. Vines were left to acclimatise outdoors for 3 weeks, before being transferred to individual weighing lysimeters in the plastic greenhouse. Fans were used to assist natural air flow ventilation. Pots were irrigated with individual sprinklers; vines received three applications of 6 L of water, equally distributed throughout the day. The pots were covered with transparent plastic to prevent soil evaporation.

Treatments were applied on 12 February 2009, with three replicates, ensuring vines from the same treatment were not positioned next to each other. Timing of treatment application was chosen to coincide with one of the recommended commercial timings for trunk girdling (Currie et al. 2008). Trunk girdling involved the use of a modified blunt chainsaw blade to remove a band of bark including the phloem tissue, approximately 5 mm in width, from around the trunk. Care was taken to ensure that all phloem tissue was removed whilst minimising damage to the xylem tissue. Root pruning was performed by cutting vertically to the base of the pot, along two sides of the trunk, approximately 150 mm away from the trunk, with an electric reciprocating saw (CR18DS, Hitachi, Tokyo). This removed approximately 50% of the root ball; the severed portion was left in place.

### 2.2.3 Hydraulic conductance

Transpiration ($E_{plant}$) was estimated based on weight loss after correcting for drainage. Drainage from each pot was measured using individual tipping bucket rain gauges (Rain-O-Matic, Scott Technical Instruments Ltd., Hamilton). Weighing lysimeters consisted of a load-cell (Model 60060C1000KG-300M, Sensortronics, California) sandwiched between two 1 $m^2$ aluminium plates; load-cells and rain gauges were connected to a datalogger via a multiplexer and a switch closure input module respectively (CR10X; AM416; SDM SW8A, Campbell Scientific, Utah). Average weight of each potted vine and total drainage were recorded at 15 minute intervals.

Xylem pressure potentials ($\Psi$, MPa) were measured using a pressure chamber (model 3005, Soil Moisture Equipment Corp., Santa Barbara); measurements were made on three leaves per vine at pre-dawn, on uncovered leaves ($\Psi_{soil}$) and at midday on both covered ($\Psi_{shoot}$) and un-covered ($\Psi_{leaf}$) leaves. Pre-dawn measurements were assumed to be equivalent to soil water potential ($\Psi_{soil}$) (Ritchie & Hinckley 1975). Midday assessments of non-transpiring covered leaves approximate xylem pressure within the shoot (Meinzer et al. 2001). Covered leaves were enclosed in foil coated plastic bags, a minimum of 2 hours prior to measurement. Midday $\Psi_{shoot}$ and $\Psi_{leaf}$ were measured on clear sunny days, between 1100 h and 1500 h, using mature fully expanded leaves; measurements were made at six timings post-treatment. Soil water potential was assessed at four timings post-treatment.

Hydraulic conductance was calculated for the soil to shoot ($K_{shoot}$) and soil to leaf ($K_{plant}$) pathways as:

$$K = E_{plant} / \Delta\Psi \qquad (2.1)$$

where $E_{plant}$ is the mean rate of $E_{plant}$ between 0900 h and 1500 h per vine for the days when $\Psi$ was measured. $\Delta\Psi$ is the difference between $\Psi$ at points along the hydraulic pathway ($\Psi_{soil} - \Psi_{shoot}$ for $K_{shoot}$ and $\Psi_{soil} - \Psi_{leaf}$ for $K_{plant}$).

Sapwood area specific conductance ($K_s$) was calculated to account for differences in trunk cross-sectional area;

$$K_s = K / A_{sw} \qquad (2.2)$$

Trunk sapwood area was estimated from measurements of trunk diameter inclusive of bark and bark thickness at points around the trunk, above and below the visible graft union. Sapwood area ($A_{sw}$, cm²) for rootstock and scion was calculated, assuming all tissue below the bark was conducting.

### 2.2.4 Photosynthesis and stomatal conductance

Stomatal conductance ($g_s$) and net photosynthetic rate ($A$) were measured and leaf internal $CO_2$ concentration ($c_i$) calculated using a portable gas exchange system (LI-6400, LI-COR Inc., Nebraska), at times to coincide with measurements of $\Psi_{shoot}$ and $\Psi_{leaf}$. Measurements were taken on four to six mature, fully expanded,

sunlit leaves per vine, using natural light. Chamber $CO_2$ concentration was controlled at 400 µmol $mol^{-1}$ and vapour pressure deficit was maintained at an average of 2.5 kPa. Leaf samples were collected for $\delta^{13}C$ isotope analysis from each vine at weekly intervals; two samples were taken per vine. Each sample comprised six newly emerging leaves selected from each vine, based on the assumption that expanding leaves are representative of recently fixed carbon (Meinzer et al. 1993). Samples were dried at 80°C to a constant weight; finely ground and 2.8 mg samples weighed into foil cups. A mass spectrometer (20/20 isotope analyser, Europa Scientific, Crewe) at the University of Waikato Stable Isotope Unit was used to measure $\delta^{13}C$ (‰) discrimination in comparison with the Pee Dee belemnite standard.

### 2.2.5 Fruit measurements

Percentage fruit DM was assessed at weekly intervals, from samples of four fruit per vine. Actual fruit fresh weight (FW), fruit length and maximum and minimum diameters at the equatorial region were recorded for each fruit. A 2 mm thick cross-sectional slice was taken from the equatorial region of each fruit, weighed and dried in a dehydrator at 65°C until constant weight was achieved. Dried slices were re-weighed (DW) and DM content was calculated as:

$$DM = (DW / FW) * 100 \quad (2.3)$$

Length and diameter measurements (mm) and actual FW (g) of the sampled fruit were used in a regression analysis to obtain a calibrated multiplier for non-destructive fruit FW estimates as described by Minchin et al. (2003). The slope of the regression line, used as the multiplier was 0.54, with an $R^2$ of 97.5%. Fruit length ($L$), and maximum and minimum diameters at the equatorial region ($D_{max}$ and $D_{min}$ respectively) were measured weekly post-treatment on six marked fruit per vine and fresh weight calculated as:

$$FW = (((L * D_{max} * D_{min}) / 1000) * 0.54) \quad (2.4)$$

### 2.2.6 Statistical analysis

Statistical analyses were carried out in R version 2.10.01 (Comprehensive R Archive Network, cran.r-project.org). Data were tested for normal distribution using the Shapiro-Wilk test and log transformations performed to normalise data

where required for ANOVA analyses. Treatment differences were tested using ANOVA. Pairwise Least Significant Difference (LSD) tests were used to compare treatments where significant differences were found. Dieback of a single root-pruned vine towards the end of the study led to the exclusion of data from this vine.

## 2.3 Results

Effects on $\Psi$ differed with treatment (Table 2.1). Pre-dawn xylem pressure potential was between -0.01 to -0.1MPa, remaining comparable in control and trunk-girdled vines. Reductions were evident in root-pruned vines. Midday $\Psi_{shoot}$ and $\Psi_{leaf}$ in trunk-girdled vines were comparable to controls. In root-pruned vines midday $\Psi_{shoot}$ and $\Psi_{leaf}$ were significantly reduced within one day of treatment, with reductions maintained for 7 weeks. Midday $\Psi_{shoot}$ and $\Psi_{leaf}$ (±SEM) in root-pruned vines were approximately 0.2 ± 0.02 MPa lower than in control vines.

Midday transpiration in control vines averaged $2.0 \times 10^{-4}$ kg $s^{-1}$, reaching a maximum rate of $3.2 \times 10^{-4}$ kg $s^{-1}$. All vines demonstrated a decline in $E_{plant}$ 1 day prior to treatment, which was maintained throughout the experiment. A treatment effect on $E_{plant}$ was not observed (Figure 2.1A). In general, $K_{shoot}$ and $K_{plant}$ (Table 2.2) in trunk-girdled vines remained comparable to controls. Maintenance of $E_{plant}$, combined with an increase in the soil to shoot pressure gradient in root-pruned vines, indicated reductions in $K_{shoot}$ (Figure 2.1B). Reductions in $K_{shoot}$ ($P < 0.05$) were evident during the 2 weeks post-treatment; however, treatment effects were not maintained to 4 and 5 weeks post-treatment. Whole-vine hydraulic conductance was less affected (Table 2.2) with small reductions ($P > 0.05$) evident in the first week post-treatment. Sapwood area was similar for all vines, averaging 14.0 ± 0.6 $cm^2$ for rootstock and 12.4 ± 0.6 $cm^2$ for scion cross-sections. Whole-vine sapwood area specific conductance ($K_{s\text{-}plant}$) showed close agreement with $K_{plant}$, with no significant differences between treatments. Significant reductions in soil to shoot sapwood area specific conductance ($K_{s\text{-}shoot}$) were observed in root-pruned vines, showing agreement with $K_{shoot}$ (Table 2.2).

**Table 2.1 Post-treatment xylem pressure potentials (MPa) for pre-dawn leaf ($\Psi_{soil}$), midday shoot ($\Psi_{shoot}$), and leaf ($\Psi_{leaf}$). Data for $\Psi_{soil}$ are means $n$ = 3 vines at 3 timings ($n$ = 2 vines for root-pruned), data for $\Psi_{shoot}$ and $\Psi_{leaf}$ are means $n$ = 3 vines at 7 timings ($n$ = 2 vines for root-pruned), ± 1SEM. Values in the same column sharing the same letter are not significantly different ($P$ < 0.05). Xylem pressure potentials were measured with a pressure chamber at pre-dawn ($\Psi_{soil}$) and at midday on covered non-transpiring leaves ($\Psi_{shoot}$) and on openly transpiring leaves ($\Psi_{leaf}$).**

| | $\Psi_{soil}$ | $\Psi_{shoot}$ | $\Psi_{leaf}$ |
|---|---|---|---|
| | | MPa | |
| Control | -0.04 ± 0.003 a | -0.34 ± 0.01 a | -0.54 ± 0.02 a |
| Root-pruned | -0.05 ± 0.005 b | -0.52 ± 0.02 b | -0.71 ± 0.02 b |
| Trunk-girdled | -0.03 ± 0.003 a | -0.36 ± 0.02 a | -0.59 ± 0.03 a |
| *P*-value | 0.0001 | <0.01 | <0.01 |

**Table 2.2 Post-treatment hydraulic conductance ($K$; kg MPa$^{-1}$ s$^{-1}$ × 10$^4$) and scion sapwood area specific hydraulic conductance ($K_s$; kg MPa$^{-1}$ m$^{-2}$ s$^{-1}$ × 10$^4$). Data are means $n$ = 3 vines at 6 timings ($n$ = 2 vines for root-pruned), ± 1SEM. Values in the same column sharing the same letter are not significantly different ($P$ < 0.05).**

| | $K_{shoot}$ | $K_{plant}$ | $K_{s\text{-}shoot}$ | $K_{s\text{-}plant}$ |
|---|---|---|---|---|
| | (kg MPa$^{-1}$ s$^{-1}$ × 10$^4$) | | (kg MPa$^{-1}$ m$^{-2}$ s$^{-1}$ × 10$^4$) | |
| Control | 6.62 ± 0.52 a | 3.97 ± 0.27 a | 0.57 ± 0.07 a | 0.30 ± 0.03 a |
| Root-pruned | 4.89 ± 0.37 b | 3.26 ± 0.18 a | 0.39 ± 0.02 b | 0.25 ± 0.01 a |
| Trunk-girdled | 5.38 ± 0.42 ab | 3.63 ± 0.24 a | 0.47 ± 0.04 ab | 0.30 ± 0.02 a |
| *P*-value | 0.0152 | 0.1655 | 0.0392 | 0.3485 |

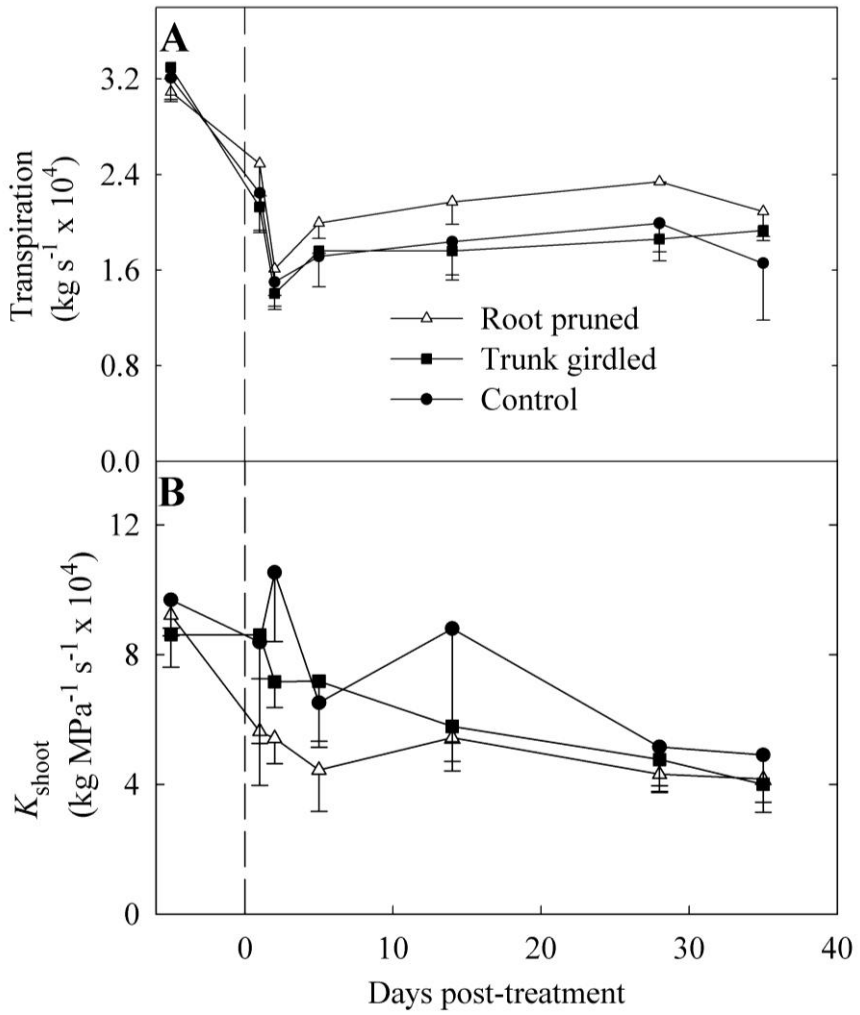


**Figure 2.1 A) Average transpiration rate ($E_{plant}$; kg $s^{-1}$ × $10^4$) between 0900 h and 1500 h for root-pruned, trunk-girdled and control vines as used to calculate hydraulic conductance. B) Hydraulic conductance for the soil to shoot pathway ($K_{shoot}$; kg $MPa^{-1}$ $s^{-1}$ × $10^4$). Treatments were applied on 12 February 2009 (dotted line). Data are means *n* = 3 vines (*n* = 2 for root-pruned), - 1SEM.**

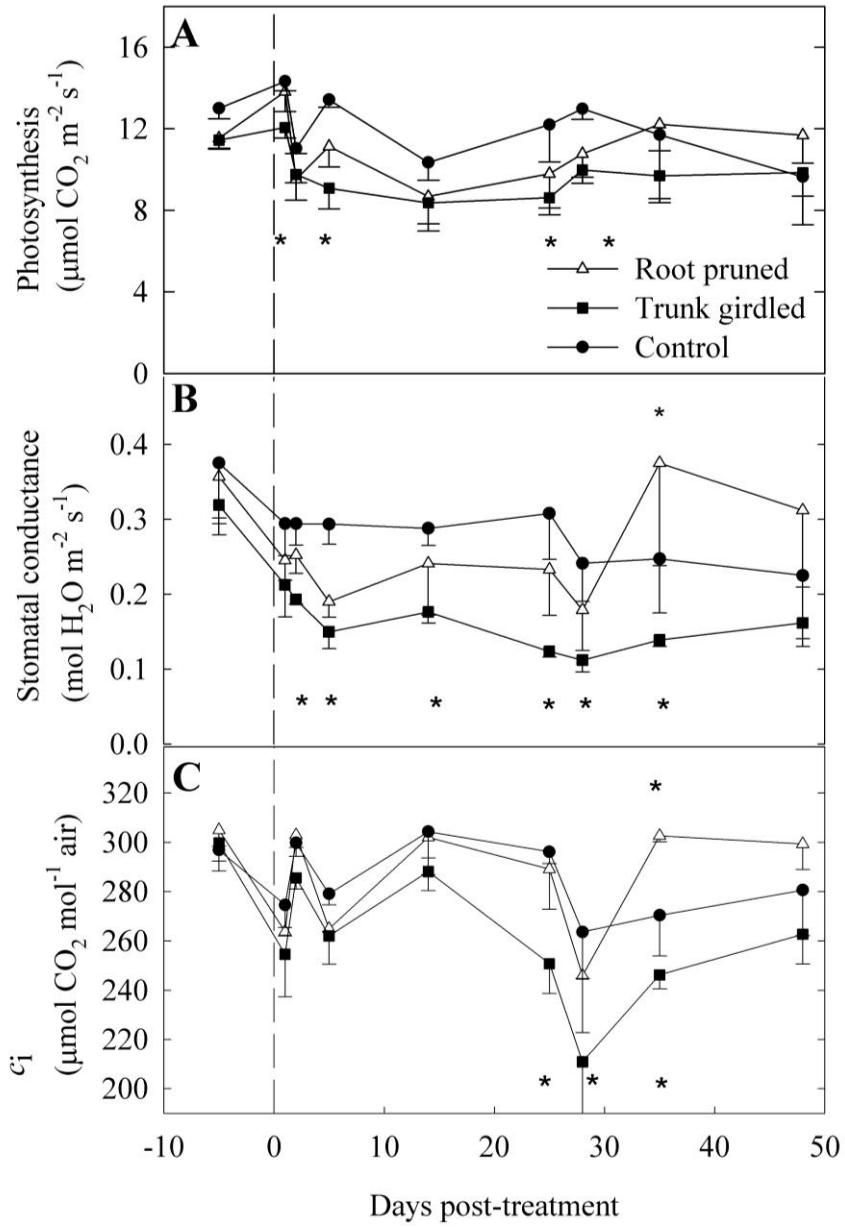


**Figure 2.2 Leaf gas exchange characteristics of root-pruned, trunk-girdled and control vines. A) Photosynthetic rate (µmol $CO_2$ $m^{-2}$ $s^{-1}$). B) Stomatal conductance (mol $H_2O$ $m^{-2}$ $s^{-1}$). C) Leaf internal $CO_2$ concentration ($c_i$; µmol $CO_2$ $mol^{-1}$ air). Treatments were applied on 12 February 2009 (dotted line). Data are means *n* = 6 - 12 leaves, - 1SEM. '*' indicates a significant difference in comparison with the control at that time.**

Trunk girdling had a larger effect on $A$ (Figure 2.2A) and $g_{s,}$ (Figure 2.2B) than root pruning, resulting in consistent and often significant reductions. In trunk-girdled vines $c_i$ was significantly reduced; however, the response was delayed in relation to effects on $A$ and $g_s$, with significant differences emerging 25 days post-treatment (Figure 2.2C). Net photosynthesis and $g_s$ were reduced in root-pruned vines compared with control vines, though not significantly. Final measurements, at 5 and 7 weeks post-treatment, showed an increase to rates higher than that of control vines. Leaf internal $CO_2$ concentration of root-pruned vines was maintained at levels comparable to controls. Trunk-girdled vines demonstrated consistently higher $\delta^{13}C$ than control vines, with significant differences evident at 4 weeks post-treatment (Figure 2.3). Leaf sample analysis of $\delta^{13}C$ content from 1 to 5 weeks post-treatment showed little difference between control and root-pruned vines.

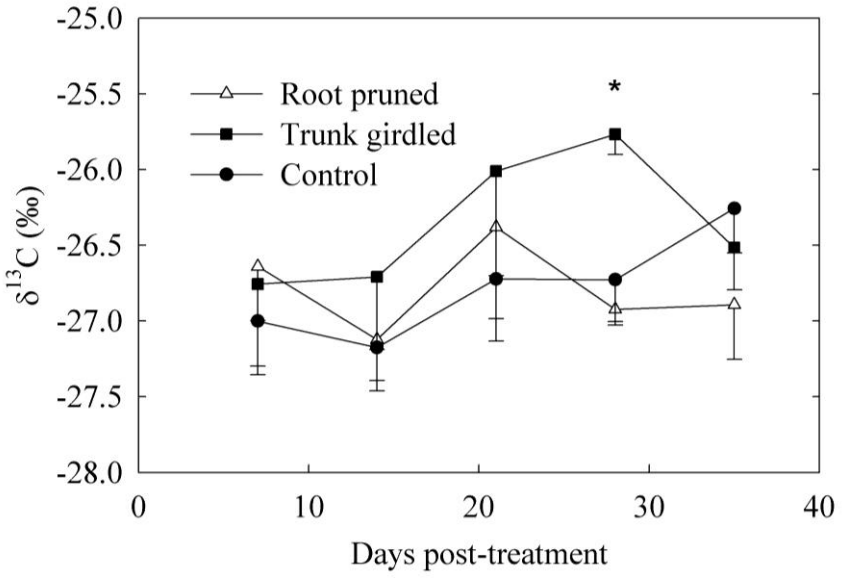


**Figure 2.3 $\delta^{13}C$ discrimination (‰) at weekly intervals post-treatment for root-pruned, trunk-girdled and control vines. Data are means $n$ = 6 samples, - 1SEM. '*' indicates a significant difference in comparison with the control at that time.**

Mean fruit weight increased from 72.1 ± 2.0 g to 85.3 ± 1.8 g over the 7 week period, with no significant differences between treatments (Figure 2.4). Rate of fruit growth (g $day^{-1}$), calculated from FW estimates using equation (2.4), showed a steady decline for all vines throughout the experiment, with little evidence of a treatment response (data not shown). Mean fruit DM increased from 15.3 ± 0.13% to 19.5 ± 0.16% over the 7 week period; fruit DM increased at a faster rate in root-pruned and trunk-girdled vines (Figure 2.5). Four and 5 weeks post-treatment, fruit DM content was significantly increased in trunk-girdled vines in comparison with control vines. Neither treatment had a visible effect on plant health or leaf area.

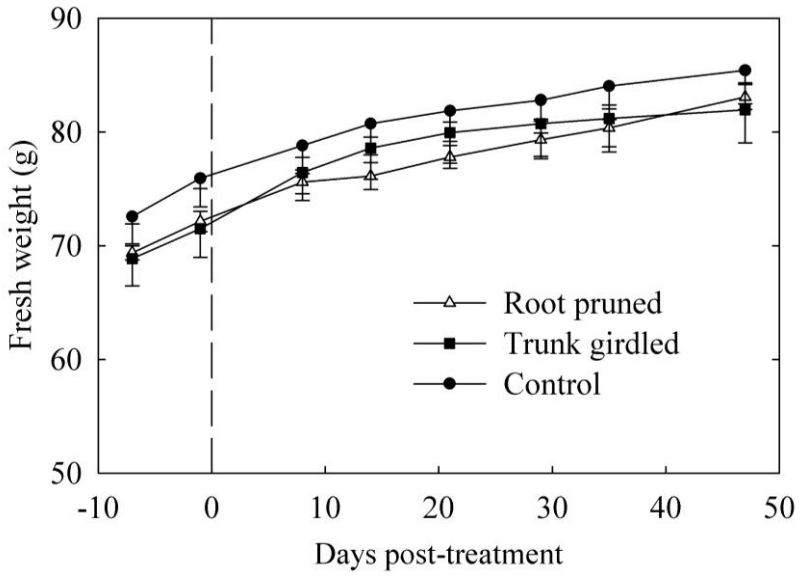


**Figure 2.4 Fruit fresh weight (g) from fresh weight calculations following measurements of fruit length and maximum and minimum diameter at the equatorial region of marked kiwifruit for root-pruned, trunk-girdled and control vines. Treatments were applied on 12 February 2009 (dotted line). Data are means *n* = 18 fruit (*n* = 12 for root-pruned), - 1SEM.**

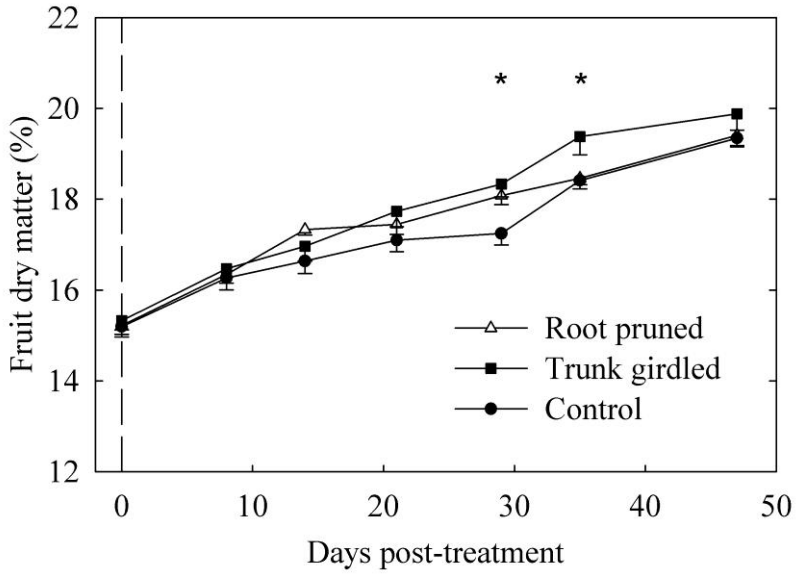


**Figure 2.5 Fruit dry matter content (% of fruit fresh weight) of periodically sampled kiwifruit from root-pruned, trunk-girdled and control vines. Treatments were applied on 12 February 2009 (dotted line). Data are means *n* = 12 fruit (*n* = 8 for root-pruned), - 1SEM. '*' indicates a significant difference in comparison with the control at that time.**

## 2.4 Discussion

Physiological responses to trunk girdling and root pruning differed markedly in these kiwifruit vines. The physiological response to root pruning demonstrated a primary hydraulic response, with significant reductions in $\Psi_{shoot}$, $\Psi_{leaf}$ and $K_{shoot}$. Reductions in $\Psi_{leaf}$ agreed with previous observations in grape (Smart et al. 2006). There was evidence of lower *A*; however, differences were rarely significant and were smaller than expected. The reduction in $K_{shoot}$ (Figure 2.1B) was in line with what might be expected from removing 50% of the root system. In control vines, the soil to shoot pathway accounted for 52% of whole-plant hydraulic resistance (Table 2.2), and the root system accounts for a large proportion of the resistance along this pathway (McAneney & Judd 1983). Assuming the majority of this resistance resides in the root, removing 50% of root area would be expected to double this resistance, resulting in an approximate 34% reduction in $K_{plant}$. During the first 5 days post-treatment, root-pruned vine $K_{plant}$ was reduced by 22 – 26%;

deviation from the estimated 34% could be attributed to resistance along the trunk and shoot. Effects on $K_{shoot}$ appeared transitory, with $K_{shoot}$ similar in root-pruned and control vines by 4 weeks post-treatment. However, this appears to be due to reduced $K$ in control vines over time, with root-pruned vine $K_{shoot}$ remaining lower than pre-treatment.

Hydraulic conductance and $E_{plant}$ were low in these root bag-grown vines in comparison with observations for orchard-grown kiwifruit vines (Judd et al. 1986; Green et al. 1989; Clearwater et al. 2004). Treatments appeared to have little effect on whole-vine $E_{plant}$, even when $g_s$ was reduced. This may be a result of environmental conditions within the plastic greenhouse, with restricted air movement reducing stomatal control of transpiration (Jarvis & McNaughton 1986).

Root pruning was expected to have a significant effect on photosynthesis, through stomatal limitation of leaf gas exchange. The lack of a consistent effect on $g_s$ in kiwifruit conflicts with previous findings in other species. In grape, Smart et al. (2006) observed significant reductions in $g_s$ that persisted for at least 2 months. In the present experiment, reductions in $\Psi_{leaf}$ without significant reductions in $g_s$ suggest that kiwifruit vines are anisohydric (Tardieu & Simonneau 1998) and that reductions in $\Psi_{leaf}$ observed were insufficient to prompt a stomatal response. The lack of a significant effect on $c_i$ or $\delta^{13}C$ following root pruning supports the observation of only minor reductions in $g_s$.

The photosynthetic response to trunk girdling was more pronounced than that in root-pruned vines (Figure 2.2). Consistent, significant reductions in $g_s$ without consistent reductions in $\Psi_{shoot}$ and $\Psi_{leaf}$ suggest that the stomatal response is not hydraulic. This agrees with previous suggestions that trunk girdling does not directly affect water transport (Noel 1970; Goren et al. 2003), instead supporting the hypothesis that stomatal closure is coordinated with a biochemical down-regulation of $A$. The delayed reduction in leaf $c_i$ and increase in $\delta^{13}C$ (Figure 2.3) suggests that during the 3 weeks post-treatment, $A$ was limited by combined biochemical down-regulation and stomatal limitation, after this time stomatal limitation appears to have been dominant. Differences in leaf $\delta^{13}C$ content between control and girdled vines became less evident 5 weeks post-treatment.

Wounds of trunk girdles applied to orchard-grown vines in spring and early summer generally callus within 4 weeks, similar to the healing time observed in grape (Williams et al. 2000). Commencement of photosynthetic recovery at 5 weeks may indicate re-connection of phloem vasculature at this time.

Reduced $g_s$ and $A$ following trunk girdling have been observed previously (Williams et al. 2000) and the role of feedback inhibition of $A$ following reduced photosynthate export from leaves has been proposed (Iglesias et al. 2002). Trunk girdling temporarily removes the root system from the source:sink ratio, increasing availability of photosynthates to parts above the girdle. A proportion of these newly available photosynthates may be utilised for growth; however, girdling has been shown to result in accumulation of carbohydrates within leaves, reducing $g_s$ and $A$ (Iglesias et al. 2002; Domec & Pruyn 2008). In contrast, Williams et al. (2000) suggested that the accumulation of carbohydrates in girdled grapevine leaves was insufficient to result in feedback inhibition of $A$ and suggested the involvement of abscisic acid (ABA) effects on stomatal aperture. This hypothesis is supported by previous observations of increased ABA accumulation in leaves, coinciding with stomatal closure following girdling (Düring 1978; Setter et al. 1980).

Fruit fresh weight and growth rate were not affected by either treatment. Root pruning in other fruit crops has resulted in negligible or negative effects on fruit growth, with reductions in fruit size and overall yield (Khan et al. 1998; Ferree et al. 2000). Previous observations of kiwifruit fresh weights following root pruning have been variable (Patterson et al. 2009). Trunk girdling was expected to increase kiwifruit fruit fresh weights following previous observations (Currie et al. 2005). Increases in fruit DM have previously been obtained from both treatments (Currie et al. 2005; Patterson et al. 2009), and trunk girdling results from this experiment concur. Inconsistent effects on fruit DM following root pruning in the present experiment suggest that the technique has the potential to increase DM; however, timing and severity may be important considerations.

In summary, hydraulic and photosynthetic responses of kiwifruit vines were investigated in response to trunk girdling and root pruning. These two practices can result in increased fruit size and quality, so it was of interest to see how they

compared in their physiological effects. The observed responses differed markedly. The response to root pruning was hydraulic, with significant reductions in $\Psi$ and $K$, supporting our hypothesis. Photosynthetic responses following root pruning were less severe than expected, attributed to kiwifruit vines demonstrating an anisohydric stomatal response. Trunk girdling resulted in a significant photosynthetic response, with little evidence of an effect on plant hydraulics. The photosynthetic response is suggested to be a combination of biochemical down-regulation and stomatal limitation, as a result of reduced carbohydrate demand, supporting our hypothesis. Both these techniques are considered to manipulate the carbon balance of the kiwifruit vine, increasing the availability of carbohydrates to the developing fruit. The results of this experiment suggest that trunk girdling had the most direct effect on the plant carbon balance, possibly explaining the variable results of root pruning on fruit quality parameters in previous studies.

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# 3 Hydraulic responses of whole-vines and individual roots of kiwifruit following root severance

## 3.1 Introduction

Hydraulic conductance of roots has been shown to vary in response to changes in soil conditions and plant requirements. Root hydraulic conductance exhibits a diurnal pattern (Henzler et al. 1999), thought to relate to the diurnal demand of transpiration (Bramley et al. 2007). Treatments including soil drying and re-wetting (Siemens & Zwiazek 2003; Vandeleur et al. 2009) and NaCl toxicity (Martínez-Ballesta et al. 2003) have induced rapid changes in root hydraulic conductance.

These rapid changes in root hydraulic conductance, that occur within minutes to hours, differ from longer term changes in root activity that can be observed after a number of days, and can be attributed to growth and anatomical changes (Bramley et al. 2007). Rapid changes in root hydraulic conductance have largely been attributed to root aquaporin activity (Javot & Maurel 2002). In the roots, aquaporins facilitate water transfer across membranes either by gating or by changing in their abundance (Bramley et al. 2007). The diurnal trend of root hydraulic conductance, peaking around midday and declining through the afternoon to an overnight low, has been associated with diurnal patterns in the expression of genes encoding aquaporins (Henzler et al. 1999; Martínez-Ballesta et al. 2003; Vandeleur et al. 2009). Drought conditions have been shown to reduce root hydraulic conductance, and these reductions are often rapid and reversible upon re-wetting. The rapidity of the response suggests the involvement of aquaporins (Siemens & Zwiazek 2003; North et al. 2004). Roots of grapevines exposed to drought demonstrated changes in root hydraulic conductance and aquaporin transcript expression. Isohydric and anisohydric cultivars showed different responses to the water shortage, with isohydric cultivars exhibiting a larger effect on the diurnal expression of aquaporin transcripts and larger reductions in midday root hydraulic conductance. Upon re-watering, root

hydraulic conductance failed to return to pre-drought values, and this was attributed to anatomical changes in the root, including suberisation (Vandeleur et al. 2009).

What is the trigger for these dynamic and rapid changes in root hydraulic conductance? Are roots responding to a hydraulic signal in the form of a change in pressure gradient? If a hydraulic signal is involved, induced by changes in plant water status, then how will the remaining roots respond when part of the root system is damaged? Plants are considered to develop a functional equilibrium between root and shoot area (Geisler & Ferree 1984a), ensuring sufficient root area to meet canopy demand for water and nutrients. If this equilibrium is disrupted by damaging the root system, do intact roots respond with increased hydraulic conductance in order to maintain water supply to the canopy?

Studies on sunflower (*Helianthus annuus*) and maize (*Zea mays*) have suggested that if part of the root system is damaged, the permeability of remaining roots is increased (Aston & Lawlor 1979). In hydroponically grown, single leafed wheat (*Triticum aestivum*)seedlings, removing four out of five roots led to increased hydraulic conductivity in the remaining root, to the extent that transpiration and stomatal conductance were not limited (Vysotskaya et al. 2004). Hydraulic conductivity observed 5 minutes after treatment was increased, and was attributed to increased flow through the apoplast since there was no increase in membrane permeability when measured under an osmotic gradient. Hydraulic conductivity measurements 90 minutes after treatment had increased further, and were attributed to increased cell-to-cell transport. It was suggested that the abscisic acid (ABA) concentration within the roots, which increased 60 minutes after root excision, might be related to aquaporin activity (Vysotskaya et al. 2004). Few studies exist regarding root level hydraulic responses to root damage, and existing research has tended to involve small potted plants, usually monocots and herbaceous eudicots. Little is known regarding the response in woody perennial plants such as the kiwifruit vine.

Root pruning is a technique used in perennial fruit crops for the control of vegetative vigour (Schupp & Ferree 1990; Maas 2008). A blade is dragged through the soil severing roots along the vine row, reducing the size of the root

system. Root pruning is considered to cause reduced vegetative growth through re-allocation of resources to the root system, following disruption of the root: shoot ratio (Geisler & Ferree 1984a). An alternative explanation is that reduced vegetative vigour is a result of reduced mineral ion supply post-root pruning; however, this theory is not widely supported (Geisler & Ferree 1984a; Schupp & Ferree 1990). Significant reductions in vegetative growth have been observed in apple trees (Ferree 1989; Schupp & Ferree 1990); however, root pruning kiwifruit vines has not demonstrated appreciable reductions in vegetative vigour. The procedure has, however, resulted in increased fruit size and improved quality (Patterson et al. 2009), and so scientific interest in the technique has been maintained.

The hydraulic system of kiwifruit is typical of other lianoid species, with low axial xylem resistance, high whole-plant hydraulic conductance and high rates of transpiration in comparison to tree species, such as olive (McAneney & Judd 1983; Dichio et al. 1999). Low axial resistance is achieved by semi-ring porous wood with xylem vessels averaging 140 – 160 mm in length and up to 0.5 mm diameter, connected by simple perforation plates (Condon 1992). The high water requirements of kiwifruit make this an interesting plant in which to study hydraulic responses to root damage. Research in other fruit crops have shown that root pruning causes responses similar to water stress. Root pruning container-grown apple trees reduced transpiration rates and leaf water potentials, and caused visible leaf wilting (Geisler & Ferree 1984b), with similar findings in container and orchard-grown grapes (Ferree et al. 1999; Smart et al. 2006). Observations of such hydraulic responses is interesting in regards to increased fruit size observed in kiwifruit, as kiwifruit fruit growth is sensitive to water stress (Judd et al. 1989). This suggests that vines are able to overcome the negative impact of root pruning on water supply, possibly through increased water transport in the remaining roots.

This experiment investigated kiwifruit vine transpiration and hydraulic conductance following root pruning. Whole-vine and individual root hydraulic conductance were observed to investigate the hypothesis that following root pruning, these mature woody plants acclimate to the reduction in root surface area through rapid increases in the hydraulic conductance of the remaining intact roots.

Leaf gas exchange responses were monitored with the expectation that photosynthetic rate and stomatal conductance would be reduced until there was a complete recovery in whole-plant hydraulic conductance. Because of the severity of the root pruning treatment being tested, it was also expected that shoot and fruit growth rates would be reduced.

## 3.2 Materials and Methods

### 3.2.1 Plant material

The experiments were conducted at the Plant & Food Research Te Puke Research Orchard, New Zealand. Vines were clonal green kiwifruit (*Actinidia deliciosa* (A.Chev.) C.F. Liang et A.R. Ferguson var. *deliciosa* 'Hayward') rootstocks with yellow kiwifruit (*Actinidia chinensis* Planch. var. *chinensis* 'Hort16A') scions. Rootstocks were planted in 1991 and grafted in 1997, 1 m above the ground with two vertical scion stems trained in opposite directions along the row, in a pergola system with 6 m spacing between plants within the row and 5 m spacing between rows. The soil type was Te Puke sandy loam. Cultural management was standard to the cultivar, adapted from recommendations for *A. deliciosa* (Sale & Lyford 1990). Fertiliser regime for the block included an annual granular application of potash, potassium, sulphur and magnesium followed by two nitrogen and potassium sulphate applications timed at budburst and petal fall. Crop load was maintained at around 42 fruit $m^{-2}$. Vines were not irrigated prior to or during the experiment; weather and soil water conditions were not considered to be water limiting. Weather data were collected from a weather station on site; rainfall for the period of August 2009 to February 2010, inclusive, was 1007 mm, with 111 mm of rainfall during the experiment period of January and February 2010. Soil volumetric water content of a neighbouring kiwifruit block, approximately 50 m from test vines, averaged 20% to a depth of 0.7 m and 38% between 0.7 m and 1.3 m during the same period. Field capacity for this soil type is on the order of 38% along a soil profile to a depth of 1 m (Cotching 1998).

### 3.2.2 Root pruning

Vines were selected from two rows, selecting for vines of similar size and avoiding neighbouring vines. Four vines, two from each treatment, were in row 1, with the remaining two vines in row 2. A hole approximately 0.8 m in diameter

was excavated around the base of each vine using a high pressure water gun, exposing the junction between the root collar and all roots. One root pruning treatment was studied, severing approximately 80% of roots in early January, in comparison with controls with zero roots severed, with three vines per treatment. The timing of treatment application (approximately 80 days post-50% full bloom) was selected to coincide with the phase of increasing starch accumulation in the fruit (Richardson et al. 1997). Cutting off 80% of roots was considered representative of the more severe treatment likely to be encountered with commercial root pruning. Sapwood area of all major roots was estimated close to the root collar from measurements of individual root diameters inclusive of bark, and bark thickness. Roots were selected as representing 80% of sapwood area with an approximately even azimuth distribution around the circumference of the stem. Selected roots were severed around midday, 5 January 2010, using a hand pruning saw, with a section of root removed to prevent healing. The vine side of the severed roots were painted with pruning paint, and the root collar left excavated.

### 3.2.3 Whole-vine hydraulic conductance

Hydraulic conductance was measured in trunks and roots of vines using the evaporative flux method (Tsuda & Tyree 1997). Trunk sap flux was measured to determine transpiration ($E_{plant}$) using the heat dissipation technique (Granier 1987; James et al. 2002), with two sets of probes installed per vine, one in each leader trunk. Probes were installed at least 1 m above ground, in the vertical part of the scion. Bark plugs were removed using a cork borer and the 12 mm long aluminium tip of the probes inserted flush with the cambium. It was assumed that all sapwood contained conducting xylem. Installation in the outer 10 mm of sapwood does not account for radial variation in flow rates; however, the purpose of the probes was to identify a treatment response. Effects of water stress on sap flow are suggested to be most noticeable in outer xylem and heat dissipation probes have been found to identify such a response accurately (Gonzalez-Altozano et al. 2008). Probes were connected to a multiplexer (AM416, Campbell Scientific, Utah) and datalogger (CR10X, Campbell Scientific, Utah). Average temperature difference between the heated and un-heated probes ($\Delta T$, °C) was

recorded at 30 minute intervals. Probes and the surrounding trunk were insulated from solar radiation with reflective foil insulation.

Xylem pressure potentials ($\Psi$, MPa) were measured periodically pre- and post-treatment, using a pressure chamber. Measurements were made between 1100 h and 1500 h on clear, sunny days, selecting fully expanded, mature leaves. Leaves were selected from different locations within the canopy: non-transpiring leaves, covered by foil bags, positioned at the top of the trunk were used to measure $\Psi_{trunk,}$ non-transpiring and transpiring leaves were selected at the end of shoots to be representative of $\Psi_{shoot}$ and $\Psi_{leaf}$, respectively (Clearwater et al. 2004). Pre-dawn $\Psi$ were recorded as a measure of $\Psi_{soil}$. Three leaves were assessed per vine for each component of $\Psi$; average $\Psi$ for each vine, location within the canopy and time of sampling was then used along with the corresponding whole-vine sap flux to calculate whole-vine hydraulic conductance ($K$, kg MPa$^{-1}$ s$^{-1}$) as:

$$K = E_{plant} / \Delta\Psi \qquad (3.1)$$

where $\Delta\Psi$ is the difference between $\Psi$ at points along the hydraulic pathway ($\Psi_{soil}$ - $\Psi_{trunk}$ for $K_{trunk}$, $\Psi_{trunk}$ - $\Psi_{shoot}$ for $K_{shoot}$, $\Psi_{shoot}$ - $\Psi_{leaf}$ for $K_{leaf}$ and $\Psi_{soil}$ - $\Psi_{leaf}$ for $K_{plant}$; Clearwater et al. 2004).

Leaf area measured at the start of the experiment was used to calculate leaf area specific hydraulic conductance ($K_{l,}$ kg MPa$^{-1}$ m$^{-2}$ s$^{-1}$) for the same portions of hydraulic pathway as $K$. Total leaf area was estimated as the count of short (<300 mm), medium (300 – 700 mm) and long (>700 mm) shoots per vine, multiplied by the average number of leaves per shoot for each length class. The sum of leaves per vine was multiplied by the average leaf area as calculated from a 200 leaf sample measured using an area meter (LI-3100, LI-COR Inc., Nebraska). Leaf area specific hydraulic conductance was calculated as:

$$K_l = K / A_{leaf} \qquad (3.2)$$

### 3.2.4 Calibration of heat dissipation probes

Heat dissipation probes underestimate sap velocity if part of the heated probe is in contact with non-conducting xylem or if sap flux density is highly heterogeneous within the sapwood (Clearwater et al. 1999). Spatial variability in sap flux is

likely in a semi-ring porous species with large vessels, including kiwifruit (Phillips et al. 1996), and a previous study has found that heat dissipation sap flux measurements under-estimate kiwifruit orchard transpiration (Silva et al. 2008). To calibrate the technique, a 10 year-old yellow kiwifruit vine, grown in the orchard in a root control bag, was transferred to a 150 L pot on a weighing lysimeter located inside a glasshouse. The pot was sprinkler irrigated at 8 hour intervals and covered to prevent evaporation from the soil. Drainage from the pot was recorded with a tipping bucket rain gauge and two sets of heat dissipation probes were installed in the trunk. Average $\Delta T$ from the heat dissipation probes, lysimeter weight and total drainage were recorded at 30 minute intervals using a datalogger, and transpiration recorded as weight loss after accounting for drainage.

Sapwood $\Delta T$ was estimated from measured $\Delta T$ as:

$$\Delta T_{sw} = \frac{\Delta T - \Delta T_m(1-a)}{a} \tag{3.3}$$

where 'a' is the proportion of the probe in contact with active xylem and $\Delta T_m$ is the maximum $\Delta T$ recorded when sap flux is expected to be minimal (Clearwater et al. 1999). In this case, the proportion 'a' was unknown, and was found as the value that gave the best agreement between sap flow estimates of $E_{plant}$, calculated using equation (3.3) and the original empirical calibration for heat dissipation probes (Granier 1987), and lysimeter measurements of $E_{plant}$ (Figure 3.1). Without correction, heat dissipation sap flow measurements underestimated $E_{plant}$ by 60%. The fitted value of 'a' (equation 3.3) was 0.65, close to the average estimate of 0.60 arrived at by Silva et al. (2008). Equation (3.3) was subsequently used to correct all heat dissipation sap flow estimates of vine $E_{plant}$ made in the orchard.

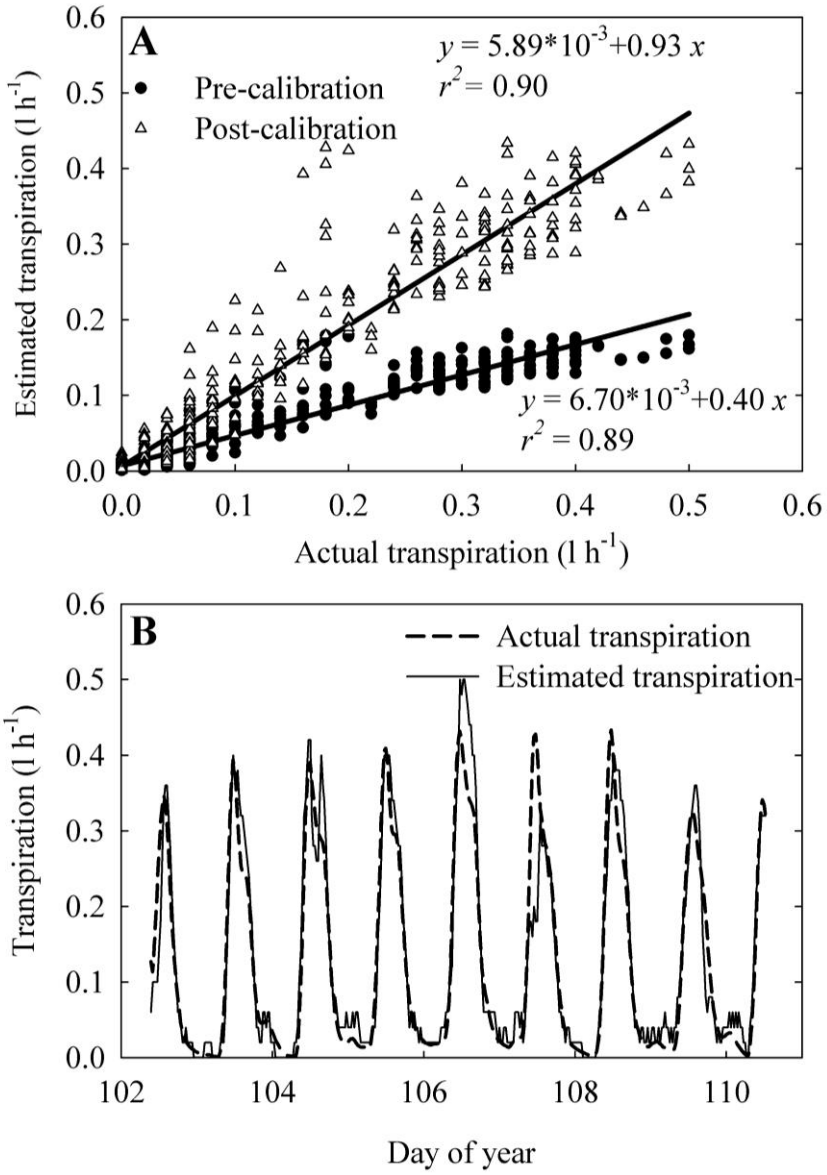


**Figure 3.1 Transpiration data used for calibration of heat dissipation probes. Data were collected from a single vine over 8 days. A) Pre- and post-calibration relationships between actual (lysimetry measurements) and estimated transpiration (heat dissipation probe measurements). B) Post-calibration diurnal pattern of actual and estimated transpiration. Estimated transpiration is the mean of two heat dissipation probes.**

### 3.2.5 Root hydraulic conductance

Root sap flux was measured using the compensation heat pulse method as described by Green and Clothier (1995). Sap flow was measured in two roots for each of four vines, two control and two 80% root-pruned. Roots were selected with approximate diameters of 33 – 56 mm inclusive of bark and sap flow probes were installed flush with the cambium after removing bark plugs. Heater probes received a 1.5 second heat pulse every 30 minutes, with temperature cross-over times recorded using a multiplexer and datalogger (AM25T and CR10X, Campbell Scientific, Utah). Sap flux density was calculated in accordance with Green and Clothier (1988), using a wound diameter of 2 mm, as previously verified for kiwifruit roots (Green & Clothier 1995), and scaled to a whole-root system flux based on measurements of total root xylem cross sectional area, measured close to the trunk.

To calculate hydraulic conductance of individual roots ($K_{root}$), $\Psi_{trunk}$ was used in equation (3.1) to obtain $\Delta\Psi$ and root flux was substituted for $E_{plant}$. The soil to root pathway is thought to present the greatest resistance to water movement between the soil and the top of the stem (McAneney & Judd 1983), and test measurements of $\Psi$ on non-transpiring shoots close to the base of the trunk did not differ significantly from measurements on non-transpiring leaves at the top of the trunk (data not shown). It was therefore considered appropriate to use $\Psi_{trunk}$ in place of $\Psi_{root}$ to calculate $K_{root.}$

### 3.2.6 Leaf gas exchange

Net photosynthesis ($A$) and stomatal conductance ($g_s$) under ambient conditions were measured using a portable gas exchange system (LI-6400, LI-COR Inc., Nebraska). Measurements were made pre-treatment, at two timings; and post-treatment; within 1 and 2 hours and five timings within the following 3 weeks. Measurements were taken around midday on sunny, cloudless days using natural light. Four to six leaves were assessed per vine, selecting for fully expanded mature leaves in a full sun position. Chamber $CO_2$ concentration was controlled at 400 µmol $mol^{-1}$ and the vapour pressure deficit was maintained at an average of 2.4 kPa.

Leaf samples were collected at weekly intervals, pre- and post-treatment for $\delta^{13}C$ isotope analysis, to determine the relative stomatal limitation of *A*. Two samples of five to eight young, newly emerging leaves were collected per vine, based on the assumption that expanding leaves were representative of recently fixed carbon (Meinzer et al. 1993). Samples were dried at 80°C to a constant weight, finely ground, and 2.8 mg samples weighed into foil cups. A mass spectrometer (20/20 isotope analyser, Europa Scientific, Crewe) at the University of Waikato Stable Isotope Unit was used to measure $\delta^{13}C$ (‰) discrimination in comparison with the Pee Dee belemnite standard.

### 3.2.7 Growth

Vegetative growth was observed by measuring the length of three actively growing shoots per vine at weekly intervals, commencing pre-treatment, on 1 December 2009. Measurements continued until the majority of shoots terminated growth in late January. Fruit growth was measured on four randomly selected and tagged fruit per vine at weekly and then fortnightly intervals from pre-treatment until harvest, on 7 May 2010. Fruit length, minimum and maximum diameter at the equatorial region were measured with digital callipers ('IP67', Sylvac, Crissier). Measurements were used to estimate fruit fresh weight as described by Minchin et al. (2003). At harvest, eighteen fruit were sampled per vine and weighed to obtain actual fresh weight. Fruit firmness (kgf) was measured using a penetrometer ('Fruit Texture Analyser', GUSS, Strand) and soluble solid content (%) was measured using a refractometer ('Master', Atago, Tokyo). Dry matter (DM) content was calculated by taking a 2 mm slice from the equatorial region of each fruit, weighing the fresh slice (FW), drying at 65°C to a constant weight and re-weighing (DW). Dry matter content (%) was calculated as:

$$DM = (DW / FW) * 100 \qquad (3.4)$$

### 3.2.8 Statistical analysis

Statistical analyses were carried out in R, version 2.10.1 (Comprehensive R Archive Network, cran.r-project.org). Data were tested for normal distribution using the Shapiro-Wilk test, and log transformations performed to normalise data where required for analyses. Treatment differences were tested using ANOVA. Where repeated measurements were collected from individual plants over time,

the ANOVA model included timing as an error term. Linear regression was used to compare the pre- and post-calibration relationship between actual and estimated transpiration. Correlations between nocturnal sap flux and vapour pressure deficit were tested using Pearson's correlation.

## 3.3 Results

### 3.3.1 Root hydraulic conductance

Hydraulic conductance ($K_{root}$) of individual intact roots was not affected by root pruning, in comparison with pre-treatment. The large reduction in root cross sectional area combined with no change in intact $K_{root}$ resulted in a significant ($P < 0.05$) reduction in whole-root system leaf area specific hydraulic conductance ($K_{l\text{-}rootsystem}$), with no clear evidence of a recovery during the month following treatment (Figure 3.2).

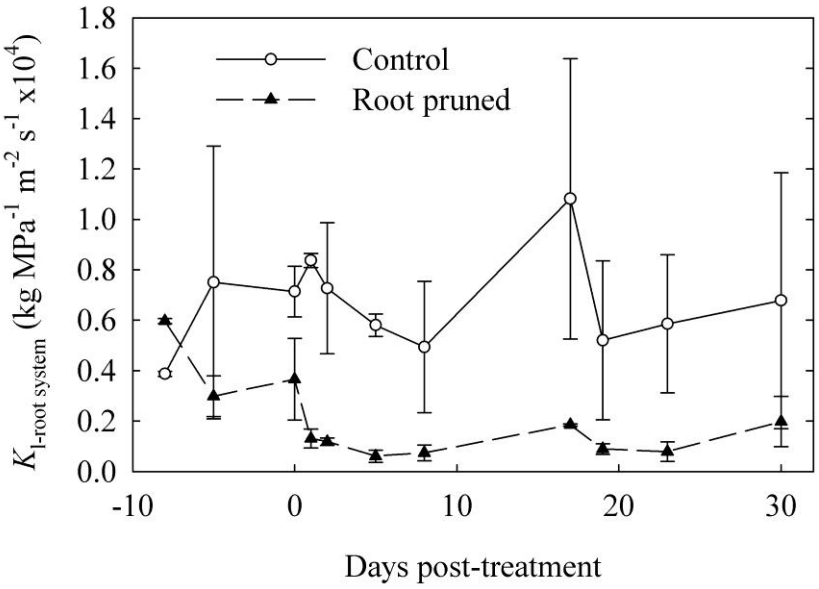


**Figure 3.2 Whole-root system, leaf area specific hydraulic conductance for control and root-pruned vines. Root pruning was performed on 5 January 2010. Data are means, *n* = 4 roots, ± 1SEM.**

Root sap flux pre-treatment was lowest ($P$ = 0.28) in the roots chosen for monitoring in root-pruned vines. Pre-treatment average root sapwood area specific flux (± SEM) was on the order of 29.4 ± 3.3 g $m^{-2}$ $s^{-1}$ for control vines and 23.4 ± 4.1 g $m^{-2}$ $s^{-1}$ for root-pruned vines. On the day of pruning, peak flux estimates in intact roots of root-pruned vines increased by almost 400%, then decreased to 150% of pre-treatment values within 4 days (Figure 3.3A). When root sap flux was scaled to the entire root system based on root sapwood area, whole-root system flux increased briefly on the day of treatment (Figure 3.3B), but from 1 day post-treatment onwards was 72% lower than pre-treatment. Individual root sap flux post-treatment in root-pruned vines was generally higher during periods of low flow, such as overnight (Figure 3.3A) or following periods of rainfall. At a given vapour pressure deficit, nocturnal root flux was higher ($P$ < 0.05) in root-pruned vines pre-treatment, averaging 3.6 ± 0.1 g $m^{-2}$ $s^{-1}$ more than in control vines. Post-treatment, the difference between control and root-pruned vines more than doubled, with root-pruned vine nocturnal flux averaging 9.9 ± 0.2 g $m^{-2}$ $s^{-1}$ higher than control vines in the 2 weeks post-treatment (Figure 3.4B). Higher nocturnal root sap flux was not associated with higher rates of nocturnal transpiration, measured as sap flow in the upper trunk (Figure 3.4A). Nocturnal root flux was better correlated with vapour pressure deficit in control vines ($r$ = 0.93, $P$ < 0.05), and less so in root-pruned vines ($r$ = 0.71, $P$ < 0.05). Estimates of daily root system flux over the 4 weeks post-treatment ranged from 6.8 to 42.3 ± 1.6 kg $day^{-1}$ in control vines and 6.3 to 20.4 ± 0.6 kg $day^{-1}$ in root-pruned vines. Absolute values for daily root system flux were not identical to trunk flux, as root flux was an average of two rather than three vines, and was measured using a different method.

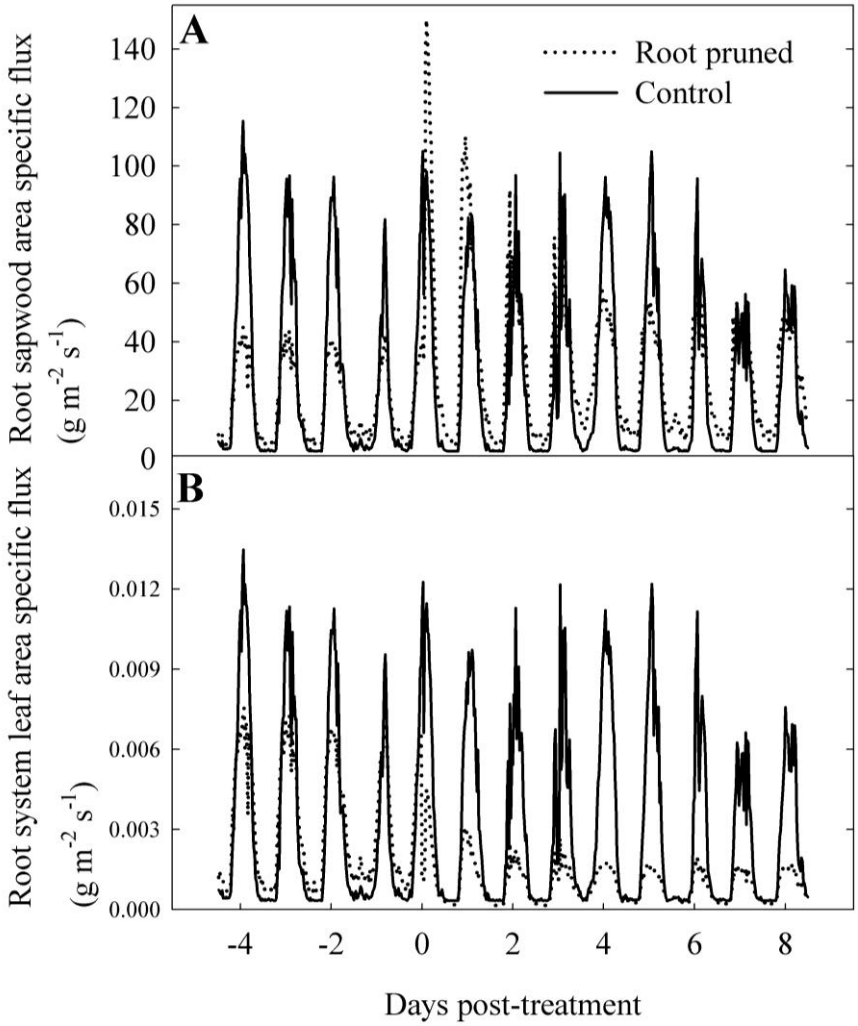


**Figure 3.3 Root sap flux for control and root-pruned vines; A) sapwood area specific flux and B) whole-root system leaf area specific flux. Root pruning was performed on 5 January 2010, peaks occur around midday. Data are means, *n* = 4 roots.**

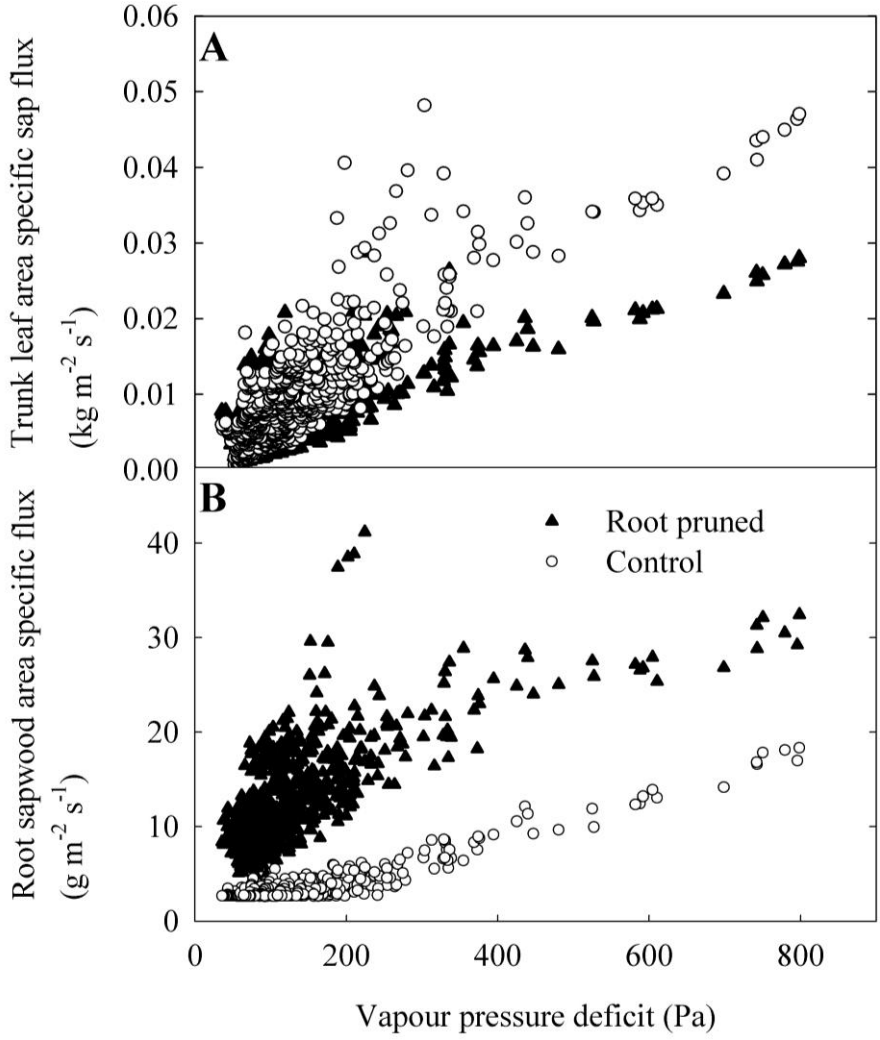


**Figure 3.4 Post-treatment correlation between nocturnal hourly sap flux rate and nocturnal vapour pressure deficit; A) trunk leaf area specific sap flux, *n* = 3 vines, and B) root sapwood area specific sap flux, *n* = 4 roots.**

### 3.3.2 Whole-vine hydraulic conductance

Pre-treatment trunk sap flow rates were similar for all vines, with daily $E_{plant}$ averaging 39.6 kg day$^{-1}$. A clear decline in root-pruned vine $E_{plant}$ ($P < 0.05$) was evident within one day of treatment and was maintained throughout the experiment (Figure 3.5). During the 5 days pre-treatment, leaf area specific $E_{plant}$ averaged 0.33 ± 0.01 kg m$^{-2}$ day$^{-1}$, declining to an average of 0.22 ± 0.01 kg m$^{-2}$ day$^{-1}$ during the 5 days post-treatment. Over the same time period, the drop in control vine transpiration was 0.01 kg m$^{-2}$ day$^{-1}$. Root pruning had a rapid and significant ($P < 0.05$) effect on midday xylem pressure potential ($\Psi$). Midday leaf,

shoot and trunk $\Psi$ (Figure 3.6B) were consistently more negative for root-pruned vines from 1 day post-treatment for the duration of the experiment. Pre-dawn $\Psi$ in root-pruned vines were reduced significantly ($P < 0.05$) 2 days post-treatment by an average of 0.02 MPa; measurements taken at other timings showed no significant difference. Pre-dawn $\Psi$ was not measured as frequently as midday $\Psi$, therefore, an average per vine was used for calculating $K$. Root-pruned vines demonstrated reduced $E_{plant}$ and increased $\Delta\Psi$, leading to significant reductions in $K_l$ (Figure 3.6C). Comparing the week pre- with the week post-treatment, control vines demonstrated a 0.3% decline in $E_{plant}$, whilst $E_{plant}$ in root-pruned vines declined by approximately 29% (Figure 3.5); reductions in root-pruned vine $\Psi_{trunk}$ post-treatment resulted in increased $\Delta\Psi$ by an average of 52%. Day to day variation in $E_{plant}$ and $\Psi$ were attributable to variable weather conditions, typical of New Zealand's maritime climate, and the trend for increased midday $E_{plant}$ post-root pruning (Figure 3.6A) was a result of increased temperatures and light levels post-treatment. On the day of treatment, leaf area specific hydraulic conductance ($K_{l\text{-}trunk}$) was calculated pre-treatment and at 1 hour and 2 hours post-treatment. Reductions in $K_{l\text{-}trunk}$ were evident in root-pruned vines within 1 hour of treatment. Values remained reduced and relatively stable for the following 8 days; from 17 days post-treatment onwards, a slight, non-significant increase was evident. Significant reductions in $K_l$ were observed for the soil-trunk, shoot-leaf and soil-leaf pathways ($K_{l\text{-}plant}$; Table 3.1).

### 3.3.3 Leaf gas exchange

Root pruning resulted in consistent and significant ($P < 0.05$) reductions in $A$ and $g_s$ (Table 3.2). Reductions in $\Psi_{trunk}$ within 1 hour of treatment were associated with reduced $g_s$. Two hours post-treatment, $g_s$ reduced further, whilst $\Psi_{trunk}$ was maintained. One day post-treatment, no further reductions in $g_s$ were evident, although $\Psi_{trunk}$ declined further, reaching its lowest value. In control vines, $A$ and $g_s$ demonstrated a general trend of increasing post-treatment, with a 23% increase in $A$ and a 32% increase in $g_s$. Root-pruned vines demonstrated a minimal increase in $A$, and $g_s$ was reduced in comparison with pre-treatment. Reductions were maintained until final measurements were taken, 24 days post-treatment. During the same period $c_i$ was not significantly affected. After root pruning, expanding

leaf $\delta^{13}C$ was generally higher for root-pruned vines, although not significantly so ($P$ = 0.074).

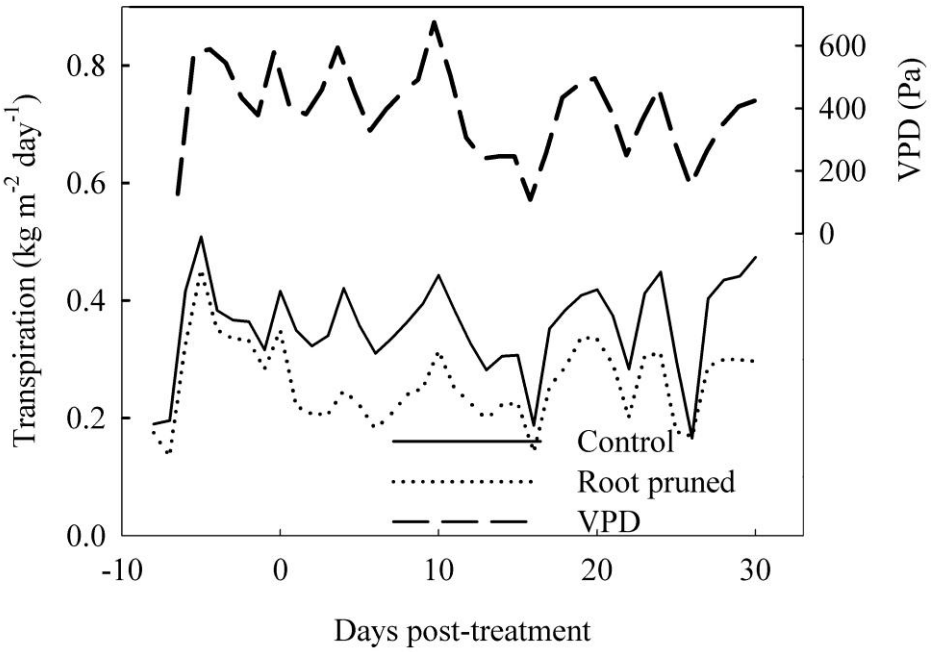


**Figure 3.5 Daily leaf area specific transpiration for control and root-pruned vines, and average daily vapour pressure deficit (VPD). Root pruning was performed on 5 January 2010. Data are means, *n* = 3 vines.**

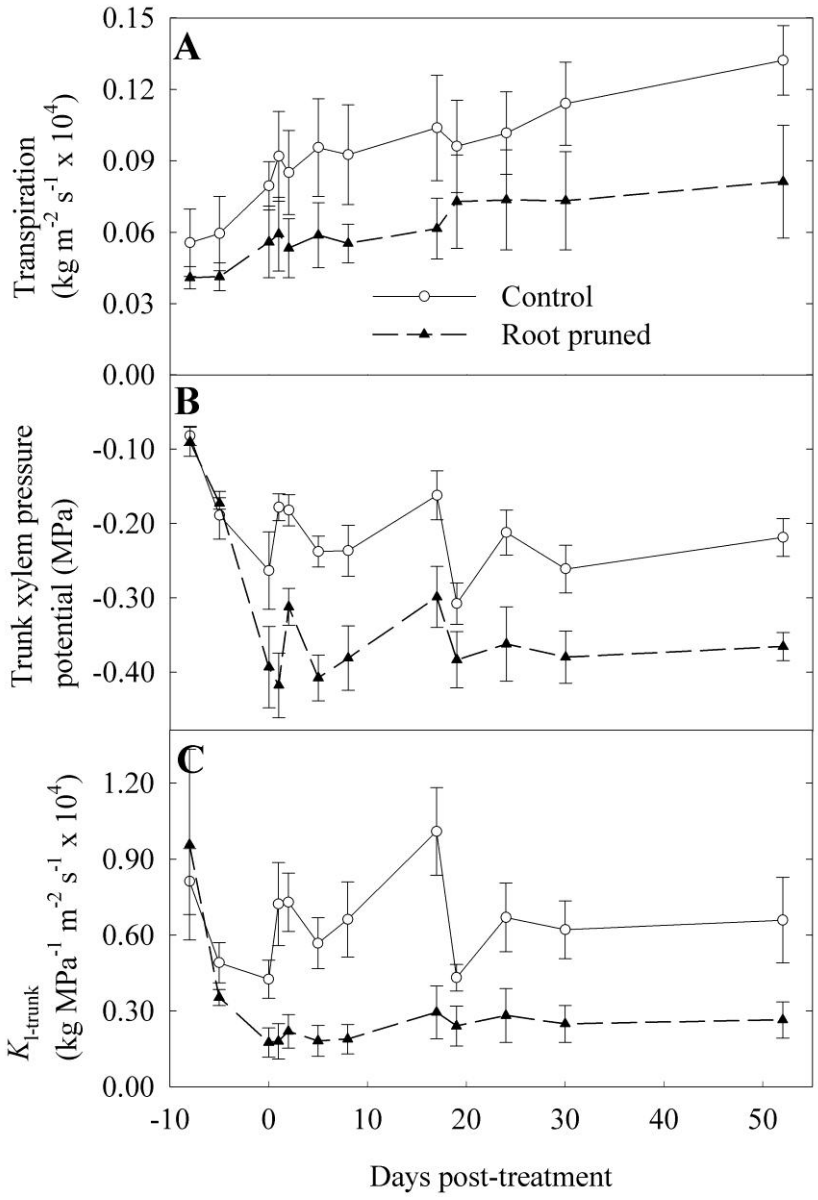


**Figure 3.6 A) Midday hourly transpiration rate and B) midday trunk xylem pressure potentials used for calculation of C) leaf area specific hydraulic conductance for the soil to trunk pathway. Root pruning was performed on 5 January 2010. Data are means, *n* = 3 vines, ± 1SEM.**

**Table 3.1 Effect of root pruning on leaf area specific hydraulic conductance ($K_l$) for the soil-trunk ($K_{l\text{-}trunk}$), trunk-shoot ($K_{l\text{-}shoot}$), shoot-leaf ($K_{l\text{-}leaf}$) and whole-plant ($K_{l\text{-}plant}$) pathways. Values are means, $n$ = 3 vines at 9 timings post-treatment, ± 1SEM. Differences are considered significant at $P < 0.05$.**

| | $K_l$ (kg MPa$^{-1}$ m$^{-2}$ s$^{-1}$ ×10$^4$) | | | |
|---|---|---|---|---|
| | soil-trunk | trunk-shoot | shoot-leaf | plant |
| Control | 0.651 ± 0.063 | 0.746 ± 0.072 | 0.427 ± 0.037 | 0.178 ± 0.012 |
| Root-pruned | 0.222 ± 0.020 | 0.553 ± 0.091 | 0.211 ± 0.015 | 0.093 ± 0.008 |
| *P*-value | <0.01 | 0.111 | <0.01 | <0.01 |

**Table 3.2 Effect of root pruning on photosynthesis ($A$), stomatal conductance ($g_s$), leaf internal $CO_2$ concentration ($c_i$) and leaf isotopic $^{13}C$ concentration. Pre-treatment differences between root-pruned and control vines were not significant, pre-treatment values are means, $n$ = 12 leaves, ± 1SEM. Post-treatment control and root-pruned values are means, $n$ = 6 leaves at 7 timings, ± 1SEM. Differences are considered significant at $P < 0.05$.**

| | $A$ (µmol $CO_2$ m$^{-2}$ s$^{-1}$) | $g_s$ (mol $H_2O$ m$^{-2}$ s$^{-1}$) | $c_i$ (µmol $CO_2$ mol$^{-1}$ air) | $^{13}C$ (‰) |
|---|---|---|---|---|
| Pre-treatment | 13.28 ± 0.51 | 0.255 ± 0.011 | 273.40 ± 3.34 | -26.74 ± 0.09 |
| Control | 14.78 ± 0.43 | 0.304 ± 0.013 | 272.46 ± 1.77 | -25.84 ± 0.09 |
| Root-pruned | 13.12 ± 0.42 | 0.255 ± 0.010 | 271.37 ± 2.35 | -25.51 ± 0.11 |
| *P*-value (control & root-pruned) | 0.001 | 0.003 | 0.690 | 0.074 |

### 3.3.4 Growth rates

Leaf area of the six vines averaged 120.5 ± 11 $m^2$ for root-pruned vines and 112.7 ± 14 $m^2$ for control vines. Vegetative growth was active at the time of treatment; termination of vegetative growth was evident in all vines in mid – late January. During the experiment there were no visible symptoms of stress (including leaf wilting, leaf drop or fruit drop). Extension of actively growing canes on root-pruned vines was maintained at rates equivalent to that of control vines (Table 3.3). Fruit growth rate was increased in root-pruned vines following treatment (Figure 3.7), with significantly ($P < 0.05$) higher growth rates evident in April and May. At harvest fruit fresh weight, DM content and soluble solids content were all significantly ($P < 0.05$) increased by root pruning (Table 3.3).

**Table 3.3 Effect of root pruning on shoot growth and fruit quality parameters. Shoot growth data are means, *n* = 9 shoots at 3 timings, ± 1SEM. Fruit data are final measurements taken at harvest, data are means, *n* = 54 fruit, ± 1SEM. Differences are considered significant at *P* < 0.05.**

| | Shoot growth (cm $day^{-1}$) | Final fruit fresh weight (g) | Final fruit DM content (%) | Fruit firmness (kgf) | Soluble solids (%) |
|---|---|---|---|---|---|
| Control | 3.76 ± 0.4 | 103.41 ± 2.3 | 16.98 ± 0.1 | 6.13 ± 0.1 | 11.11 ± 0.2 |
| Root-pruned | 3.65 ± 0.9 | 118.78 ± 2.9 | 18.72 ± 0.2 | 5.92 ± 0.1 | 14.15 ± 0.3 |
| *P*-value | 0.9101 | <0.01 | <0.01 | 0.1403 | <0.01 |

Abbreviations; DM – dry matter.

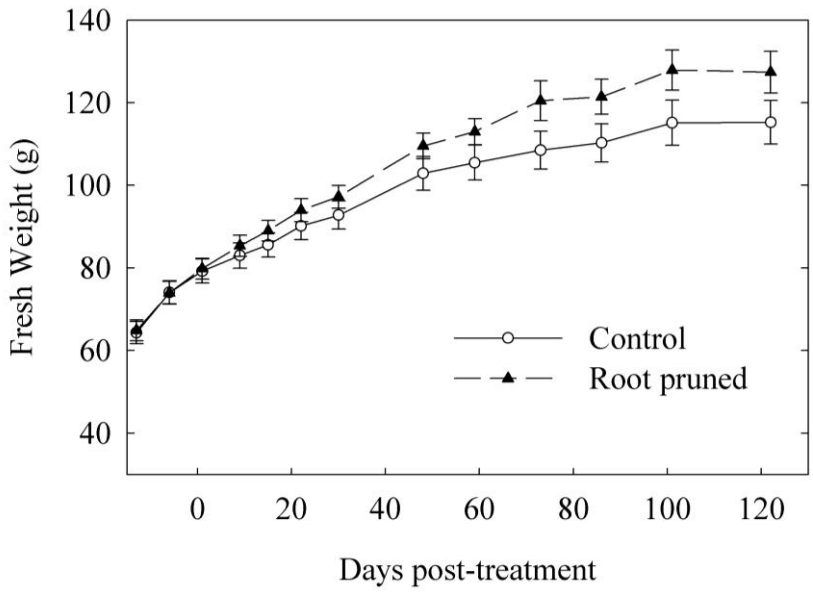


**Figure 3.7 Estimated fruit fresh weight growth curve calculated from fruit length and diameter measurements. Root pruning was performed on 5 January 2010. Data are means, *n* = 12 fruit, ± 1SEM.**

## 3.4 Discussion

Pruning 80% of root sapwood area close to the kiwifruit vine trunk did not have a significant effect on the hydraulic conductance of remaining intact roots. Following observations of dynamic root responses to treatments such as soil drying and re-wetting (Vandeleur et al. 2009), and severing of roots (Vysotskaya et al. 2004), it was expected that the reduction in water supply to the canopy, caused by this severe root pruning treatment, would be compensated by increased hydraulic conductance in the remaining intact roots. Our hypothesis that acclimation to reduced root surface area would involve increased hydraulic conductance in intact roots must, therefore, be rejected. The large reduction in whole-root system hydraulic conductance is the result of combined reductions in root surface area without a change in individual root hydraulic conductance. There was no clear evidence of root system hydraulic conductance recovering in the short term; 1 month post-treatment, slight recovery was evident, which in this time frame might be attributed to new root growth. New root growth has been

suggested as a long-term recovery mechanism post-root pruning in apple trees (Geisler & Ferree 1984b).

Increased sap flux through intact roots of root-pruned vines was clearly evident within 1 day of treatment. Post-treatment, the contribution of measured roots to whole-vine water use in the two root-pruned vines increased from 2.8% and 9.1% to 8.26% and 16.56%. Previous studies investigating the effects of root excision on sap flow in woody species are few. Studying conifer trees, Nadezhdina and Cermak (2000) observed root sap flow, when roots upstream of the sensor were severed. Depending on the severity of the root excision, they observed small, temporary increases in flow through the root closest to the trunk. It was suggested that following root excision there was a temporary compensatory increase in water uptake in the remaining root (Nadezhdina & Cermak 2000). In the present experiment, root hydraulic conductance was not increased, suggesting the increase in sap flux was not related to physiological changes in the roots, such as aquaporin expression, but rather was a response to the increased pressure gradient between root and canopy. These findings differ from responses observed following root excision in monocot seedlings (Vysotskaya et al. 2004) and to the drought response of roots of grapes, another lianoid species (Vandeleur et al. 2009). Failure to identify a change in root hydraulic conductance in these kiwifruit vines following root pruning may be a consequence of plant material. In the studies mentioned above, plants were smaller and younger, either being potted grape vines (Vandeleur et al. 2009) or hydroponic wheat seedlings (Vysotskaya et al. 2004). Root system structure would, therefore, be very different to that of the mature kiwifruit vines used in this study, which would have had root systems largely comprised of roots that have undergone secondary development (Hughes & Gandar 1989). Aquaporin involvement would only be evident if the cell-to-cell pathway was a significant component to radial water movement into the roots (Bramley et al. 2007), therefore it may be hypothesised that in kiwifruit roots, the apoplastic pathway is a considerable component of the radial movement of water. Compared with pre-treatment fluxes, increased root sap flux in root-pruned vines was maintained throughout the 5 weeks of measurement, suggesting that there is redundancy in the hydraulic function of kiwifruit vine roots under normal growing conditions, with the potential for increased water transport under stress conditions.

This supports the suggestion that kiwifruit vines have an excessive root:canopy ratio (Reid & Petrie 1991). Redundant hydraulic capacity could be a cavitation avoidance mechanism, allowing xylem pressures to remain above the cavitation threshold, despite a large reduction in root system hydraulic conductance. Kiwifruit vessels have been found to embolise at low xylem tensions, when xylem pressure potentials fall below -0.5 MPa (Clearwater & Clark 2003).

Nocturnal transpiration is common in kiwifruit (Green et al. 1989), and was clearly evident in the present experiment. Nocturnal root sap flux was generally highest in root-pruned vines. Nocturnal root flux may be attributed to nocturnal transpiration, driven by vapour pressure deficit (Green et al. 1989), or nocturnal rehydration of stem tissues (Bucci et al. 2004). Lower nocturnal transpiration and weaker correlation of root sap flux and vapour pressure deficit suggest more reliance on capacitance to meet diurnal transpiration demands. The contribution of nocturnal sap flux to tissue rehydration has been suggested previously in olive (Fernández et al. 2001) and nocturnal root pressure may be involved in maintaining kiwifruit vine water status (Clearwater et al. 2007).

Root pruning had a rapid effect on vine water use and leaf water status, resulting in symptoms similar to those imposed by water stress. Pre-treatment daily transpiration for control vines averaged 40 L $day^{-1}$, which concurs with previous estimates for kiwifruit in the order of 31 L $day^{-1}$ (Green & Clothier 1995) to 60 L $day^{-1}$ (Prendergast et al. 2007). Whole-vine daily $E_{plant}$ in root-pruned vines was reduced immediately post-treatment, with reductions in stem flux of approximately 27%. Over the 2 month observation period there was no clear evidence of a recovery in $E_{plant}$ as seen in other species (Ferree et al. 1999); this could be because of treatment severity (Ferree et al. 1999). These findings agree with previous research where root pruning has led to reduced $E_{plant}$, $\Psi_{leaf}$ and $K$, as well as visible wilting in apple trees (Geisler & Ferree 1984b) and grape vines (Ferree et al. 1999; Smart et al. 2006).

In control vines, the soil to trunk pathway contributed in the order of 30% to total plant resistance (Table 3.1). Assuming the majority of this resistance is in the root system, removing 80% of roots would result in a five-fold increase in root system resistance, resulting in an approximate doubling of total plant resistance, and

therefore a 50% reduction in $K_{l\text{-}plant}$. This is what was observed in root-pruned vines. Slight variances in the reduction of $K_{l\text{-}plant}$ between individual vines would be explained by the inability of our technique to accurately determine the amount of root biomass being removed. Reductions in $K_{l\text{-}leaf}$ were also observed and are considered to be associated with the reduction in $\Psi_{leaf}$, as $K_{leaf}$ has been found to be responsive to $\Psi_{leaf}$, assumed to be the result of cavitation (Brodribb & Holbrook 2003).

Xylem pressure potentials were reduced by root pruning. Reductions in $E_{plant}$ and $g_s$ were evident, with a rapid stomatal response observed on the day of treatment. However, stomatal closure was insufficient to maintain pre-pruning $\Psi$ values, suggesting that kiwifruit vines are anisohydric (Tardieu & Simonneau 1998). Stomatal closure following root pruning is considered to be a response to changes in plant hydraulic conductance rather than a root-initiated signal such as ABA (Hubbard et al. 2001). Stomatal responses in anisohydric species are less responsive to reduced *K*, demonstrating a curvilinear response (Hubbard et al. 2001). It has been shown that kiwifruit stomata show little response until $\Psi_{leaf}$ becomes very negative, in the order of -2.9 MPa (Dick 1987).

Reductions in $g_s$ indicate that stomatal limitation was responsible for reduced *A*. However, $c_i$ and leaf $\delta^{13}C$ were not reduced, suggesting involvement of biochemical down-regulation. Coordinated reductions in *A* and $g_s$ without a clear drop in $c_i$ have also been observed following water stress in kiwifruit (Chartzoulakis et al. 1993). It was suggested that reduced *A* may be a result of a combined reduction in $g_s$ and chloroplast activity (Chartzoulakis et al. 1993). Reductions in *A* following root pruning may also result from feedback inhibition caused by accumulation of photosynthates in leaves following reduced carbohydrate demand (Iglesias et al. 2002). Kiwifruit vines under drought conditions can have larger specific leaf areas (Montanaro et al. 2007), which indicates the association of reduced *A* and photosynthate accumulation in kiwifruit vine leaves. Maintenance of vegetative growth and increased fruit size and DM content suggest that *A* was not reduced in proportion to the reduction in sink demand, so that more carbohydrates became available to above-ground growth.

Previous root pruning research has demonstrated clear reductions in vegetative growth across a range of species (Ferree et al. 1999; Maas 2008). This severe root pruning in kiwifruit did not, suggesting that vegetative growth in kiwifruit vines is able to withstand negative effects imposed by root pruning. Increased fruit size and DM content are intriguing, as results in other fruit crops have been variable. Negative effects on fruit have been reported, including reductions in size and yield in apple (Khan et al. 1998), cluster and berry weight in grape (Ferree et al. 1999) and yield in peach (Kurtural & Taylor 2001). Few reports of positive responses such as those seen here exist. In kiwifruit there is a close link between fruit growth and vine water status (Judd et al. 1989), with even mild water shortage negatively affecting fruit growth (Prendergast et al. 1987). It was therefore expected that the reduction in water supply to the canopy following root pruning would reduce fruit growth. Results suggest that the signal for reduced fruit growth under drought conditions is not necessarily hydraulic, and is therefore absent from the response to root pruning. Mingo et al (2003) suggested that reduced fruit growth in plants experiencing low soil water availability is a result of a chemical signal produced in roots exposed to drying soil, rather than an actual reduction in plant water status.

In summary, severing a large number of structural roots resulted in coordinated reductions in $K_{\text{l-rootsystem}}$, $K_{\text{l-trunk}}$ and $K_{\text{l-leaf}}$, $E_{\text{plant}}$, $\Psi$, $A$ and $g_{\text{s}}$. Sap flux in individual intact roots was increased with no change in $K_{\text{root}}$; there was no evidence of $K_{\text{plant}}$ recovering in the short-term. Negative effects on the hydraulic status of root-pruned kiwifruit vines did not reduce vegetative growth, and fruit growth was increased. It is suggested that roots of the kiwifruit vine exhibit redundant hydraulic capacity, enabling tolerance to severe root system damage.

## 3.5 References

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# 4 Seasonal trends in root growth and soil respiration of kiwifruit vines: considerations for carbohydrate partitioning

## 4.1 Introduction

A large research effort has gone into understanding whole-plant carbon balances. This is particularly important in horticultural crops where growers aim to manipulate carbohydrate partitioning to increase biomass accumulation of the economically important components. The root system is a significant component of whole-plant biomass, but has sometimes been overlooked due to difficulties with access. Whole-plant carbon balances need to take into account seasonal variation in demand by different organs. Seasonal trends in the carbohydrate demands of the root system will be indicated by seasonal variation in root growth and biomass accumulation. Root growth in perennial fruit plants is often seasonal, with one or two pronounced peaks (Eissenstat et al. 2001; Eissenstat et al. 2006). The seasonality of root growth in kiwifruit vines is debatable. Previous experiments have observed a single peak in root growth in summer that was maintained until early autumn (Buwalda & Hutton 1988; Buwalda 1991b). In contrast, Reid et al. (1993) were unable to identify seasonal trends and suggested that growth occurred throughout the year. Conflicting findings between these two studies may be a result of the measuring technique. Further research is required to resolve these inconsistencies if the root system is to be included in future considerations of the whole-plant carbon balance for the kiwifruit vine.

Soil conditions such as temperature and moisture have a strong influence on root growth, particularly under extreme conditions (McMichael & Burke 2002; Comas et al. 2005). Variations in soil conditions are likely to account for some of the seasonality in root growth; though they are not considered to be the controlling factor (Buwalda & Hutton 1988). Carbohydrate availability is also strongly related to seasonal trends in root growth (Comas et al. 2005). Roots are considered to be a low priority sink when carbohydrate supply is limited, with the

carbohydrate demands of fruit and shoot growth being met first (Wardlaw 1990). Peaks in root growth are expected to occur after peaks in above ground growth or after fruit have been harvested. In grapevines peaks in root growth have been attributed to the combined effects of soil conditions and carbohydrate availability (Comas et al. 2005). Peaks in root growth in kiwifruit vines were found to coincide with the end of active shoot and fruit growth (Buwalda & Hutton 1988; Buwalda 1991b), conforming with the priority hierarchy defined by Wardlaw (1990).

Soil respiration may also indicate seasonal trends in root carbohydrate demand. Total soil respiration is comprised of that originating from microbial decomposition of organic matter (heterotrophic respiration) and that originating from the roots and root:microbial associations (autotrophic respiration; Ryan & Law 2005). A large proportion of root carbohydrate consumption is involved in respiration (Valentini et al. 2000). Daily root respiration consumes 8 – 52% of daily carbohydrate production (Lambers et al. 2002). Autotrophic respiration is a considerable proportion of total soil respiration. Estimates of autotrophic contributions to total soil respiration vary from 10 to 70% in forestry and pasture situations (Högberg et al. 2001; Lee et al. 2003; Andersen et al. 2005; Zhang et al. 2009). Few studies have been carried out in horticultural systems (Ceccon et al. 2011). Fluctuations in soil respiration can therefore be related to changes in plant metabolism and carbohydrate supply to the roots (Ryan & Law 2005). The reliance of root respiration on the continued supply of carbohydrates from the canopy has been demonstrated with trunk girdling of forestry trees resulting in rapid reductions in soil respiration (Högberg et al. 2001; Andersen et al. 2005; Domec & Pruyn 2008).

Distinction between autotrophic and heterotrophic respiration is ambiguous, due to mycorrhizal associations and the reliance of soil fauna on root exudates (Baggs 2006). Further, the two components may not be equally affected by changing soil conditions (Boone et al. 1998; Lee et al. 2003). A number of methods have been used for quantifying the contribution of autotrophic respiration, including component integration, root exclusion, isotope distribution, girdling and biomass regression analysis. The procedures, benefits and pitfalls of these techniques have been reviewed elsewhere (Hanson et al. 2000; Baggs 2006; Kuzyakov 2006). The

biomass regression technique has been used in a range of environments with varying results (Behera et al. 1990; Wang et al. 2008; Zhang et al. 2009; Ceccon et al. 2011). This technique makes the assumption that the relationship between root biomass and soil respiration is linear and that the heterotrophic component is spatially constant.

In New Zealand kiwifruit orchards the practice of trunk girdling is widely used for increasing fruit size and carbohydrate content (Currie et al. 2008). Trunk girdling has been demonstrated as a physiological treatment to manipulate carbohydrate partitioning, temporarily removing the root system as a carbohydrate sink. Girdling involves a band of bark including the phloem being removed from around the trunk, preventing the downward transport of carbohydrates. Girdles heal by forming a callus; differentiation of cambial cells enables the reconnection of the phloem vasculature (Goren et al. 2003). In grapevines girdles took approximately 5 weeks to heal (Williams et al. 2000) and phloem connection is suggested to resume within a few days of complete callus bridging (Sidlowski et al. 1971; Pang et al. 2008). Little is known regarding the effects of annual trunk girdling on the growth and functionality of the kiwifruit vine root system and questions have arisen regarding the sustainability of this technique. In Japanese persimmon trunk girdling reduced root growth (Fumuro 1997). Repeated annual application of girdles may therefore result in reduced root biomass. The period of carbohydrate starvation may also result in unseasonal utilisation of carbohydrate reserves. This has been observed in grape and peach (Roper & Williams 1989; Jordan & Habib 1996). The significance of this reduction in carbohydrate reserves will depend on the plants ability to replenish reserves after the girdle has healed.

This study used a modification of the mini-rhizotron to monitor root growth in kiwifruit vines. The aim was to identify seasonal trends in root carbohydrate demand, determined as seasonal variation in root biomass accumulation and respiration. The following hypothesis was investigated: maximum root growth will occur during times when canopy demand for carbohydrates is low, with the seasonal trend in root growth demonstrating peaks after peaks in fruit and shoot growth. The mass regression technique was used to estimate the contribution of root respiration to total soil respiration at different times throughout the growing season. It was hypothesised that soil respiration would be strongly related to root

growth and root biomass. Further, it was hypothesised that trunk girdling would temporarily disrupt carbohydrate partitioning to the roots resulting in temporary reductions in root growth and respiration. It was hypothesised that with the annual use of trunk girdling the reduction in root growth rates would gradually reduce root biomass.

## 4.2 Materials and Methods

### 4.2.1 Plant material

The experiment was conducted at a research orchard (Plant & Food Research Ltd.) in Te Puke, New Zealand. Vines were green kiwifruit (*Actinidia deliciosa* (A.Chev.) C.F. Liang et A.R. Ferguson var. *deliciosa* 'Hayward') scions grafted onto seedling rootstocks derived by open-pollination of *Actinidia deliciosa* (A.Chev.) C.F. Liang et A.R. Ferguson 'Bruno'. Rootstocks were planted in 1981, grafted in 1982 and trained to a pergola system with 6 m spacing between plants within the row and 3 m spacing between rows. The soil type was Te Puke sandy loam. Cultural management was standard to the cultivar (Sale and Lyford 1990). The annual fertiliser regime included a winter application of phosphate, potassium, sulphur and magnesium followed by a spring application of nitrogen, two applications of potassium and sulphur, applied in early and mid-summer and a second nitrogen application in summer. Full bloom stage of flowering was on the 26 November in 2009 and 2010. Crop load was maintained at around 40 fruit $m^{-2}$. Fruit were harvested on the 20 May 2010. Vines had not been irrigated since 1990. Weather data (Figure 4.1A) were collected from a weather station on site. Six vines were selected, randomly distributed within the kiwifruit block. Three vines were trunk-girdled annually and three had never been girdled. Trunk-girdled vines had been girdled for the 3 years prior to this experiment, with girdles applied in January and February 2007, February, March and April 2008, and January and February 2009. During this experiment a single girdle was applied each year, on the 4 February 2010 and the 4 of February 2011.

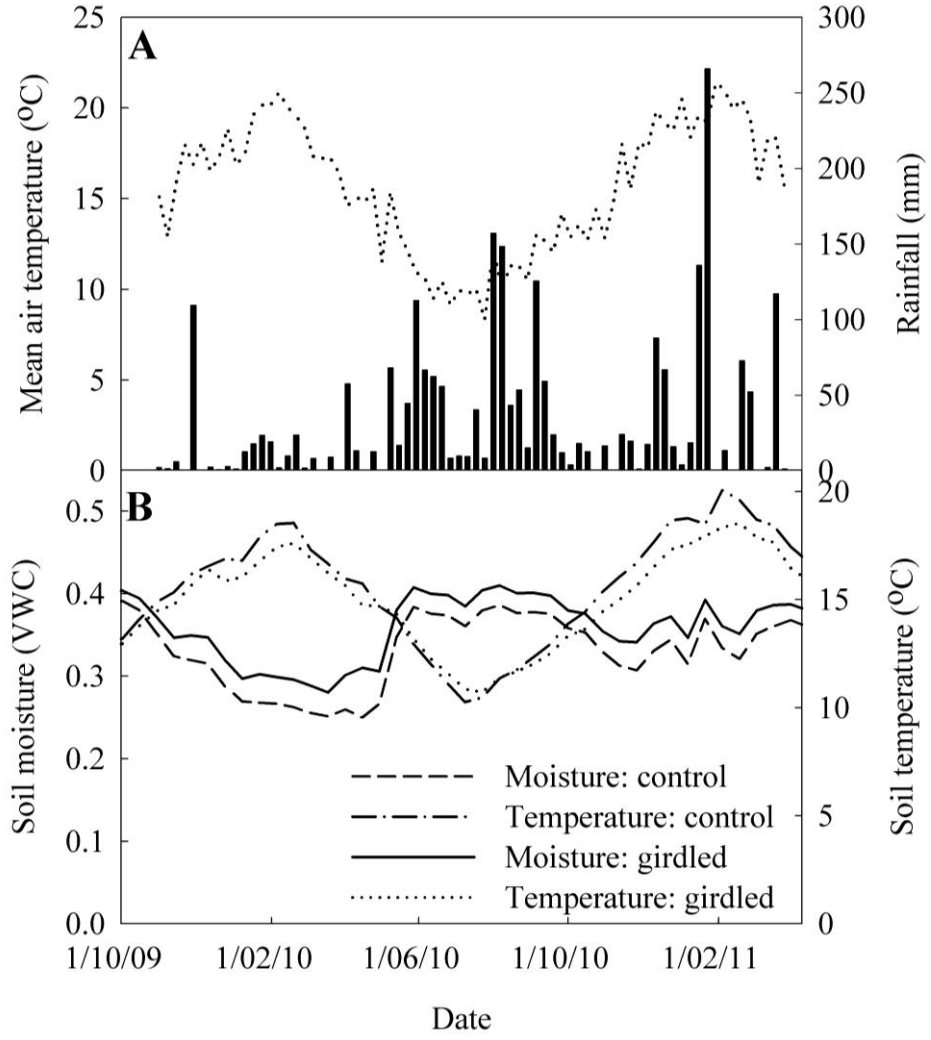


**Figure 4.1 A) Weekly mean air temperature and rainfall for the experimental period. B) Fortnightly soil moisture and soil temperature for control and trunk-girdled vines. Data are medians, *n* = 8 probes, with two at 100 mm, 400 mm, 700 mm and 1000 mm.**

### 4.2.2 Root growth observations

Twelve rhizotron sleeves were constructed of 8 mm thick sheet acrylic (Anything Acrylic Ltd., Auckland). Internal dimensions measured 60 mm deep by 270 mm wide and 1500 mm long. Exterior joins were coated with waterproof silicone. The top 200 mm of the rhizotrons, which was not buried, was made impermeable to light using a layer of black then white duct tape. Caps were made out of 6 mm thick acrylic, with internal dimensions of 80 mm deep by 295 mm wide and 150 mm long, fitting over the top of each rhizotron. Caps were also coated with duct

tape. Each rhizotron was fitted with a scale bar, which was visible in the scanned images to ensure that the same location was scanned on each date. Between scanning events the rhizotrons were filled with a removable rectangular strip of polystyrene to act as insulation against temperature gradients.

Two rhizotrons were installed per vine, at a 1 m radius from the trunk, with one to the north and one to the south of the vine, on the 11 and 12 March 2009. Holes were dug using a 300 mm diameter post-hole borer to a depth of 1300 mm. Front and side walls of the hole where then squared-off using a spade, to minimise the depth of disturbed soil in front of the rhizotron. Rhizotrons were installed vertically. Soil was carefully repacked returning soil to the depth it was extracted. Approximately 2 months were allowed for settlement, after which image collection commenced at monthly intervals. Once root growth was evident in multiple rhizotrons, image collection frequency was increased to fortnightly. Image collection continued until 31 March 2011. Images collected from October 2009 onwards were considered to be representative of root growth, having allowed a 6 month settlement phase to minimise the effects of soil disturbance, resulting in 18 months of root growth data.

Images of root growth were collected using a flat bed scanner (Canoscan D646u ex, Canon, California), fitted in an acrylic case. The case was fitted with a long indexing handle, allowing the scanner to be lowered to the base of the rhizotron and moved up in 300 mm increments. The handle slid through a custom-made cap that covered the top of the rhizotron during scanning preventing the entry of external light. Previous work using flatbed scanners for collecting root growth images has shown that charge coupled device (CCD) technology provides better image depth (Dannoura 2008), therefore a scanner was selected that incorporated this technology. The scanner was powered by a 12 volt battery and USB connection to a laptop computer. Images were collected at four depths, 100 mm, 400 mm, 700 mm and 1000 mm, at 300 dpi.

Image analysis was performed using the software package ‘RootFly’ (Clemson University, Clemson). The length and diameter of each root was measured; root birth was taken as the date halfway between the first observation and the previous scanning (Bauerle et al. 2008). Root death was determined as darkening and

shrivelling or disappearance (Wells & Eissenstat 2001). The time of root death was taken as the date midway between when death was determined and the previous observation. From the rhizotron data the following parameters were calculated: daily mean growth rates of individual roots and all roots per rhizotron (total growth rate); relative root production (Eissenstat et al. 2006) and relative root death during the first year of the experiment and root turnover. Turnover was calculated for the first year of the experiment as the ratio of annual cumulative root growth and mean net root length (Aerts et al. 1992). Survival times were estimated for roots born between October 2009 and March 2010. Roots that appeared at each scanning event during this time were placed into cohorts and their death during the remainder of the experiment was monitored to determine the percent survivorship. The number of days from the start of a cohort to the date when 50% of the roots in that cohort had died was taken as the median survival time (Wells & Eissenstat 2001).

### 4.2.3 Soil Parameters

Soil volumetric water content (VWC) was measured using time domain reflectometry (TDR) probes (CS616-L, Campbell Scientific, Utah). Probes were installed in close proximity to four rhizotrons, two at trunk-girdled vines and two at control vines. Probes were installed to four depths to coincide with the depths of image collection, 100 mm, 400 mm, 700 mm and 1000 mm. An 80 mm diameter post-hole borer was used to core to the desired depth. For the three deeper probes an access tube, consisting of 80 mm drainage pipe, was installed and capped. Probes were installed vertically so that the metal rods were inserted into un-disturbed soil. Soil temperature was measured at the same locations and depths as VWC using a combination of temperature probes (Model 107, Campbell Scientific, Utah) and custom-made copper-constantan thermocouples. Sensors were wired to multiplexors and dataloggers (AM416; CR10, Campbell Scientific, Utah) and average VWC and soil temperature recorded at 3 hour intervals.

### 4.2.4 Soil Respiration

Soil respiration was measured at fortnightly intervals to coincide with scanning events, using a LI-COR portable gas exchange system fitted with a soil chamber (LI-6400-09, LI-COR Inc., Nebraska). Plastic collars (100 mm diameter × 30 mm deep) were installed with four per vine. Collars were positioned approximately

500 mm from the trunks, inserted to a depth of 20 mm and left in place throughout the experiment. Three measurement cycles were performed per collar at each assessment, recording the average of each cycle.

### 4.2.5 Biomass regression analysis

To determine the contribution of autotrophic respiration to total soil respiration the mass regression technique (Kucera & Kirkham 1971) was used at six times during the 18 month period. A second set of collars were installed a minimum of 24 hours before measurement. Sixteen to 32 collars were installed at random around the kiwifruit block. Total soil respiration was measured as described above and then a soil core was taken to a depth of 300 mm directly below each collar using an 88 mm diameter soil corer. Soil samples were sieved to extract all root material; roots were washed and then dried at 80ºC until constant weight was achieved. Dried roots were weighed and dry weight biomass per unit soil surface area calculated (g dw $m^2$). A linear regression was then performed, with root biomass as the independent variable and total respiration as the dependant variable. The *y*-intercept was taken as the heterotrophic contribution to total soil respiration in the absence of roots (Behera et al. 1990; Wang et al. 2008). The autotrophic contribution to total soil respiration in the orchard was estimated by deducting the heterotrophic contribution (*y*-intercept) from total soil respiration. Further, the heterotrophic component (*y*-intercept) was used to estimate the autotrophic contribution around control and trunk-girdled vines by deducting the heterotrophic component from the fortnightly measurements of soil respiration.

### 4.2.6 Statistical analyses

Statistical analyses were carried out in R, version 2.13.0 (Comprehensive R Archive Network, cran.r-project.org). Data were tested for homogeneity using the Bartlett test and for normal distribution using the Shaprio-Wilk test. ANOVA and Wilcoxon Signed Rank Test were used to test treatment differences. Correlations between soil parameters, root growth, root numbers and soil respiration were performed using the non-parametric Spearmans correlation. Multiple linear regression was used to relate soil respiration with root and soil parameters at quarterly intervals.

## 4.3 Results

### 4.3.1 Root growth

Root numbers demonstrated a population build up in summer (January/February) 2010 and then started to decline in winter (June/July) 2010 (Figure 4.2). The total number of roots observed throughout the experiment (± SEM) averaged 447 ± 110 roots per rhizotron, with considerable variation between rhizotrons. Root numbers varied with depth (Figure 4.2). The highest numbers of roots were found in the top 700 mm of soil, particularly during the initial population increase. The total number of roots observed per rhizotron was generally lower around girdled vines, though not significantly ($P > 0.05$) lower (Figure 4.2). Girdled vines had approximately 70% lower root populations below 700 mm in comparison with control vines until summer 2010 – 2011.

The mean balance of visible root length is shown in Figure 4.3. Cumulative root growth (Figure 4.3A) was most rapid during January to June 2010 and November 2010 to January 2011. Cumulative root growth was consistently lower around girdled vines. Over the 18 month experiment mean cumulative root growth reached approximately 10 m for control vines and approximately 8 m for girdled vines (Figure 4.3A). Root biomass estimates performed at the end of the experiment, showed significantly ($P < 0.05$) lower root biomass around girdled vines. Total root biomass around girdled vines was 59% lower than that around control vines (Table 4.1).

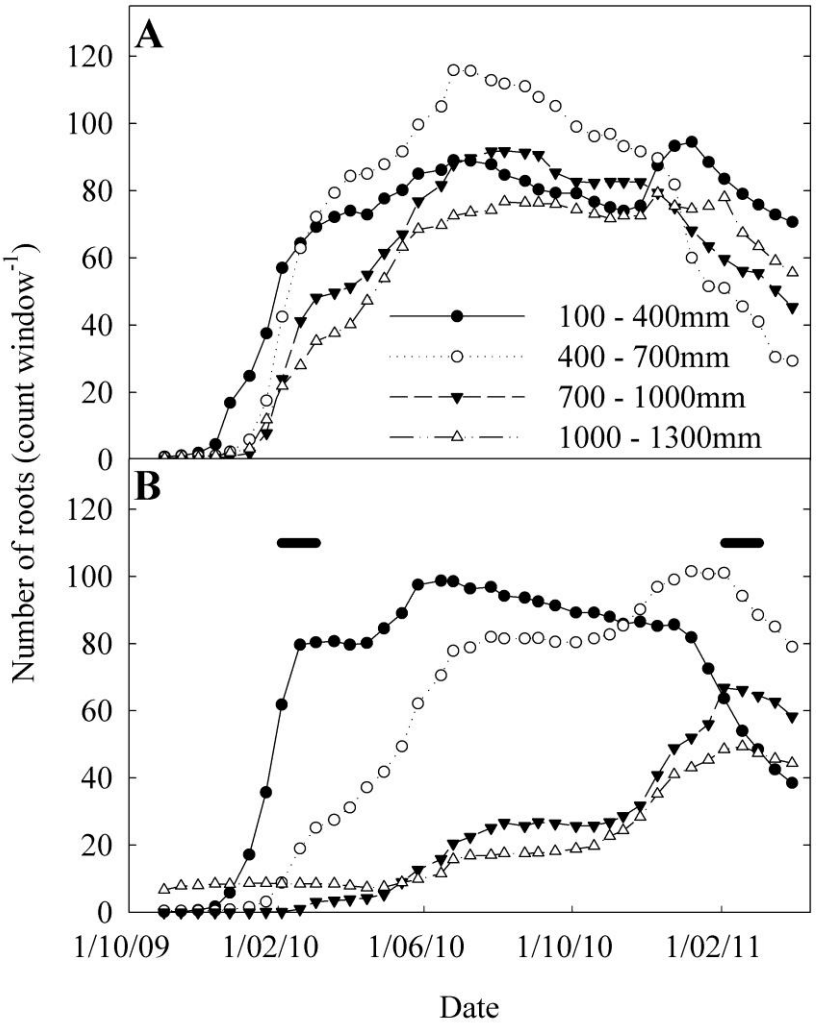


**Figure 4.2 Mean number of roots observed per rhizotron at each window (100 – 400 mm, 400 – 700 mm, 700 – 1000 mm, and 1000 – 1300 mm) for A) control and B) trunk-girdled vines. Black bars represent the period the girdle was assumed open. Data are means, *n* = 3 vines.**

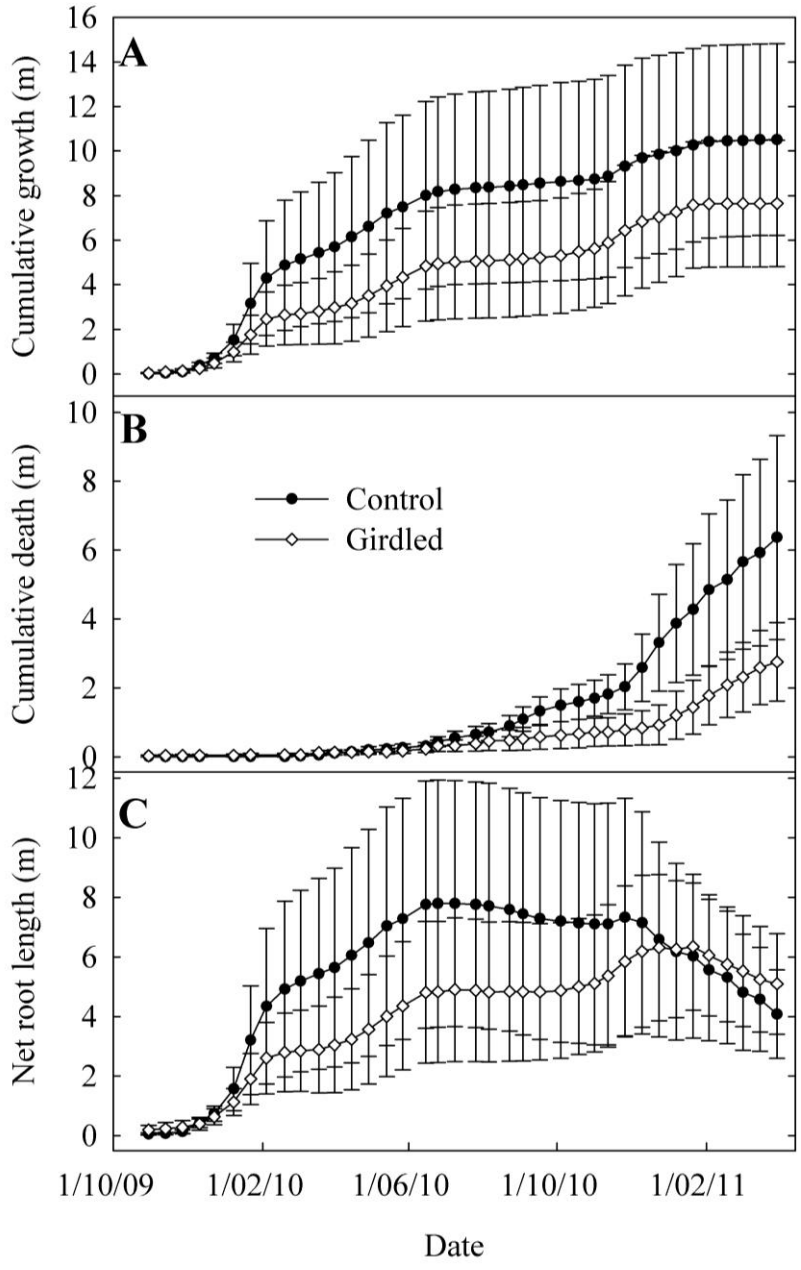


**Figure 4.3 Root balance per rhizotron for control and trunk-girdled vines. A) Cumulative root growth (m), B) cumulative root death (m) and C) net root length (m). Data are means, *n* = 3 vines, ± 1SEM.**

**Table 4.1 Root biomass for control and trunk-girdled vines. Data were collected by soil coring at the end of the experiment, in March 2011. Fine roots are those less than 2 mm diameter and coarse roots those larger than 2 mm diameter. Data are means, *n* = 24, ± 1SEM.**

| | Fine | Coarse | Total |
|---|---|---|---|
| Control | 236 ± 29 | 580 ± 115 | 984 ± 218 |
| Trunk-girdled | 109 ± 26 | 146 ± 64 | 407 ± 136 |
| *P* -value | 0.002 | 0.002 | 0.029 |

Total root growth rates showed a seasonal trend. Summer peaks in growth were observed in both years. During the 2009 – 2010 growing season a second peak was observed in autumn (Figure 4.4A). The current data set ends before autumn in the second year. Mean total root growth rates peaked at approximately 120 mm $day^{-1}$ for control vines in January 2010. The seasonal trend in root growth rates (Figure 4.4A) showed minimal growth during winter months when soil temperatures were between 10 and 14°C and soil moisture was between 0.37 and 0.42 VWC (Figure 4.1B); maximum root growth occurred in summer when soil temperatures were between 16 and 18°C and soil moisture was between 0.28 and 0.3 VWC. However, root growth rates showed little correlation to soil temperature (Figure 4.5A) or soil moisture (Figure 4.5B). Total root growth rates (Figure 4.4A) were consistently lower around girdled vines for the first six months, this resulted from lower root numbers per rhizotron, as the mean rate of growth of individual roots of girdled vines was significantly ($P < 0.05$) higher (0.23 ± 0.01 mm $day^{-1}$) compared with control vines (0.19 ± 0.01 mm $day^{-1}$). During the girdling periods of 2010 and 2011 there was no evidence of reductions in root growth rates resulting from the girdle. In 2010 root growth rates declined between February and March in girdled and control vines. In 2011, a small insignificant reduction in growth rates was observed in girdled vines. Relative root length production during the first growing season was at its highest between late January and early February, with 11 – 13% of annual root production occurring during this

period; followed by 8 – 11% of annual root production during May and June (Figure 4.6A). Girdling did not affect the timing of peak root production.

Root death was evident from June 2010 onwards and the largest losses in root length occurred from November 2010 onwards (Figure 4.3B). Cumulative root death reached approximately 6 m for control vines and 3 m for girdled vines; resulting in mean net root lengths of approximately 4 m for control vines and 5 m for girdled vines (Figure 4.3C). Relative root death (Figure 4.6B) during the first growing season showed a gradual increase in control vines with maximum root death occurring in October 2010. A peak in root mortality was observed in control and girdled vines in March 2010. Root death in girdled vines was variable throughout the year. Annual root turnover during the 2009 – 2010 growing season was estimated at 1.8 ± 0.13 $year^{-1}$ for control vines and 1.7 ± 0.17 $year^{-1}$ for girdled vines. A higher proportion of roots born in summer, autumn and winter months (61 – 71%) died in comparison with roots born in spring of either year (41 – 45%). Examples of survival curves for root cohorts born during the first six months of the experiment are shown in Figure 4.7. Median survival time for the cohorts ranged 315 – 455 days (median 364 ± 16.9) for control vines and 153 – 447 days (median 383 ± 31.4) days for girdled vines. Although data indicates longer surviving roots around girdled vines the differences were not significant. Longevity of roots in these cohorts did not differ significantly with soil depth.

All of the roots visible in the rhizotrons were classified as fine roots. Approximately 50% of roots were in the diameter class 1.0 – 1.5 mm. Less than 1% of roots had a diameter less than 0.5 mm or more than 2.5 mm (Figure 4.8). Mean root diameter was relatively constant throughout the experiment. Between spring 2009 and winter 2010 there was some evidence of larger diameter roots being found at shallower depths, though differences did not persist. Root diameter was not related to the season in which roots were born or the girdling treatment. Very few roots increased in diameter; those that did also tended to undergo pigmentation and produce laterals. The majority of roots remained white throughout the experiment until they died. Dying roots often showed discolouration, which was later followed by shrivel or complete disappearance.

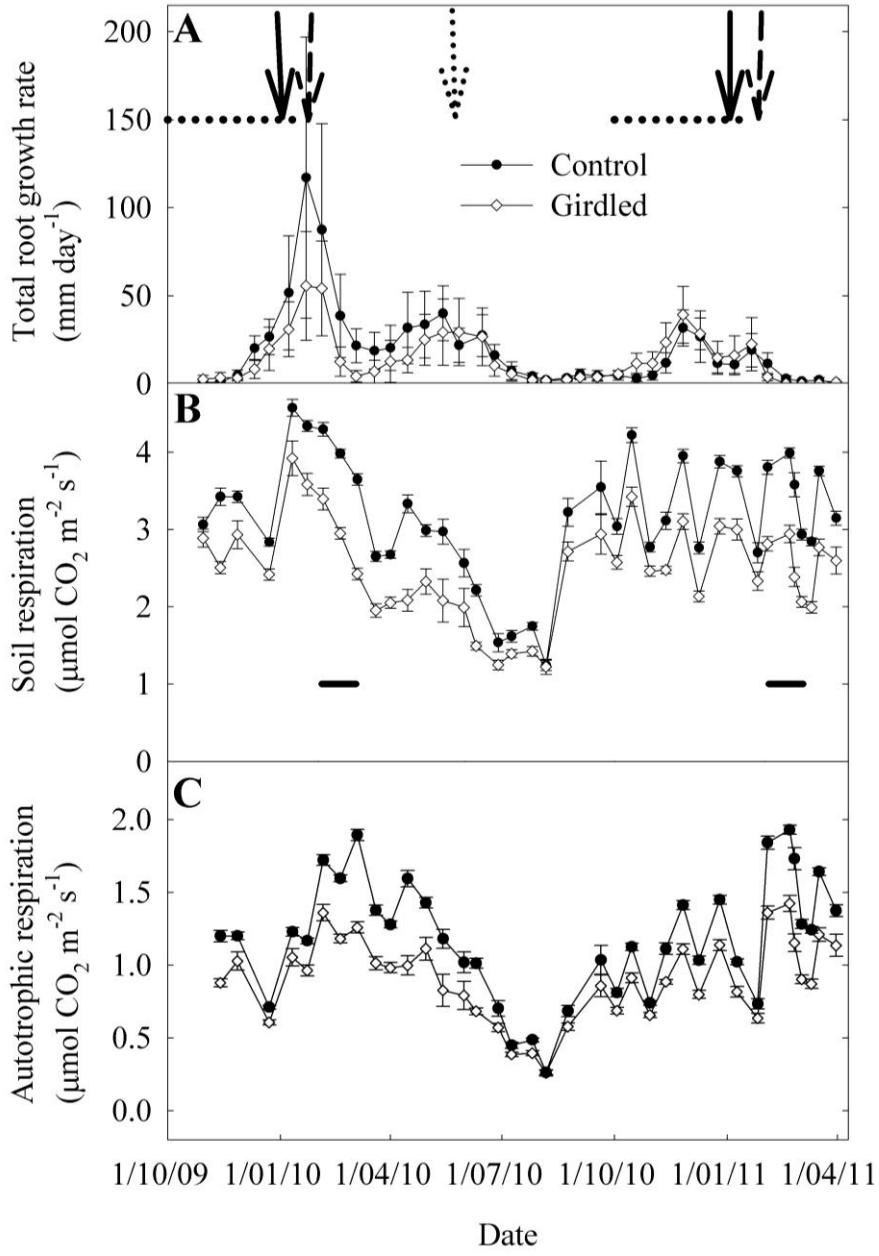


**Figure 4.4 A) Mean daily total root growth rate (growth of all roots per rhizotron per day) for trunk-girdled and control vines. Arrows mark the timing of maximum fruit fresh weight increases (solid; (Minchin et al. 2003), maximum fruit dry weight increases (dashed; (Snelgar et al. un-published), and fruit harvest (dotted); dotted lines mark the main period of vegetative growth (Davison 1990). B) Total soil $CO_2$ efflux for trunk-girdled and control vines. Black bars represent the period the girdle was assumed open. C) Autotrophic respiration estimated from differences in soil respiration and total root biomass between control and trunk-girdled vines. Data are means, *n* = 3 vines, ± 1SEM.**

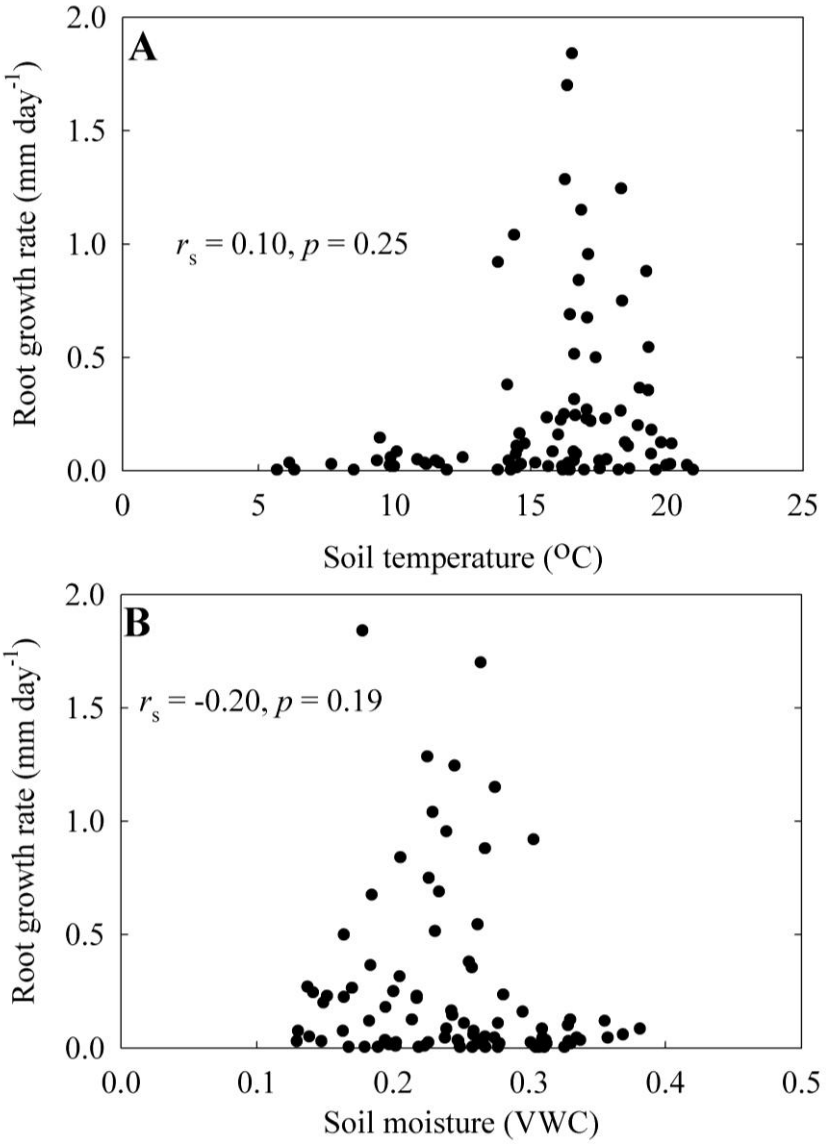


**Figure 4.5 Correlation plot and Spearman correlation coefficient for individual root growth rates in the top 100 – 400 mm of soil with, A) soil temperature at 100 mm and B) soil moisture at 100 mm.**

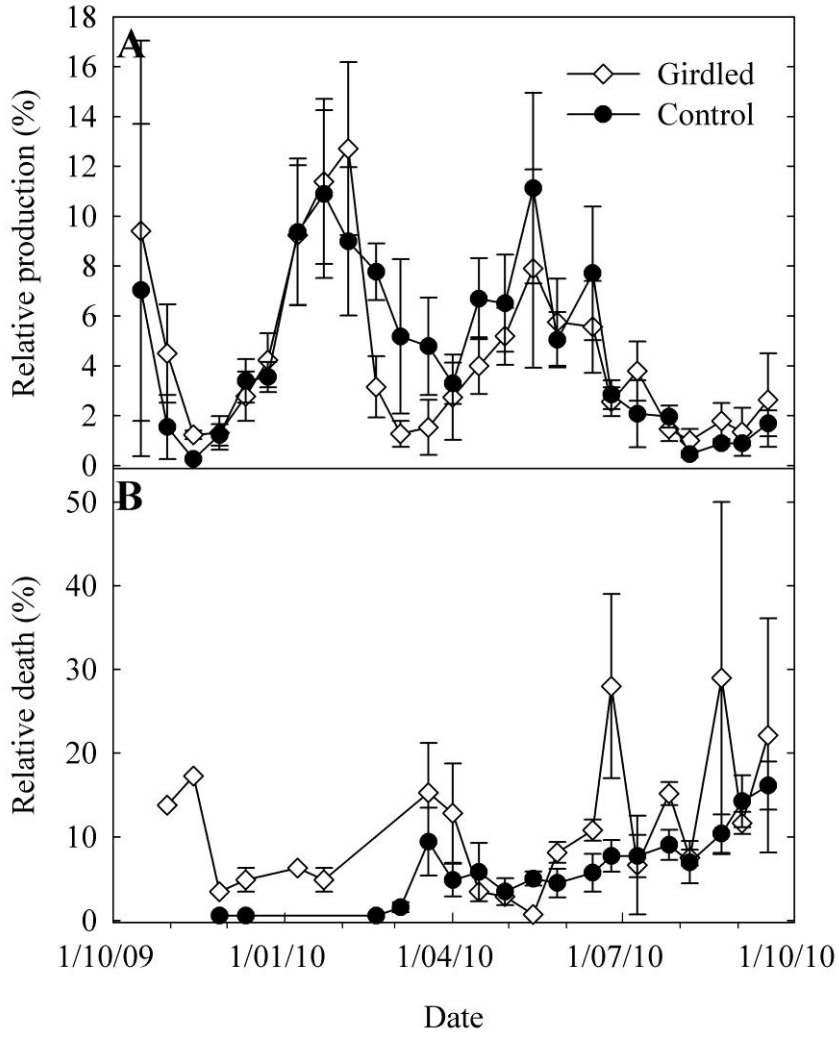


**Figure 4.6 Seasonal trends in A) relative root production and B) relative root death during the 2009 – 2010 growing season. Data is for the first 12 months of the experiment for control and trunk-girdled vines. Data are means, *n* = 3 vines, ± 1SEM.**

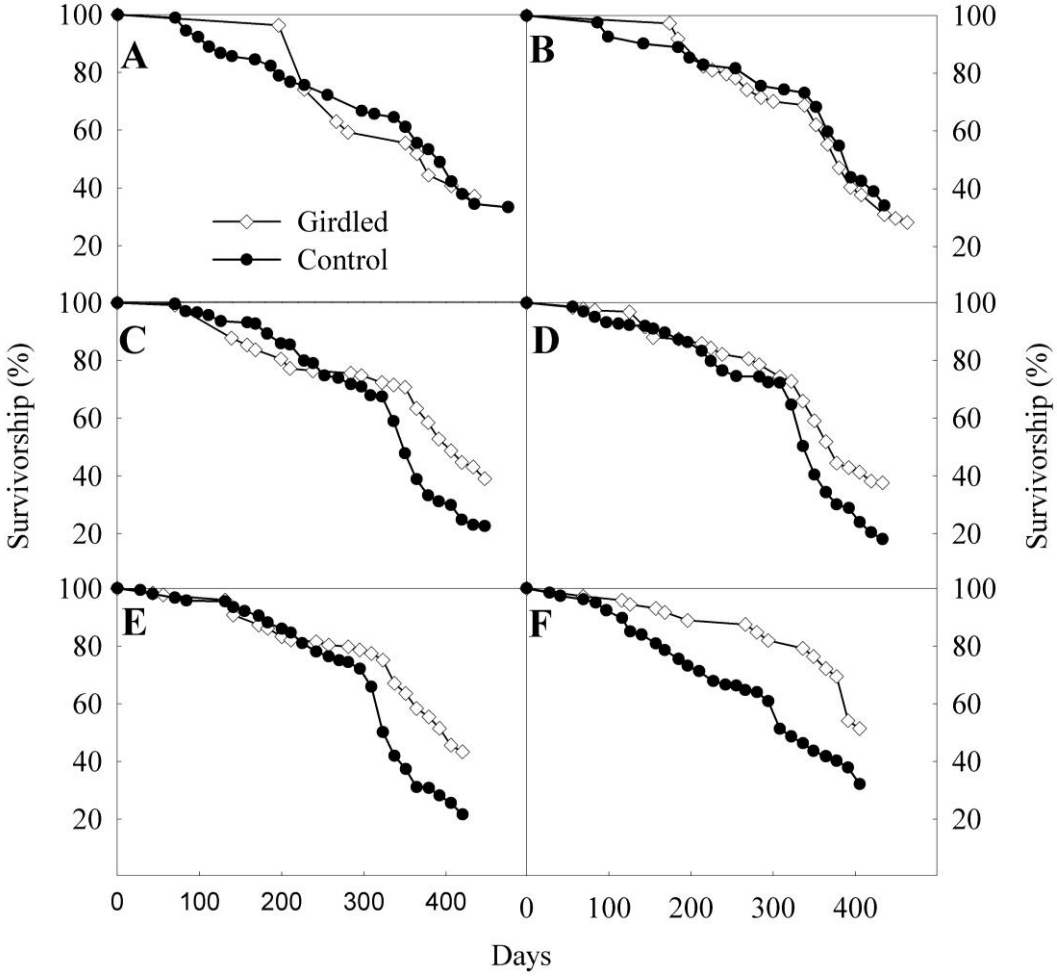


**Figure 4.7 Root survivorship (%) for cohorts born during October 2009 and March 2010. A) Cohort 4 born 11/12/09, B) cohort 5 born 23/12/09, C) cohort 6 born 08/01/10, D) cohort 7 born 22/01/10, E) cohort 8 born 04/02/10 and F) cohort 9 born 19/02/10. *n* = 3 vines.**

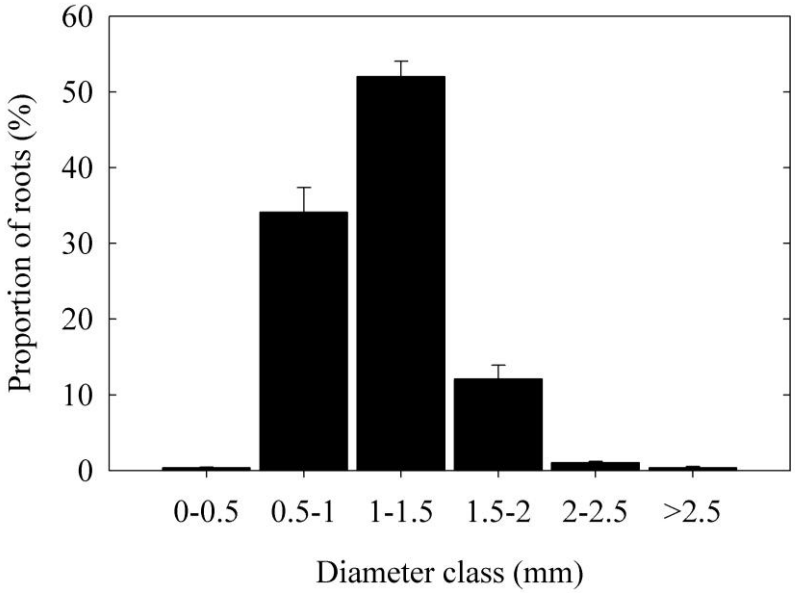


**Figure 4.8 Diameter distribution of all roots observed using the rhizotrons during the 18 month experiment, *n* = 6 vines, ± 1SEM.**

### 4.3.2 Respiration

Soil respiration also showed a seasonal trend, particularly during the 2009 – 2010 growing season when trends in soil respiration resembled trends in root growth (Figure 4.4). Peak soil respiration occurred in summer at approximately 5 µmol $m^{-2}$ $s^{-1}$. In autumn respiration declined rapidly, reaching winter lows of approximately 1 – 1.5 µmol $m^{-2}$ $s^{-1}$, before rapidly increasing again in early spring (Figure 4.4B). Soil respiration was poorly correlated with growth rates of roots in the top 100 – 400 mm of soil ($r_s$ = 0.22, $P$ = 0.01; Figure 4.9A) and there was no evidence of a correlation with root numbers. Soil respiration demonstrated stronger correlation with soil conditions than root growth. Soil respiration was positively correlated with soil temperature ($r_s$ = 0.53, $P$ < 0.01; Figure 4.9B) and negatively correlated with soil moisture ($r_s$ = -0.48, $P$ < 0.01; Figure 4.9C).

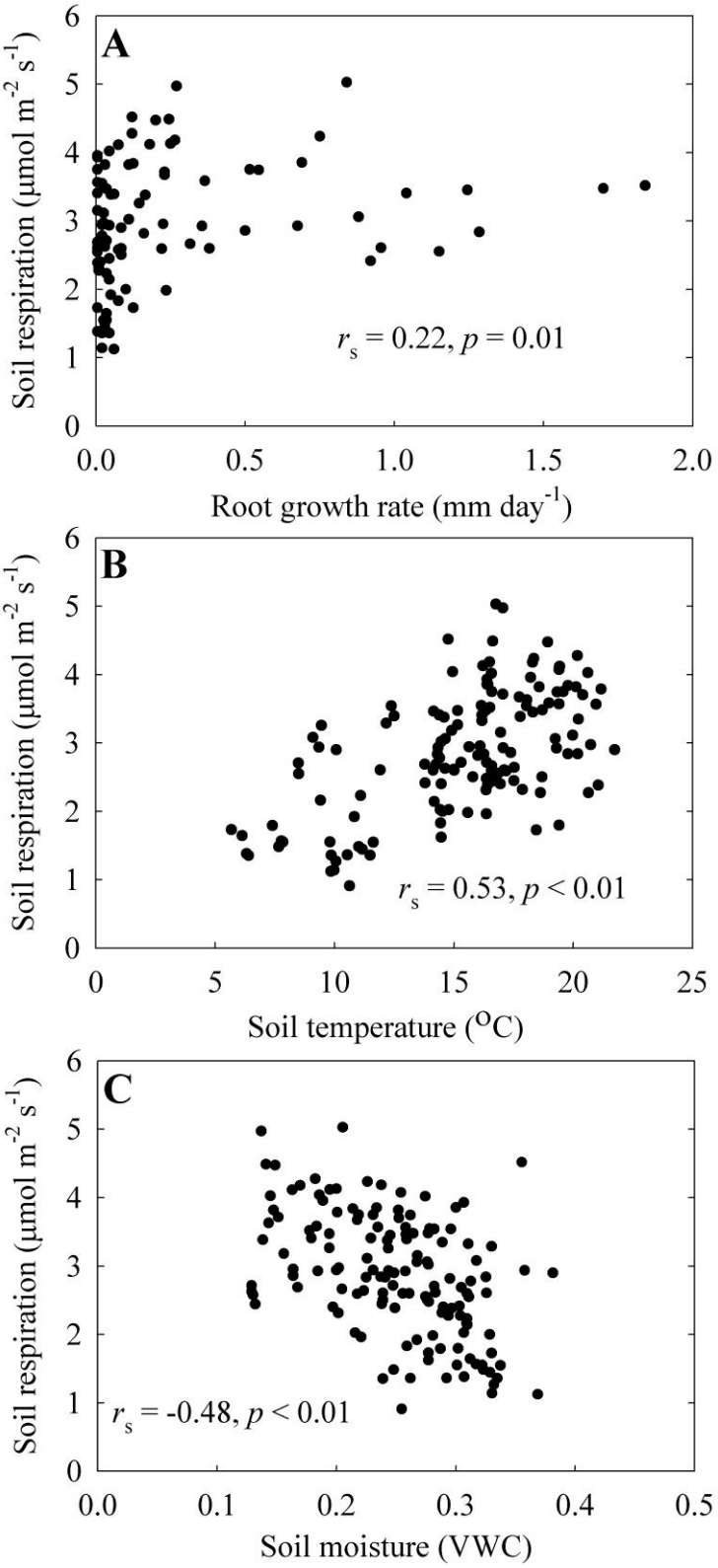


**Figure 4.9 Correlation plot and Spearman correlation coefficient for soil respiration with A) individual root growth rates in the top 100 – 400mm of soil, B) soil temperature in the top 100mm, and C) soil moisture in the top 100mm.**

The significance of soil conditions influencing soil respiration varied with season (Table 4.2). For control vines, root growth was significantly related to soil respiration in summer and winter 2010 and spring 2011, soil moisture was a significant factor in summer and autumn 2011 and soil temperature in autumn 2010 and summer 2011. A difference in these relationships between control and girdled vines was evident. Soil moisture was higher around girded vines and soil conditions were more influential on soil respiration for those vines, with moisture significantly related to respiration at six out of seven timings (data not shown).

Soil respiration was consistently and significantly ($P < 0.05$) lower around girdled vines (Figure 4.4B). Respiration was reduced further following girdle application. In 2010 significant ($P < 0.05$) reductions in soil respiration around girdled vines were observed between January and February (during-girdling; 13%), and February and March (post-girdling; 41%). Reductions were also evident around control vines, but these reductions were smaller (7 and 29%, respectively). In 2011 respiration was increased in February, though increases were lower around girdled vines (Figure 4.10).

Mass regression analysis demonstrated significant positive relationships between fine root biomass and total soil respiration at four of the six timings (Table 4.3). A seasonal trend was evident in the relationship; significant relationships were observed in autumn and spring 2010 and summer and autumn 2011, with no evidence of a relationship over winter. The contribution of autotrophic respiration to total soil respiration was estimated to range between 2.3 ± 0.6% in winter and 19.3 ± 2.0% in autumn (Table 4.3). Application of the heterotrophic component to total soil respiration measured fortnightly at test vines frequently resulted in negative estimates of autotrophic respiration. This indicated that the heterotrophic contribution was overestimated by the mass regression technique. Therefore an alternative technique was devised to estimate autotrophic respiration around the six test vines. At the end of the experiment total root biomass was 59% lower around girdled vines in comparison with control vines (Table 4.1). Assumptions were made that the effects of girdling on heterotrophic respiration were minimal in comparison with effects on autotrophic respiration. Further, it was assumed that the biomass difference between control and girdled vines was relatively constant throughout the experiment. The difference in soil respiration between control and

girdled vines was calculated for each month; the difference in soil respiration was attributed to the 59% reduction in root biomass. This enabled the estimation of the percent autotrophic respiration for each month. Using this technique autotrophic respiration was estimated to range between 21% in August 2010 (winter) and 52% in March 2010 (early autumn; Table 4.4). These contributions were applied to total soil respiration data to obtain seasonal autotrophic respiration (Figure 4.4C). Autotrophic respiration ranged from 0.25 to 2.0 µmol $CO_2$ $m^{-2}$ $s^{-1}$, and was at its highest during March to May of both years.

**Table 4.2 Quarterly multiple linear regressions for control vines. Soil respiration was the dependent variable and individual root growth rates in the top 100 – 400 mm of soil and soil moisture and temperature at 100 mm depth were the independent variables. *P*-value is the statistical significance of the model. Relationships are considered significant at *P* = 0.001'***', 0.01'**', 0.05'*'.**

| Quarter | Season | Months | Factors | *P* - value | $R^2$ | DF |
|---|---|---|---|---|---|---|
| 1 | Spring | Oct – Nov 09 | Growth<br>Soil temperature<br>Soil moisture | 0.162 | 0.15 | 32 |
| 2 | Summer | Dec 09 – Feb 10 | Growth ***<br>Soil temperature<br>Soil moisture | < 0.01 | 0.27 | 86 |
| 3 | Autumn | Mar – May 10 | Growth<br>Soil temperature *<br>Soil moisture | 0.072 | 0.06 | 116 |
| 4 | Winter | Jun – Aug 10 | Growth ***<br>Soil temperature<br>Soil moisture | 0.006 | 0.12 | 98 |
| 5 | Spring | Sep – Nov 10 | Growth **<br>Soil temperature<br>Soil moisture | 0.058 | 0.06 | 132 |
| 6 | Summer | Dec 10 – Feb 11 | Growth<br>Soil temperature ***<br>Soil moisture *** | < 0.01 | 0.25 | 140 |
| 7 | Autumn | Mar11 | Growth<br>Soil temperature<br>Soil moisture *** | 0.009 | 0.16 | 68 |

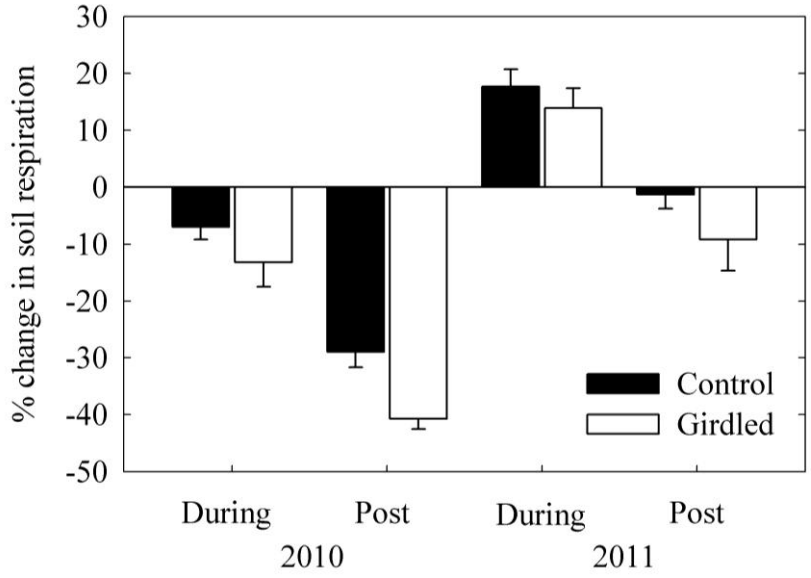


**Figure 4.10 Percent change in soil respiration from the month pre-girdling (January) to the month during (February) and the month post-girdling (March) for control and trunk-girdled vines. Girdles were applied in February 2010 and 2011. Data are means, *n* = 3 vines, +/- 1SEM**

**Table 4.3 Regression results of soil respiration against fine root biomass in the top 300 mm of soil, measured by soil coring. Measurements were taken at six timings throughout the experiment period at random locations around the kiwifruit block. *n* ranged from 16 to 32 respiration collars. The mean percentage root contribution is calculated by deducting the *y*-intercept value, assumed to be the heterotrophic component of soil respiration at that time, from total soil respiration.**

| Date | Intercept | Slope | *P* - value | $R^2$ | DF | % root contribution |
|---|---|---|---|---|---|---|
| 12/04/2010 | 2.07 | 0.0027 | <0.001 | 0.25 | 14 | 19.3 ± 2.02 |
| 16/07/2010 | 2.06 | -0.0013 | 0.404 | 0.05 | 14 | 2.25 ± 0.57 |
| 24/09/2010 | 2.73 | -0.0004 | 0.923 | 0.00 | 14 | 6.47 ± 2.05 |
| 08/10/2010 | 3.01 | 0.0035 | 0.004 | 0.27 | 29 | 16.02 ± 1.71 |
| 11/01/2011 | 3.39 | 0.0032 | 0.014 | 0.19 | 30 | 14.12 ± 1.40 |
| 17/03/2011 | 3.2 | 0.0025 | <0.001 | 0.35 | 22 | 15.2 ± 1.57 |

**Table 4.4 Monthly differences in total soil respiration between control and trunk-girdled vines (% reduction) and the estimated autotrophic contribution to total soil respiration. Autotrophic respiration is based on the reduction in respiration being attributed to a 59% reduction in root biomass around trunk-girdled vines.**

| Month | Reduction in respiration (%) | Autotrophic contribution (%) |
|---|---|---|
| November 2009 | 20.6 | 35.0 |
| December 2009 | 14.7 | 25.1 |
| January 2010 | 15.8 | 26.9 |
| February 2010 | 23.6 | 40.1 |
| March 2010 | 30.5 | 51.9 |
| April 2010 | 28.1 | 47.8 |
| May 2010 | 23.4 | 39.7 |
| June 2010 | 26.9 | 45.7 |
| July 2010 | 16.3 | 24.8 |
| August 2010 | 12.5 | 21.2 |
| September 2010 | 17.2 | 29.2 |
| October 2010 | 15.7 | 26.7 |
| November 2010 | 21.0 | 35.7 |
| December 2010 | 22.0 | 37.4 |
| January 2011 | 16.0 | 27.2 |
| February 2011 | 28.4 | 48.3 |
| March 2011 | 25.7 | 43.7 |

## 4.4 Discussion

The seasonal trend of root growth rates demonstrated minimal growth in the cooler, wetter winter months and peak growth in the warmer, drier summer months. Root growth rates showed little direct correlation to soil conditions; however, growth rates were minimal at temperatures below 13°C or when soil moisture was above 0.3 VWC. These values indicate a threshold at which soil parameters become the controlling influence on root growth. It has been suggested that an optimum temperature exists for root growth and that deviation from that temperature affects growth and function. Temperatures below optimum may reduce root system size and water and nutrient uptake efficiency whereas

temperatures above the optimum may reduce enzyme activity (McMichael & Burke 2002). The optimum temperature for kiwifruit root growth is 20°C and temperatures lower than 10°C may halt growth (Smith et al. 1989). Surface soil layers briefly experienced temperatures close to the optimum during summer months and fell close to the minimum in winter. The second peak in growth in autumn occurred when soil temperature had fallen between 13°C and 16°C; suggesting that fluctuations in temperature between 13°C and 20°C may not have a strong influence on root growth rates.

Correlations between soil respiration and soil conditions suggest that soil moisture and temperature influenced the seasonal trend in soil respiration. The positive effect of increasing temperature and the negative effect of increasing moisture on soil respiration agree with observations in an apple orchard (Ceccon et al. 2011). Soil moisture is most influential on soil respiration when deficient or in excess (Epron et al. 1999a; Andersen et al. 2005). Summer soil water deficit is not unusual in the Te Puke region (Cotching 1998) and the 2009 – 2010 summer season was dry (Figure 4.1). Visible symptoms of water stress are rarely observed in kiwifruit vines in this region, possibly due to the deep rooting ability of the vines (Greaves 1985). Excess moisture appeared to have a larger effect on soil respiration than water deficiency, this is supported by the significance of relationships between soil moisture and soil respiration for the wetter summer and autumn of 2011 (Table 4.2).

Factors other than soil conditions must be influencing root growth; such as, the availability of carbohydrates within the vine. It was hypothesised that peaks in root growth would be evident after peaks in above ground growth, due to the low priority of the root system for carbohydrate supply (Wardlaw 1990). Root growth peaked in January and then declined rapidly until March. Hayward kiwifruit fresh weight increases are highest approximately 40 days after mid-bloom (Minchin et al. 2003), in these vines this corresponds to the 2 January in both years. Increases in fruit dry weight peak approximately 30 days later (Snelgar et al. un-published). Therefore, root growth was high in both years when fruit demand for carbohydrates would have been high (Figure 4.4A). The main period of shoot growth in Hayward vines ends in early January (Davison 1990). Fruit growth is

generally thought to have a higher priority for carbohydrate supply than vegetative growth (Wardlaw 1990); however, in kiwifruit there is conflicting evidence (Minchin et al. 2010). The timing of peak root growth may be linked with increased availability of carbohydrates as shoot growth slows. Peak root growth occurred around 100 – 150 days after vegetative bud-break. At this time the photosynthetic capacity of the kiwifruit canopy is suggested to meet and exceed vine demand (Buwalda 1991a). Around this time starch accumulation in the leaves plateaus, accumulation in the fruit is rapid and rapid accumulation in the shoots commences (Smith et al. 1992). Accumulation of carbohydrate reserves in shoots does not normally occur until carbohydrate supply is in excess of that required by growing organs (McQueen et al. 2004). Also around this time starch reserves accumulate in the roots (Smith et al. 1992), indicating that more carbohydrates are being supplied than are required for growth and maintenance. Competition between fruit and root growth may be enabled at this time due to carbohydrate production exceeding the demands of the growing organs. The second smaller peak in root growth in autumn occurs during the later stages of fruit development. At this time the starch content of most tissues is close to its annual maximum (Smith et al. 1992) and carbohydrate accumulation by fruit has reduced, as starch conversion to sugars commences (Boldingh et al. 2000).

Seasonal trends in soil respiration are generally related to plant carbohydrate supply to the rhizosphere (Ryan & Law 2005). The weak correlation between root growth and soil respiration does not support this theory. This suggests that growth alone is not a good indication of carbohydrate supply to the roots. Metabolic function and its associated respiration may increase without increases in root growth. In spring 2010 a transient increase in soil respiration was observed before any evidence of increasing root growth. This is attributed to increased root metabolic activity prior to the onset of spring shoot growth. Kiwifruit accumulate considerable root pressure in spring, with pressures of 0.1 MPa recorded for *A. deliciosa* vines (Clearwater et al. 2007). Spring root pressure indicates the onset of root activity in water and mineral ion uptake (Kramer & Boyer 1995; Clearwater et al. 2007). Future estimates of the autotrophic contribution to soil respiration should include destructive measurements of root metabolism.

Trunk girdling had a clear impact on the root system of these kiwifruit vines. Root numbers, cumulative root growth and total daily root growth rates were consistently reduced from the onset of the observations. This indicated an effect derived from the application of girdles in previous years. It was hypothesised that girdling would have a temporary effect on the growth rates of individual roots for the period the girdle was open. There was no evidence of reduced root growth rates, turnover or survivorship. Reduced root biomass around girdled vines was attributed to the reduction in root numbers.

Soil respiration was consistently and significantly lower around trunk-girdled vines throughout the 18 month experiment. This reduction is attributed to lower autotrophic respiration due to lower root numbers. There was evidence of further reductions in soil respiration around girdled vines during the two months post-girdling. During the girdling period soil respiration was reduced by a further 6 – 12%, suggesting the reliance of root respiration on the continued supply of carbohydrates. Reductions in respiration were generally larger one month post-girdling, suggesting that between 4 to 8 weeks post-girdling phloem transport had not resumed fully and carbohydrate reserves were depleting.

These vines had been girdled annually for the last 5 years, sometimes at multiple times annually, but they showed no visible signs of stress in regard to shoot or fruit growth. Combined with the reduced size of the root system in girdled vines it is suggested that control vines have excessive root biomass than is required under favourable growing conditions. Girdled vines maybe more efficient in the use of carbohydrates for root growth. However, lower root numbers around girdled vines, especially roots in the deeper soil profile, might be a disadvantage to vines under drought conditions.

The contribution of autotrophic respiration to total soil respiration was estimated using the mass regression technique to range between 2.3% in winter and 19.3% in autumn. These estimates are low in comparison with estimates in other vegetation types; for example: 18 – 60% for forestry (Behera et al. 1990; Epron et al. 1999b; Lee et al. 2003; Andersen et al. 2005; Wang et al. 2008), 9 – 51% in grassland (Wang et al. 2005; Zhang et al. 2009) and 35% in an apple orchard (Ceccon et al. 2011). The estimates calculated from root biomass differences

between control and girdled vines at the end of the experiment agreed more closely with these previous estimates.

The two peaks in root growth seen in 2010 disagree with observations made by Buwalda and Hutton (1988), who observed a single period of high root growth. Differences between this study and that of Buwalda and Hutton (1988) may be a result of the different experimental conditions or measurement techniques. The observations by Buwalda and Hutton (1988) were based on soil coring measurements of white root biomass, with a single core collected per vine at fortnightly intervals. Soil coring usually requires multiple cores to be taken to obtain a representative sample. This is particularly important with the uneven distribution of kiwifruit roots (Greaves 1985). The rhizotron sleeves used here allowed repeated observations of the same root populations, therefore seasonal variation in root growth would not be confused with patchy root distribution.

Longevity of roots observed in the present experiment was high in comparison to previous observations in kiwifruit. The median age of control vine roots when they died was estimated at 364 days; Reid et al (1993) estimated that 69% of kiwifruit roots died at an age of 56 days or less, with over half of the roots surviving less than 1 month. Our longevity estimates are closer to those observed in sugar maple (Eissenstat et al. 2000). Longevity estimates are likely to be affected by rootstock and scion species, soil conditions and differences in observation technique. Further, the criteria used to classify dead roots can result in overestimates of longevity (Comas et al. 2000).

In conclusion, root growth and soil respiration demonstrated seasonal trends. Seasonality of root growth is suggested to be related to the availability of carbohydrates within the plant. Rapid root growth commences in summer and is likely to be in direct competition with fruit growth for carbohydrate supply. At this time the photosynthetic capacity of the canopy must be sufficient to meet the demands of fruit growth with excess available for root growth. Further root monitoring is required to confirm the trend of a peak in root growth in both summer and autumn. Seasonality of soil respiration is likely to be affected by soil conditions and carbohydrate supply to the rhizosphere. There was evidence of a relationship between root biomass and soil respiration, which was supported by

the observation of lower respiration around girdled vines. Girdling highlighted the reliance of root metabolism on continued carbohydrate supply from the canopy. Annual trunk girdling reduced the size of the root system. Application of a single annual girdle is likely to allow sufficient time for the root system to obtain an adequate carbohydrate supply.

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# 5 *In vivo* monitoring of $^{14}C$ tracer distribution between fruit and roots of kiwifruit

## 5.1 Introduction

Carbohydrate partitioning, the distribution of photosynthetically sourced carbohydrates between competing organs within a plant, is a complex interaction of sources, transport and sinks (Minchin & Thorpe 2003). Plants consist of numerous sources and sinks. Photosynthesising leaves are the primary sources of carbohydrates. During periods when carbohydrate supply exceeds demand, reserves are accumulated in plant tissues, providing a source for periods of reduced supply. Organs that utilise or store larger quantities of carbohydrates than they can supply are referred to as sinks (Wareing & Patrick 1975). Partitioning is influenced by carbohydrate availability, the properties of the phloem pathway and the properties of the sink, including sink strength and priority. The terms sink strength and sink priority are often confused. Sink strength is related to the size and activity of an organ, and refers to the potential quantity of carbohydrates that can be utilised in growth and storage, if supply is not limited (Wareing & Patrick 1975). Priority is used to describe the order in which the carbohydrate demands of plant organs are met, when supply is the limiting factor. A widely accepted priority hierarchy has been suggested; that is, fruit growth has a higher priority than vegetative growth, which has a higher priority than root growth (Wardlaw 1990); however, there have been some conflicting findings (Minchin et al. 2010). Priority and competition between organs is likely to vary throughout the year as phenological development occurs (Wardlaw 1990).

For many years, horticultural practices such as root pruning have been used to manipulate carbohydrate partitioning in favour of the economically important components (Geisler & Ferree 1984a). Root pruning has been widely used for reducing vegetative vigour in a number of species (Ferree 1992; Poni et al. 1992; Ferree et al. 1999). Effects on fruit growth have been variable, with reductions in yield and fruit size in grape and apple (Ferree 1992; Ferree et al. 1999); little

effect in pear (Maas 2007); and both increases and decreases in fruit size of kiwifruit (Patterson et al. 2009). In kiwifruit vines, there has been no evidence of reduced vegetative vigour following root pruning, suggesting the mechanisms involved in their physiological response to the treatment differ from those of other species.

The effect of root pruning on carbohydrate partitioning is not fully understood. Reductions in vegetative growth in some species are thought to be the result of disruption of a root:shoot equilibrium reached by the plant before perturbation (Brouwer 1983; Geisler & Ferree 1984a). When part of the root system is severed, root system size is reduced, disrupting the root:shoot equilibrium. Shoot growth is reduced because additional carbohydrates are diverted to the root system, to supply increased root growth until the original root:shoot equilibrium is regained (Brouwer 1983; Geisler & Ferree 1984a). This theory has been developed in plants in the vegetative phase. How root pruning affects carbohydrate partitioning to reproductive organs is not known, and is questioned by the variable response of fruit growth. Root pruning during a plant's reproductive phase may result in carbohydrates being diverted towards the reproductive organs (Geisler & Ferree 1984a). A primary interest of this study was to determine the partitioning response in a kiwifruit plant in the reproductive phase.

Most studies of carbohydrate partitioning in horticultural species have studied growth and dry matter accumulation in plant organs (Geisler & Ferree 1984b; Poni et al. 1992). Plant growth is a direct consequence of the photosynthetic capacity of leaves and phloem transport of carbohydrates. Because of the difficulty in observing phloem transport, little work has been done to understand the mechanisms controlling carbohydrate partitioning between competing sinks in horticultural species. Phloem transport of recently fixed carbohydrates has been studied using radioactive tracers (Minchin & Thorpe 2003). These studies involve the tracer ($^{11}C$, $^{14}C$) being applied to a source leaf by exposure to labelled $CO_2$, which is fixed by photosynthesis. The distribution of the tracer through the plant via the phloem can then be monitored.

*In vivo* monitoring of tracer distribution is possible with $^{11}C$ (Williams et al. 1991); however, $^{11}C$ has a short half-life of 20.4 minutes (Minchin & Thorpe

2003) and experiments need to be conducted in close proximity to a particle accelerator for its production (Minchin & Thorpe 2003). The half-life of $^{14}C$ is very long, 5700 years (Sowinski et al. 1990), enabling longer duration experiments. The tracer $^{14}C$ is widely used; however, this technique often requires destructive sampling of plant material using liquid scintillation, solubilising the tracer to get it into a scintillation liquid (e.g. Dickson & Larson 1975; Giaquinta 1978; Tustin et al. 1992). *In vivo* monitoring of $^{14}C$ is possible and involves detecting the $\beta^-$ particles emitted during decay using a Geiger-Müller tube (Geiger & Fondy 1979), which is sensitive to charged particles. These $\beta^-$ particles can only penetrate short distances, for example 0.3 mm in water (Thorpe 1986), hence *in vivo* monitoring of $^{14}C$ has been limited to thin plant tissues such as leaves and root tips (Geiger & Swanson 1965; Fondy & Geiger 1980; Leonardos & Grodzinski 2002). Absorption of $\beta^-$ particles within plant tissues results in emission of low energy X-rays, known as Bremsstrahlung radiation. These X-rays penetrate much deeper thicknesses of plant tissue than the primary $\beta^-$ particles, enabling *in vivo* monitoring through thicker plant tissues using X-ray detectors (Sowinski et al. 1990).

The Bremsstrahlung technique has been widely used in medicine (e.g. D'Asseler 2009); but not in plant physiology. The only use of this approach in plant research has been in maize seedlings (Sowinski et al. 1990; Sowinski et al. 1998). *In vivo* measurement of tracer distribution via the Bremsstrahlung technique offers the potential for making pre- and post-treatment measurements in an individual plant. The first objective of this study was to determine the suitability of this technique for use in larger, woodier species, with larger tissue depths, through which X-rays must penetrate to be detected. Regardless of the type of detection method used, most of the previous *in vivo* studies of C ($^{11}C$, $^{14}C$) tracer partitioning have been carried out in seedlings (Minchin et al. 1994a; Dilkes et al. 2004); few studies have worked with woody species, or species in the reproductive phase.

The response of fruit growth to root pruning has been inconsistent across species. The second objective of these experiments was to determine whether root pruning increased carbohydrate supply to the root system of rooted kiwifruit cuttings. Using *in vivo* observations of tracer distribution, short-term responses to root pruning can be observed; this will indicate whether kiwifruit roots have the

capacity for a rapid response to an increase in carbohydrate availability. The following experiments investigate the hypothesis that when part of the root system is cut off, whole-root system demand for carbohydrates is reduced corresponding with the reduction in root area, and partitioning of tracer to the fruit is increased.

## 5.2 Materials and methods

### 5.2.1 Plant material

Plant material was collected from a Te Puke orchard (Plant & Food Research, New Zealand). Plants were 4 month-old *Actinidia arguta* (Siebold & Zucc.) Planch. ex Miq. hardwood cuttings, collected and propagated during winter 2010. Cuttings were approximately 300 mm in length. Flowers produced in November were hand pollinated with *Actinidia arguta* pollen collected in 2009. After fruit set, plants were continuously pruned to maintain a shoot with a single fruit towards the top of the cutting and a shoot with four leaves, approximately half way between the root system and the fruit shoot (Figure 5.1). In late November 2010, plants were transferred from potting-mix to a hydroponic growing system; a frame held plants with their roots submerged in a bath of aerated nutrient solution. The nutrient solution was specifically designed to meet the high nitrogen, potassium and calcium requirements of kiwifruit (Smith et al. 1988; Table 5.1).

### 5.2.2 Experimental protocol

On the day of experiment, a single plant was transferred to the laboratory. The plant was positioned with its roots in a custom-made chamber (Figure 5.1), constructed of acrylic, using thin (3 mm) sheet acrylic for the front wall to minimise radiation attenuation. For control plants, the root chamber was a single compartment (internal dimensions: 40 x 70 x 200 mm); a split-root chamber was used for root-pruned plants (internal dimensions: 40 x 140 x 200 mm), with roots split evenly between the two compartments. The root chamber contained the standard hydroponic solution, which was continuously aerated and the level maintained using an external reservoir. A plastic mesh sling held roots together in front of the detector to minimise disturbance caused by aeration. The root chamber was wrapped in black plastic to minimise light.

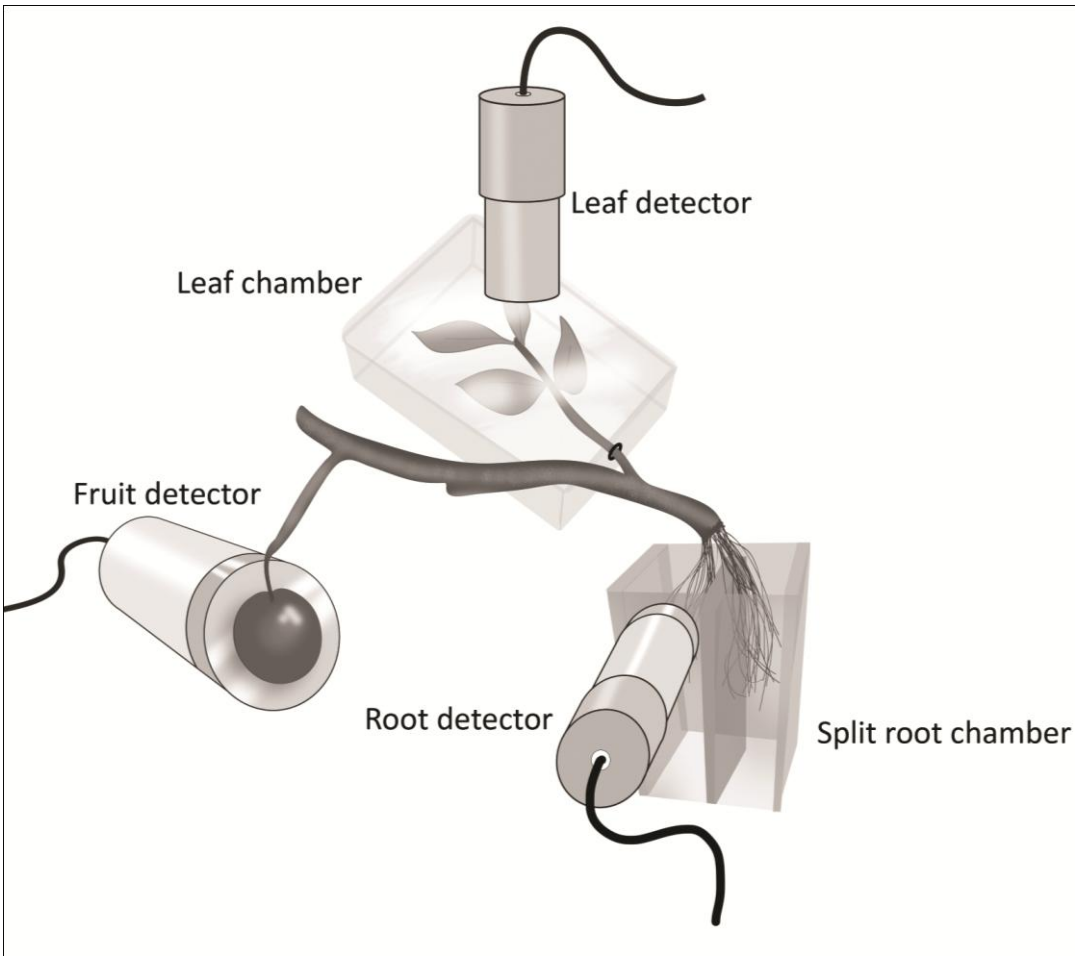


**Figure 5.1 Illustration of the experimental set-up (not to scale). Roots of root-pruned plants were split evenly between a split root chamber. One detector was positioned directly in front of the fruit, and another in front of the root chamber to monitor tracer import. For control plants, the root detector was positioned in front of the whole-root system and for root-pruned plants, in front of the half to remain intact. A third detector was positioned directly above a terminal leaf section, above the leaf chamber where tracer labelling took place to monitor tracer export.**

**Table 5.1 Final concentration of mineral nutrients in the hydroponic solution used for propagation of kiwifruit cuttings.**

| Nutrient | ppm (diluted) |
|---|---|
| Nitrate | 36 |
| Phosphorus | 14 |
| Potassium | 60 |
| Sulphur | 18 |
| Calcium | 40 |
| Magnesium | 14 |
| Iron | 0.6 |
| Manganese | 0.32 |
| Zinc | 0.2 |
| Copper | 0.2 |
| Boron | 0.08 |
| Molybdenum | 0.04 |

The source shoot was placed in a custom-made leaf chamber, which contained a mesh platform for securing the leaves to and an internal fan for air circulation. An air pump circulated air from the laboratory through the leaf chamber at all times, except during $^{14}C$ labelling. Light levels directly above the leaf chamber were maintained at around 150 µmol $m^{-2}$ $s^{-1}$ PAR (LI-185 Quantum Meter, LI-COR Inc., Nebraska), using supplementary light. Lights were turned off between 2200 h and 0600 h each day. Plants were left to acclimatise for 1 – 2 hours before labelling. Root pruning was carried out 20 – 24 hours after the first pulse label; roots were severed close to the base of the stem using a razor blade. Severed roots were left in place.

Detectors were positioned so that the plant part of interest was close to and directly in front of the detector window. Two detectors were positioned to monitor tracer import into sinks, one monitoring the fruit and the second monitoring the roots. The single fruit was located central to the detector window and secured in place using a strip of plastic mesh to prevent movement. The root detector was positioned centrally to the root section of interest, with the detector window close to the front wall of the root chamber. For root-pruned plants, the detector was positioned in front of the root half that was to remain intact. A third detector was used to monitor tracer export from a source leaf. The detector was positioned face

down against the leaf chamber lid, located directly above a leaf tip. Lead shielding (5 mm thick) was used to ensure that detectors picked up only radiation emitted from the tissues of interest.

Labelling the leaves with tracer involved turning the leaf chamber air pump off. $^{14}CO_2$ was released by addition of 0.12 ml (12 MBq) of $^{14}C$ bicarbonate (Perkin Elmer, Massachusetts) to a dish inside the leaf chamber containing 1 ml of saturated citric acid, through a hole in the lid, which was sealed immediately afterwards. Thirty minutes after introduction of the $^{14}CO_2$, the leaf chamber was vented by turning the chamber air pump on, flushing any $^{14}C$ not taken up by the plant through soda lime.

Ten experiments were performed each using a new plant; four received a single pulse label and six received two pulse labels; half the experimental plants were root-pruned. At the end of each experiment, plant dimensions and fruit and root fresh weights were recorded (Table 5.2).

**Table 5.2 Distance (mm) between the basal source leaf and the top of the root system or the top of the fruit pedicel, fresh weight (g) of roots and fruit at the end of each experiment, fruit diameter (mm) and percentage of root fresh weight removed during root pruning; for the six plants receiving two pulse labels.**

| Plant | Distance (mm) | | Fresh weight (g) | | Fruit diameter | Roots pruned |
|---|---|---|---|---|---|---|
| | Root | Fruit | Root | Fruit | (mm) | (%) |
| 1 | 235 | 173 | 32.3 | 5.6 | 21.9 | 0 |
| 2 | 150 | 270 | 46.8 | 5.0 | 20.0 | 0 |
| 3 | 250 | 232 | 25.0 | 4.8 | 21.4 | 0 |
| 4 | 288 | 250 | 39.3 | 6.8 | 21.9 | 48 |
| 5 | 145 | 167 | 31.4 | 8.5 | 23.7 | 59 |
| 6 | 235 | 248 | 40.3 | 8.7 | 23.0 | 57 |

### 5.2.3 Radiation monitoring

The Bremsstrahlung radiation produced as $^{14}C$ decays within plant tissue (Sowinski *et al.*, 1990) was monitored using NaI scintillation detectors (Saint Gobain, Cedex) with a thin beryllium window (50 µm). Detectors were modified to run on a mains power supply. Pulses from the scintillation detector were counted after pulse height analysis (PHA; Model 2360 Alpha/Beta Data Logger, Ludlum Measurements Inc., Texas). Total counts over 5 minutes were collected; pulse height analysers for each detector were connected to separate computers and data were manually downloaded. A radioactive iron source ($^{55}Fe$) was used to calibrate the PHA to allow pulses between 5 and 50 kev to be counted; the lower range was set to exclude electronic noise and the upper range to exclude background cosmic radiation.

Detector efficiency was tested before experiments using tomato (*Lycopersicon esculentum*) fruit from a plant pulse labelled with $^{14}CO_2$, using the method described above (data not shown). Counts per minute (CPM) from each of the three detectors were compared with disintegrations per minute (DPM) obtained by oxidation (Sample Oxidizer, Model 307, Perkin Elmer, Massachusetts) and liquid scintillation spectrophotometry (Tri-Carb® Liquid Scintillation Analyzer, Model 2910TR, Perkin Elmer, Massachusetts) of tomato fruit tissues. A mean efficiency of 0.017% was obtained for fruit with a mean fresh weight of 4.0 g and a mean diameter of approximately 14 mm.

### 5.2.4 Tracer losses from roots

An exhaust fan was fitted above the root chamber to collect respired $^{14}CO_2$. An exhaust hood sat over the chamber, and an air pump sucked air out of the hood and through soda lime. Sub-samples of the soda lime (0.77 – 0.87 g) were taken and the amount of radioactivity measured following oxidisation and liquid scintillation spectrophotometry. At the end of each experiment, after the plants had been removed from the root chamber, tracer activity in the bathing solution was checked with the root detector.

### 5.2.5 Tracer spatial distribution

At the end of one single labelling experiment that continued for 91 hours, the spatial distribution of tracer within the plant was determined. The stem and shoots

were cut into similar-length sections and the leaves, fruit and roots detached. X-ray counts in each plant part were then counted with a single detector, by placing the tissue segment centrally on the detector window.

### 5.2.6 Data analysis

Total counts per 5 minute interval are proportional to the amount of tracer in the field of view of the detector. The observed counts are affected by the sensitivity of the detector and the geometry of the material. Because of differences in sensitivity, count values between detectors cannot be directly compared within an experiment. Further, because plant geometry varies between experiments, a detector is not quantitatively comparable between experiments. Comparisons can be made between the first and second labelling within an individual plant, as plant:detector geometry was not disturbed and the same quantity of bicarbonate was used for each labelling. Relative count rates between detectors are quantitatively comparable within a plant. Between plants, qualitative comparisons of the shape of tracer import/export curves can be made.

Normalised accumulation was calculated to determine the proportion of tracer partitioned to the fruit and roots. Total fixed tracer was taken as the maximum counts observed in the leaf immediately after venting the leaf chamber. Normalised accumulation in the roots and fruit was calculated at different time points, by dividing the counts in each of the sinks by the total fixed tracer. This allowed partitioning to be compared between the first and second labelling within a plant. Because of differences in detector sensitivity, normalised counts can be higher than one. The gradients of import/export curves were calculated (SigmaPlot, version 8.0).

The speed of phloem sap flow (mm $s^{-1}$) from source leaves to the fruit and root sinks was calculated as:

$$S = \frac{\Delta D}{\Delta T} \qquad (5.1)$$

where $\Delta D$ is the distance between the base of the lowest source leaf and the top of the root system or the top of the fruit pedicel, and $\Delta T$ is the time between tracer export commencing and sink import first observed. The time of tracer export was determined as a visible decline in leaf counts; this time was selected as an

alternative to the time when labelling ended to remove the time delay associated with phloem loading. Sink import was determined as the time when tracer counts rose to double the level associated with background noise.

## 5.3 Results

### 5.3.1 Tracer distribution

Figure 5.2A shows an example of the raw data collected from one of the double labelling experiments. Background data collection commenced shortly after plant set-up; background values varied between detectors because of differences in sensitivity. After 60 – 120 minutes, tracer was released in the leaf chamber, resulting in a rapid increase in the leaf activity curve. After 30 minutes of labelling, the chamber was flushed with fresh air. Absence of a large drop in leaf activity when the chamber was vented indicated that the majority of available $^{14}CO_2$ had been incorporated into the leaf tissue. Tracer export was evident within 30 minutes of ending labelling. The following 48-hour section in Figure 5.2A shows the export of $^{14}C$ from the leaf, before a second label was applied to the same leaves. Export of tracer from the leaf followed a double exponential decay. A rapid phase of export was evident for approximately the first 21 hours, followed by a longer phase of slower export lasting 24 – 48 hours before reaching a plateau, as the available tracer became depleted. Tracer activity in the leaves approximately 48 hours after labelling was on average 20 – 40% of that originally fixed and remained above background values. In experiments that were continued for approximately 5 days, the proportion of total tracer fixed that was maintained in leaf tissues was in the order of 20 – 30% of that originally accumulated.

Import of tracer is seen in the fruit and root tracer accumulation curves (Figure 5.2A). Delays were evident between the time of labelling and the time of arrival in either the fruit or the root. This time delay is taken as the time for labelled photosynthates to be loaded into the phloem sieve tubes and to travel the length of the phloem pathway. The first parts of the fruit and root curves in Figure 5.2A show the import of tracer following the first labelling; import follows a sigmoid pattern, with a rapid rate of import during the first 24 hours of tracer arriving in the sink. This is followed by a slower rate of import, before curves reach a plateau after approximately 48 hours. The plateau in import is due to depletion of

available tracer, rather than a physiological change in import, indicated by the coinciding decline in leaf export. Forty-eight hours after the first labelling, import curves had not completely reached a plateau in all plants; however, the delay and rapid phase of accumulation following the second labelling were easily identified (e.g. Figure 5.2A).

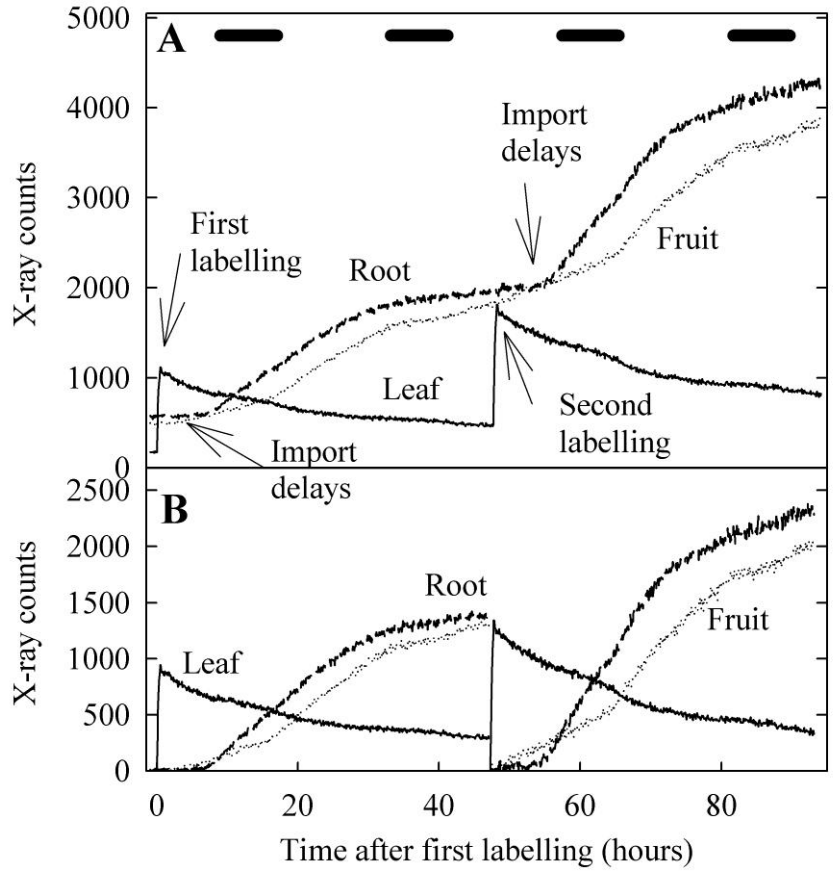


**Figure 5.2 Example data; A) raw data obtained from the pulse-height analyser, for a control plant, showing the leaf export curve and the fruit and root import curves. Two pulse labels of tracer were applied, approximately 48 hours apart. B) Background corrected import and export curves for data shown in 5.2A. Black bars represent the dark periods.**

Figure 5.2B is the background-corrected data derived from Figure 5.2A. Background counts, recorded before the first labelling, were subtracted from the subsequent import/export curves. For the second labelling a new background

value was calculated based on the curve plateau values within the 30 minutes before that labelling. All further analyses were made on data that had been background corrected.

### 5.3.2 Response of tracer partitioning to root pruning

During initial experiments, using plants that received a single tracer label (e.g. Figure 5.3), there was no evidence of an effect on tracer distribution to the fruit or roots following root pruning within the first 12 hours of treatment. Within 6 – 13 hours after root pruning, leaf export and fruit and root import curves reached a plateau as available tracer became depleted; therefore, a second pulse of tracer was applied to allow observations to continue.

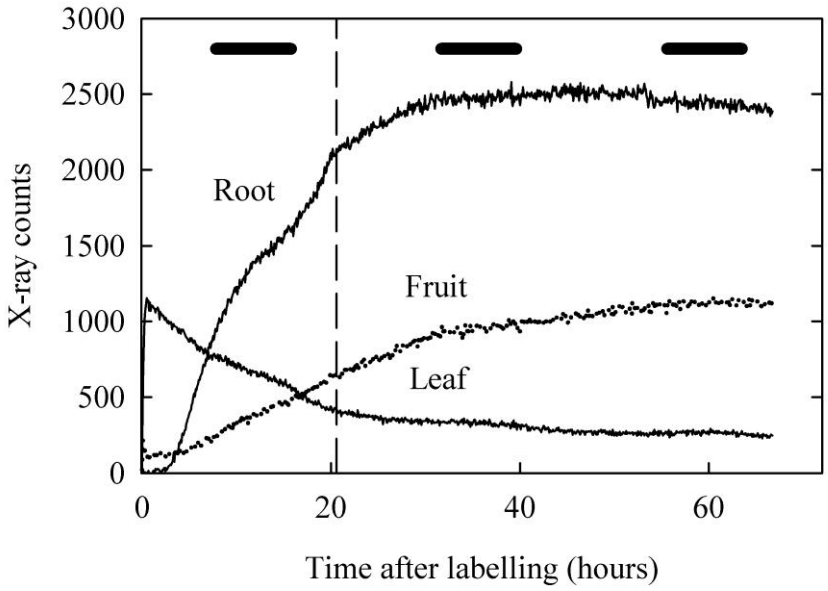


**Figure 5.3 An example of background-corrected leaf export and fruit and root import curves for a plant receiving a single pulse label. Root pruning (dashed line) was carried out approximately 20 hours after labelling. Black bars represent the dark periods.**

Tracer distribution was affected approximately 36 hours after pruning, with higher normalised accumulation in the fruit following root pruning. In the three replicate plants, normalised accumulation in the fruit 24 hours after labelling was 0.5, 3.1

and 0.9 pre-treatment and 2.8, 3.3 and 1.7 post-treatment respectively (Figure 5.4). This trend was not evident in control plants, although higher accumulation was observed approximately 36 hours after the second labelling. The maximum rate of import into the fruit was also higher following root pruning, by as much as five times (data not shown). Such increases were not observed in control plants.

There was no evidence that root pruning affected tracer distribution to the remaining root half (Figure 5.4). Normalised accumulation in the root system was generally higher following the second labelling in all plants.

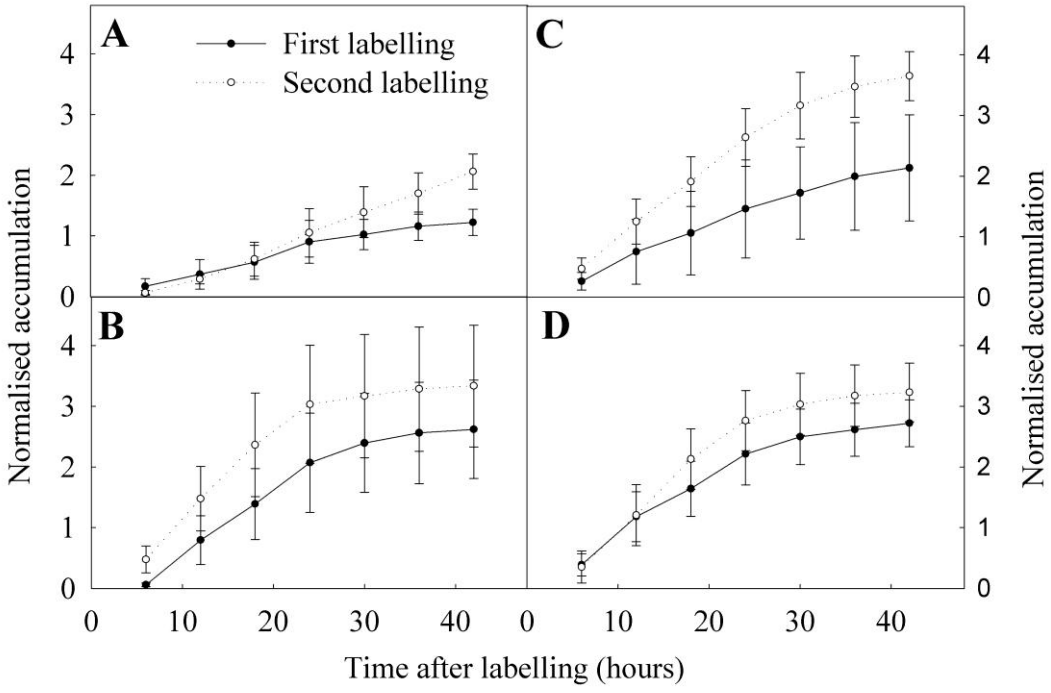


**Figure 5.4 Tracer accumulation in fruit and roots, normalised to total fixed tracer in the leaves, following the first and second labelling. A) Control plant accumulation in the fruit, B) control plant accumulation in the roots, C) root-pruned plant accumulation in the fruit, and D) root-pruned plant accumulations in the roots. Data are means, *n* = 3, ± 1SEM.**

Time delays for tracer to reach the fruit or roots varied between plants. Transit delays were not correlated with natural variation in sink size ($r_s$ = < 0.4, $P$ > 0.05),

or plant geometry ($r_s$ = < 0.7, $P$ > 0.05; data not shown). Transit delays ranged between 3 and 11 hours after the first labelling. In control plants, time delays were generally shorter after the second labelling (Table 5.3). In the fruit, this was also true for two of the three root-pruned plants, with delays reduced by as much as 2 hours. In the root, root-pruned plants showed a variable response. Transport speeds were calculated to remove differences between plants in the length of the phloem pathway between source leaves and sinks. Speed of phloem sap flow towards the fruit was generally faster following the second labelling (Table 5.3). Mean speed of tracer transport towards the fruit increased from 0.02 to 0.04 mm $s^{-1}$ between the first and second labelling. The mean speed of flow towards the root was also faster following the second labelling in control plants, increasing from 0.01 to 0.02 mm $s^{-1}$; however, in root-pruned plants mean speed was reduced.

**Table 5.3 Differences between the first and second labelling; change (%) in time delay for tracer to reach the roots and fruit and change (%) in speed of phloem sap flow (*S*) from the source leaves to the root or the fruit.**

| Treatment | Plant | Time delay root (%) | Time delay fruit (%) | *S* – root (%) | *S* – fruit (%) |
|---|---|---|---|---|---|
| Control | 1 | - 61.8 | - 115.0 | + 162.0 | + 115.0 |
| | 2 | - 37.5 | - 259.4 | + 60.0 | + 259.4 |
| | 3 | + 13.9 | - 5.7 | - 12.2 | + 5.7 |
| Root-pruned | 4 | + 121.9 | - 120.2 | - 54.9 | + 120.2 |
| | 5 | - 32.5 | - 320.9 | + 48.2 | + 320.9 |
| | 6 | + 62.8 | + 59.1 | - 38.6 | - 59.1 |

Tracer export from the leaves was not affected by root pruning. Diurnal fluctuations in the export and accumulation of tracer were evident (e.g. Figure 5.5). The rate of export from leaves was reduced during dark periods, reaching a minimum within 2 hours of the lights being turned off. The rate of export generally started to increase again during the dark period, reaching a new maximum shortly after lights were turned on. Import into sinks also showed diurnal fluctuations. Import started to reduce after lights were turned off;

minimum fruit and root import occurred approximately 0.5 – 4.5 and 1 – 7 hours, after leaf export reached a minimum, respectively. The rate of import into fruits and roots started to increase again during the dark period, continuing to increase and reaching a morning high, generally within 4 hours of the lights being turned on.

### 5.3.3 Tracer losses from the root system

In plants receiving a single labelling, import/export curves were monitored for up to 91 hours. After approximately 48 hours, tracer accumulation in the root began to decline (e.g. Figure 5.3), showing that tracer was leaving the detector's field of view. Only a small fraction of the total fixed $^{14}C$ (0.3 – 1.2%) was lost through root respiration. Tracer activity of the bathing solution at the end of experiments was close to background, excluding significant losses of tracer through root exudation.

### 5.3.4 Tracer spatial distribution

X-ray counts at the end of a single experiment showed that tracer was retained in tissues throughout the plant. Large quantities of tracer were retained in the leaves (Figure 5.6). In addition, tracer was identified along the leaf and fruit shoots and throughout the main stem of the plant.

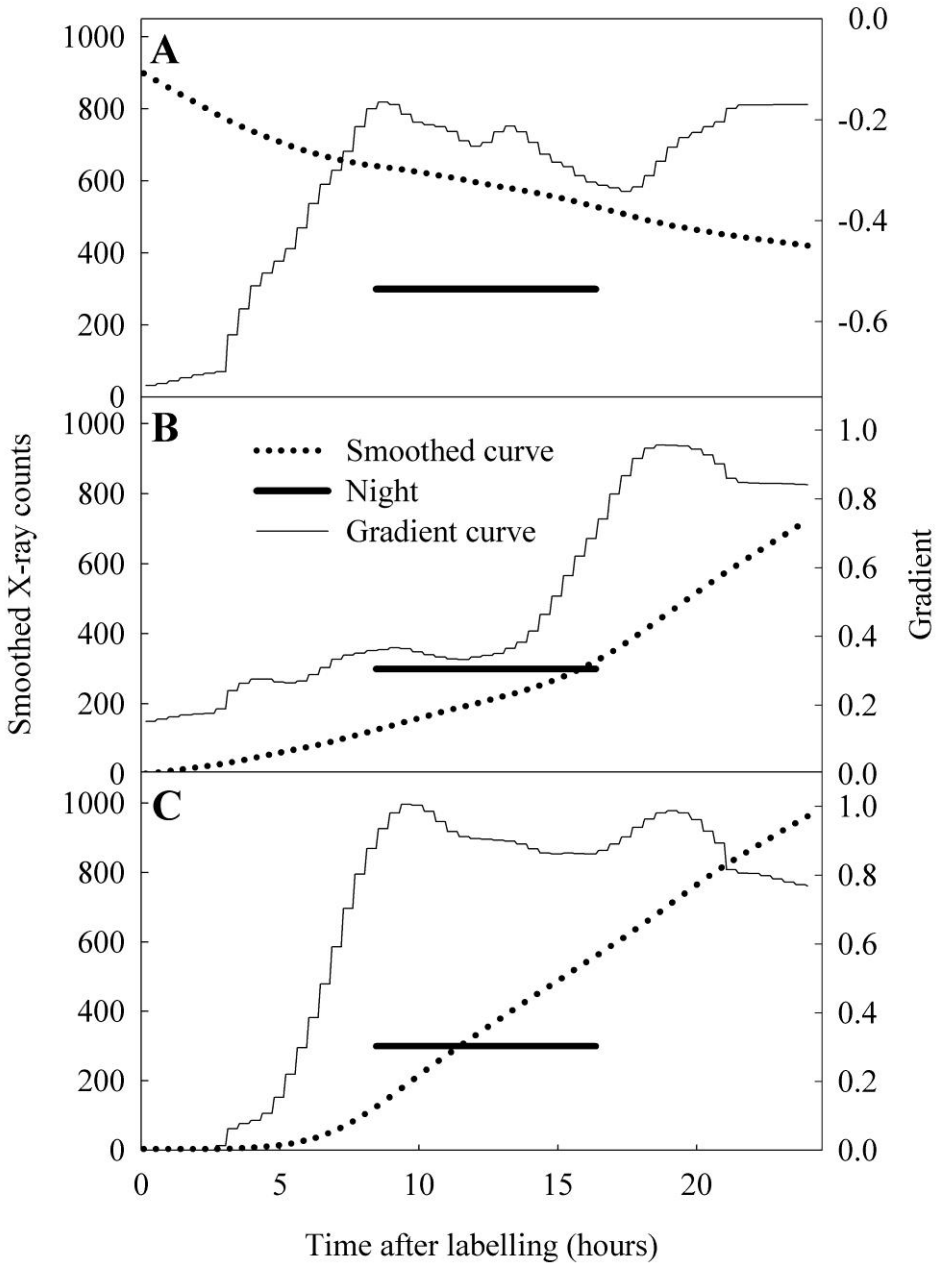


**Figure 5.5 An example of tracer export and import curves smoothed using a Lowess function and the gradient calculated as (($y_i$-$y_{i-1}$)/($x_i$-$x_{i-1}$)), where *i* = 2 – 287 (SigmaPlot). Data shown are for the first 24 hours after the first pulse label for the data shown in Figure 5.2. A) Leaf export, B) fruit import and C) root import. Black bars represent the dark-periods, highlighting the diurnal trends in tracer export/import.**

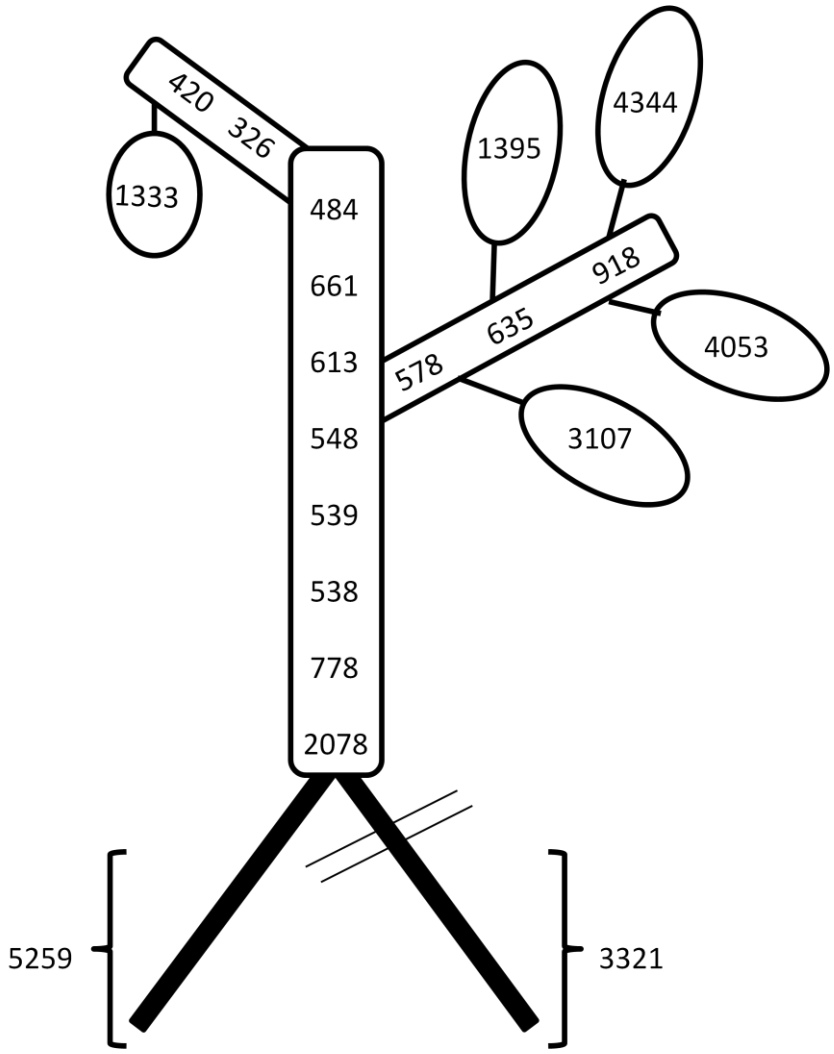


**Figure 5.6 Spatial distribution of tracer in a single plant at the end of a single-label root-pruned experiment. Measurements were taken approximately 91 hours after labelling. Data are background-corrected X-ray counts (CPM). All four leaves were enclosed in the leaf chamber. The root half that was pruned is depicted as being cut. Root pruning was carried out approximately 24 hours after labelling.**

## 5.4 Discussion

### 5.4.1 Suitability of the technique

These experiments are the first demonstration of the Bremsstrahlung technique for *in vivo* monitoring of $^{14}C$ tracer distribution in woody plants. The detection efficiency observed here (0.017%) was lower than that reported for Geiger-Müller tubes (0.5 – 1.0%; Leonardos & Grodzinski 2002) and for previous use of the Bremsstrahlung technique in maize (0.05 – 0.1%; Sowinski et al. 1990). The reduced detection efficiency is probably a result of the higher radiation attenuation through the larger tissue depths monitored in these experiments. It has been shown that although detection efficiency is low, this technique is useful for monitoring import of tracer into fleshy organs, with tissue depths up to 24 mm.

Leaf export curves can be broken down into multiple phases. The first phase, where tracer export was rapid, lasted approximately 21 hours. This was followed by a longer phase of slower export, before reaching a plateau. The occurrence of two or more phases of tracer export from leaves has been described previously and is suggested to be related to the proportions of tracer accumulated in transport and storage pools within the leaf (Hofstra & Nelson 1969a; Moorby & Jarman 1975). The length of these export phases vary between species (Hofstra & Nelson 1969b). In *Zea mays*, for example, 50% of $^{14}C$ tracer accumulated in the leaf was exported within 30 minutes, with as much as 90% exported within 24 hours (Hofstra & Nelson 1969a). Faster sieve tube loading in $C_4$ plants (Moorby & Jarman 1975) would contribute to such rapid export rates in *Zea mays.* The quantity of $^{14}C$ retained in leaves after 24 hours has also been shown to vary between species. The majority of tracer retained in leaves has been shown to be in insoluble forms (Hofstra & Nelson 1969b). With the large fractions of tracer retained in the leaves of these kiwifruit plants, it is suggested that a large proportion of the fixed tracer was accumulated in insoluble compounds, such as starch (Hofstra & Nelson 1969b). Tracer export is a result of carbohydrate accumulation exceeding leaf carbohydrate requirements, and is related to the age and photosynthetic capacity of the leaves (Moorby 1977). The leaves of these kiwifruit plants were mature, and would therefore be expected to demonstrate lower export rates and retain a larger proportion of tracer than leaves that have

recently reached their full size (Wardlaw 1968). An additional loss of tracer from the leaf is through respiration; however, this is assumed to be low in comparison to net carbon assimilation (Grimmer & Komor 1999).

Spatial distribution of tracer 91 hours after labelling (Figure 5.6) demonstrated the distribution of tracer within the plant, after import and export curves had reached a plateau. These data confirm the large amounts of tracer retained in the labelled leaves. Further, significant quantities were present within the main stem and the leaf and fruit shoots, consistent with tracer unloading along the phloem pathway (Minchin & Thorpe 1987).

This technique has promising applications for root research. A decline in tracer from the roots was observed after import curves had reached a plateau. Potential tracer losses from roots include carbon lost through root respiration and exudation (Minchin & McNaughton 1984; Dilkes et al. 2004); however, these were not found to be considerable losses in these experiments. Alternatively, $^{14}C$ may have been recycled from the roots after being used in the construction of carbon skeletons (Minchin & McNaughton 1984) in processes such as nutrient cycling and amino acid production (Clauss et al. 1964; Marschner 2005).

Phloem sap flow speeds will have been underestimated because of the low detection efficiency. The speeds calculated here are estimates of the speed for the proportion of tracer transported directly to the sink, excluding tracer involved in unloading and loading along the phloem pathway. Phloem speeds from this experiment should therefore be taken as conservative estimates of direct transport. Phloem sap flow speeds calculated in these kiwifruit cuttings (0.01 – 0.04 mm $s^{-1}$) are low in comparison with observations in maize (0.1 – 0.3 mm $s^{-1}$; Sowinski et al. 1990; Sowinski et al. 1998). Differences may be partly explained by the differences in detector efficiency as well as differences between species.

Observations within these experiments indicate effects of experimental set-up on carbohydrate partitioning, suggesting that a settlement period should be allowed before labelling, particularly if the goal is an estimate of the speed of phloem transport. Reduced time delays, increased flow speeds and higher root import after the second labelling may be attributed to recovery of phloem transport after

handling (Jaeger et al. 1988) and acclimation to the lower light levels of the laboratory (Minchin et al. 1994b).

### 5.4.2 Effects of root pruning on tracer distribution

In these kiwifruit plants, there was no evidence of a response to root pruning in the first 12 hours. This suggests that at the time of root pruning the sinks were receiving sufficient carbohydrates to meet their requirements. It may be hypothesised that by cutting off half the roots, import into the remaining half will double, as root activity increases to compensate. This was not the case; there was no evidence of increased import into the roots or the fruit in the short term. Import into fruit was still evident at the time of root pruning; therefore, tracer was available to the two sinks. A consideration is that at the time of root pruning, the tracer could have passed the point of bifurcation between the roots and fruit shoot.

Changes in carbohydrate partitioning are thought to be a result of changes within the sink, rather than an effect on the transport pathway (Minchin et al. 1994a), and the longer term response to a treatment indicates how organs acclimate to new conditions (Minchin et al. 1997). The longer term response, approximately 36 hours after root pruning, demonstrated an increase in tracer accumulation in the fruit; the fruit was able to acclimate to the increased availability of carbohydrates. This supports the hypothesis that root pruning manipulates carbohydrate partitioning so that a larger proportion is transported to the fruit. In apple, fruit have been shown to increase carbohydrate import when carbohydrate availability was increased (Minchin et al. 1997). In that study increases in import were larger the day after treatment, suggesting that the fruit acclimated to the newly available carbohydrates by increasing enzyme concentrations and metabolic rate (Minchin et al. 1997). There was no evidence of a longer term effect on tracer import into the root in these experiments. Therefore within the timeframe of these experiments, the fruit had a higher priority for carbohydrate supply than the roots. This confirms the widely accepted priority hierarchy (Wardlaw 1990).

The lack of a change in tracer import into intact roots following root pruning agrees with previous root manipulation experiments using $^{11}C$. In barley seedlings, treatments reducing tracer import into half the roots by root pruning or cooling, showed that the control half did not utilise the newly available tracer-

labelled carbohydrates, reducing total root system import (Farrar & Minchin 1991; Minchin et al. 1994a; Henkes et al. 2008). It was suggested that the roots were working at full capacity (Minchin et al. 1994a). Interestingly, the response in a woody species in its reproductive phase is similar to that of monocot seedlings. It is suggested that at the time of root pruning, the root system of these kiwifruit plants was not supply-limited.

The increase of tracer accumulation in the fruit after root pruning supports observations of orchard-based trials, where increases in fruit size and dry matter content have been achieved. Fruit responses have been variable and are likely to be influenced by severity and timing of root pruning. Fruit and root growth are seasonal in kiwifruit (Buwalda & Hutton 1988; Davison 1990; Minchin et al. 2003); therefore, it might be argued that if root pruning is carried out at a time when fruit growth is low and root growth is rapid, an increase in partitioning to the remaining intact roots may be observed. However, fruit growth demonstrated the priority for carbohydrate supply in these experiments, which were conducted at a time when fruit growth in *A. arguta* is low.

In conclusion the Bremsstrahlung technique was successfully used to observe the distribution of recently fixed carbohydrates to terminal sinks in these small woody plants. Root pruning *A. arguta* rooted cuttings increased partitioning to the fruit, though this was not detected until approximately 36 hours after treatment. In these plants, the fruit had a higher priority for carbohydrate supply than the roots. Partitioning to the remaining roots was not affected, suggesting that the intact roots did not increase in function to compensate for the loss of root area within the time-frame of these experiments. The technique of *in vivo* monitoring of $^{14}C$ tracer distribution warrants further investigation in a wider range of species.

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# 6 General discussion

In this chapter the main findings of the experimental chapters are summarised and discussed in context with general observations of root biology and function. The effects of trunk girdling and root pruning on kiwifruit vines are then discussed and possible implications for kiwifruit production proposed.

## 6.1 General root system observations

The results of this research have provided new knowledge on the importance of the root system to the kiwifruit vine. Results from the rhizotron study (chapter 4) add to the debate on seasonal root growth in kiwifruit vines. Our observations agree with those of Buwalda and Hutton (1988) that root growth in kiwifruit is seasonal; however, the timing of growth differed. Root growth showed a bimodal trend during the 2009 – 2010 growing season. The observations by Buwalda and Hutton (1988) demonstrated a single peak in summer, which then gradually declined into autumn. Both of these observations are based on data sets covering less than two years. Multiple years of growth data would be preferable to confirm the typical trend in seasonality. The rhizotron measurements have been continued with the aim of collecting a two year data set. The presence of white roots during winter months was one of the reasons given by Lemon (1986) for suggesting that root growth occurred throughout the year. Our observations demonstrate that roots may persist as white roots for significant periods of time and should not be taken as an indication of recent growth.

Peak root growth occurred in summer and was evident in both years, the second peak in root growth was in autumn, pre-harvest. The timing of the summer peak in root growth agrees with observations in other deciduous fruit crops, whereas the pre-harvest peak does not (Psarras et al. 2000; Eissenstat et al. 2006; Basile et al. 2007). The increase in root growth pre-harvest can be attributed to reduced carbohydrate demand by the fruit. Around this time fruit carbohydrate accumulation slows and starch commences conversion to sugars (Boldingh et al. 2000). There was no evidence of a peak in root growth after fruit harvest. Post-harvest peaks in root growth are thought to occur as a result of sink removal

increasing carbohydrate availability (Basile et al. 2007). The lack of a post-harvest peak in root growth in these vines may be a result of the short duration between fruit harvest and leaf senescence.

The summer peak in root growth was at a time when fruit growth would also have been high. These results indicated direct competition for carbohydrates between fruit and root growth in kiwifruit vines. Competition between fruit and root growth is also indicated by the increase in fruit size and dry matter content following summer root pruning (chapter 3). These observations disagree with the suggestion that root growth has a low priority as a sink for carbohydrates compared to fruit growth (Wardlaw 1990). It also contrasts with previous observations in other crops that have demonstrated the negative effects of crop load on root growth. Increasing fruit load has reduced vegetative growth, in particular root growth (Avery 1970; Petrie et al. 2000), to the extent that heavy crop loads reduced the size of the root system (Avery 1970). These results suggest that if a fruit crop has the potential to utilise carbohydrates, then it will, even at the expense of root growth. Competition for carbohydrate supply between fruit and root growth in kiwifruit was therefore investigated further using the tracer $^{14}C$. Import into fruit and root sinks was observed. When half the root system was cut off carbohydrate partitioning to the fruit was increased. Simultaneous import into fruit and roots demonstrated competition between these two sinks for carbohydrates. However, increased import into the fruit following root pruning, with no change in import into the remaining roots indicated that fruit growth had a higher priority for carbohydrate supply than roots. Competition between fruit and root sinks demonstrated through this research leads to the conclusion that the root system is more competitive in kiwifruit vines compared to some other fruit crops. There must be a balance within the kiwifruit vine whereby when competing organs are present fruit growth reaches an optimal rate and carbohydrate availability above the demands of that rate are made available to other organs. Reducing the competitive effects of sinks may allow remaining organs to acclimate and grow at a rate higher than that experienced prior to the manipulation.

The measurement of root hydraulic conductance in intact, mature, field grown plants has not been widely done. Root system leaf area specific hydraulic

conductance ranged between 0.4 and 1.1 × $10^{-4}$ kg $MPa^{-1}$ $m^{-2}$ $s^{-1}$ in control vines (chapter 3). This can be compared with leaf area specific root hydraulic conductance's of 3.3 × $10^{-4}$ kg $MPa^{-1}$ $m^{-2}$ $s^{-1}$ in 5 to 7 year old field grown *Quercus pubescens* (Nardini & Tyree 1999), 0.15 × $10^{-4}$ kg $MPa^{-1}$ $m^{-2}$ $s^{-1}$ in 3 year old potted olive trees (*Oleo oleaster*; Lo Gullo et al. 1998) and 0.2 to 0.9 × $10^{-4}$ kg $MPa^{-1}$ $m^{-2}$ $s^{-1}$ in potted seedlings of neotropical tree species (Tyree et al. 1998). These observations indicate that root hydraulic conductance in these kiwifruit vines was relatively average in comparison with these tree species. Considerable redundancy was observed in the hydraulic capacity of these kiwifruit vine roots. When 80% of root biomass was removed the sap flow through monitored roots showed a transitory fourfold increase and then stabilised at a rate 50% higher than pre-treatment. The increase in sap flow would have lessened the reduction in water supply to the canopy, though, the increase was insufficient to compensate for the reduction in root area. Whole-vine hydraulic conductance was reduced in proportion to the amount of roots removed. The lack of visible water stress symptoms or reduction in fruit fresh weights suggest that vines were able to obtain sufficient water to maintain growth through the remaining 20% of root biomass. Increases in root sap flow have also been observed following root pruning of conifer trees. It was suggested that a compensatory mechanism was involved, increasing the water uptake capacity of the remaining root section (Nadezhdina & Cermak 2000). In that study the monitored roots were those that were damaged by partial pruning, therefore, the transitory increase in flow may have resulted from the damage itself reducing resistance to water uptake. In our study sap flow was monitored in the remaining intact roots. The increase in sap flow must have been a result of increased radial water uptake across these roots. It is concluded that the high level of redundancy in root hydraulic capacity of kiwifruit may be a tolerance mechanism that enables the vines to withstand stresses such as localised soil water depletion, root herbivory or mechanical damage. This growth strategy may be in keeping with the natural habitat of *Actinidia* sp., which are commonly found in the forest under-storey and along forest margins (Ferguson 1990).

Attempts were made to infer the radial pathways of water transport by investigating changes in root hydraulic conductance following root pruning. Rapid

changes in root hydraulic conductance have been attributed to the involvement of aquaporins, which indicates the involvement of the transcellular pathway for water uptake (Javot & Maurel 2002; Bramley et al. 2007). It was hypothesised that with dynamic root responses to environmental stresses observed in other species (Martínez-Ballesta et al. 2003; Siemens & Zwiazek 2003; Vandeleur et al. 2009) there could be hydraulic mechanisms within the remaining roots of kiwifruit vines that compensate for the hydraulic effects of reducing root biomass. There was no evidence of a change in root hydraulic conductance in response to root pruning. This result did not support our hypothesis or agree with observations in wheat seedlings (Vysotskaya et al. 2004). The results were taken as an inference that the transcellular pathway is a small component of total radial flow in kiwifruit roots. An increase in root hydraulic conductance may only have been evident if apoplastic water transport made a minor contribution to total radial flow in the remaining intact roots. These kiwifruit vines were mature and a considerable proportion of the root system, up to 90% (Hughes & Gandar 1989), would have comprised of woody roots that had undergone secondary development. As kiwifruit roots mature they develop Casparian bands that may extend to the root tip (Considine & Lovelock 1990). The presence of Casparian bands should increase resistance to water movement through the apoplast and reduce apoplastic transport (Steudle & Ranathunge 2007). It therefore seemed reasonable to propose that the cell-to-cell and transcellular pathways could be considerable components of radial water transport and therefore the involvement of aquaporins would be probable. No evidence was found to support this proposition in this research. Radial water uptake in kiwifruit warrants further investigation as our inability to observe changes in root hydraulic conductance may be a result of the method used. Evaporative flux measurements of root hydraulic conductance on large woody plants are imprecise, but the system is more biologically meaningful than laboratory grown potted plants. Attempts were also made in the laboratory to measure root hydraulic conductivity with a root pressure probe, to investigate the involvement of aquaporins using inhibitors, such as silver nitrate (Niemietz & Tyerman 2002). This work was unsuccessful due to time limitations, but further work with the pressure probe would enable more detailed investigation of the water transport properties of kiwifruit roots.

Previous studies into whole-vine biomass distribution in kiwifruit have demonstrated high root-shoot ratios in comparison with other fruit crops. The results of this research also indicated that the kiwifruit vine has a larger root biomass than is required under favourable growing conditions. Evidence for this comes from the observation of hydraulic redundancy enabling increased root sap flow without any evidence of hydraulic failure when the size of the root system was reduced. Further, the reduction in root biomass following annual trunk girdling was not seen to negatively affect fruit or shoot growth. This suggested that the smaller root system was not affecting water or nutrient uptake or carbohydrate reserves required for spring canopy development.

## 6.2 Responses to vine manipulations

Trunk girdling and root pruning are used by kiwifruit growers with a common aim, to increase the size and dry matter content of fruit. It was of interest to compare how these two practices affected the kiwifruit vine root system in regards to structure and function. The two practices differed in their effects on vine physiology; however, they ultimately led to the same result: reduced carbohydrate demand by the roots. The primary physiological response to trunk girdling was photosynthetic (chapter 2). The reduction in photosynthesis was hypothesised to result from feedback inhibition following reduced carbohydrate export from the leaves. This agrees with observations in citrus following trunk girdling (Iglesias et al. 2002). Future research should attempt to confirm this theory, as alternative theories have been proposed, including the involvement of ABA on stomatal aperture (Williams et al. 2000).

It was hypothesised that the period of carbohydrate starvation imposed on the roots would reduce root growth rates, with annual girdling reducing root biomass. Girdling did not reduce growth rates; however, root biomass was significantly lower around girdled vines (chapter 4). The reduction in biomass was attributed to lower root numbers. The vines response to reduced carbohydrate availability was to produce less root tips; this suggests that root extension is less of a carbohydrate consumer than the production of new roots. This disagrees with the suggestion that carbohydrate supply is more important for root elongation than new root production (Lloret & Casero 2002). The reduction in root system size did not

appear to have negative effects on shoot or fruit growth. This is based on visual observations as shoot responses were not a focus of the orchard study. Lower root numbers, in particular less roots in deeper soil layers, may be a disadvantage to vines under periods of drought stress. The reduction in carbohydrate reserves should also be considered as the period of carbohydrate starvation will result in un-seasonal use of root carbohydrate reserves. In peach trees and grapevines trunk girdling has resulted in the depletion of root carbohydrate reserves (Roper & Williams 1989; Jordan & Habib 1996). Root carbohydrates were not measured as part of this research. Previous observations in kiwifruit vines showed that between January and March (girdling period) root starch concentrations were low in both girdled and non-girdled vines. Non-girdled vines showed a peak in root starch concentration in May; this was also observed in girdled vines, however, concentrations were considerably lower than those for control vines (Pers. Comm. A. Richardson, 22/07/11). This suggests that replenishment of carbohydrate reserves started from the shoots down. At the time root reserves peaked in non-girdled vines, it is probable that trunk reserves were being replenished in girdled vines.

The results of these experiments conform with the theory that girdling temporarily increases carbohydrate availability to above-ground organs at the expense of the root system (Goren et al. 2003). Fruit growth is able to utilise a proportion of the newly available carbohydrates, evidenced by increased fruit dry matter accumulation in girdled vines (chapter 2 and previous orchard studies; Currie et al. 2005). It may be suggested that the root systems of girdled vines are more efficient users of carbohydrates, as less energy was put into root production without any evidence of negative impacts on vine health or productivity.

Root pruning primarily caused a hydraulic response (chapters 2 and 3). This agreed with our hypothesis that a reduction in root biomass would reduce hydraulic conductance and supports observations in other crops (Poni et al. 1992; Smart et al. 2006). The severity of the response was related to the severity of the treatment, with larger effects on vine water status resulting from the more severe pruning treatment (80% root biomass removal compared with 50%). The more severe root pruning treatment (chapter 3) also reduced photosynthesis. The reduction in photosynthesis was attributed to stomatal limitation and feedback

inhibition following reduced sink demand. The tracer partitioning experiments (chapter 5) demonstrated that when half of the root system was removed, root system import of carbohydrates was reduced and accumulation in the fruit was increased. This result agrees with the observation of increased fruit size and dry matter content in the orchard grown vines following root pruning (chapter 3). In the short-term of the tracer experiments there was no evidence of increased carbohydrate partitioning to the remaining intact roots. It was hypothesised that the remaining roots would show an increase in carbohydrate import if physiological processes increased to compensate for the reduction in root biomass. There was no evidence of increased import into the remaining root half, indicating that there was no up-regulation of physiological processes in the remaining roots. This warrants further investigation. In particular the competitive ability of root re-growth following root pruning needs to be investigated over a longer period. The results of this research support the theory that root pruning increases fruit size by reducing the sink strength of the root system. An alternative theory is that root pruning reduces vegetative vigour through re-allocation of carbohydrates to the root system as the root:shoot balance is re-gained (Geisler & Ferree 1984). This theory was not supported by these results.

These experiments did not account for effects of manipulating the root system on hormone signalling. Hormone signals are likely to be affected by practices that manipulate the size of the root system (Skogerbo 1992). This is an area that warrants further investigation in kiwifruit.

## 6.3 Horticultural Implications

The results of these experiments give a better understanding as to the role of the root system in carbohydrate partitioning and seasonal trends in root carbohydrate demand. These results can be used to time horticultural practices with maximum potential for increasing carbohydrate availability to fruit.

Combining the observations of root growth and respiration it is suggested that root carbohydrate demand will be high between December and April, with minimal demand between June and September. Reduced demand during winter agrees with the deciduous growth habit of the kiwifruit vine. Carbohydrate use during this period should be minimal to maintain reserves. From September onwards root

carbohydrate demands will increase. Canopy demands for water and nutrients increase in spring and will increase carbohydrate partitioning towards the roots to ensure sufficient root area to meet the requirements of the developing canopy. Peak root carbohydrate demand during summer and early autumn coincides with the period during which the canopy will be at its largest and most efficient in regards to carbohydrate assimilation. However, during this time the demands of above-ground growth will also be high. Shoot growth will be a considerable carbohydrate sink until January, when growth slows in Hayward kiwifruit vines. Fruit growth will continue to accumulate carbohydrates into late autumn (Davison 1990). Therefore root growth is in direct competition with above-ground growth.

It is recommended that trunk girdles should be applied in February with the aim of increasing fruit dry matter content (Currie et al. 2008) and root pruning has been trialled for use from December to January. These timings agree with the period when competition for carbohydrates between fruit and root growth will be high. Manipulations carried out before December or after April may not increase partitioning to the fruit. A concern with increasing carbohydrate availability to above-ground growth before January is the effects on shoot growth. The main period of rapid shoot growth in Hayward kiwifruit is between October and January (Davison 1990). Increasing the availability of carbohydrates during this period may increase shoot growth, which is not a desirable outcome.

The results of these experiments indicate potentially negative effects of horticultural practices on the root system. These results can be used to determine sustainable practices. Orchard grown kiwifruit vines appeared to tolerate significant reductions in root biomass, without reducing productivity during the same growing season. Effects on vine productivity in following years need to be determined before root pruning is widely adopted. Annual trunk girdling affected root biomass with evidence of effects derived from previous years girdles. There were fewer differences in root numbers towards the end of the rhizotron study. This indicated that a single annual trunk girdle is a sustainable practice, as long as vines are not exposed to additional stresses. Root pruning will probably be a less sustainable practice. Effects on fruit growth are related to treatment severity. Root pruning will be severest in its first year of use and may not have the desired effect

on fruit growth in subsequent years. Pruning opposite sides of the row in alternate years may be an option for increasing sustainability.

The use of trunk girdling in New Zealand kiwifruit orchards has recently been questioned. In November 2010 an outbreak of the bacterial disease Psa (*Pseudomonas syringae* pv. *actinideae*) was found in kiwifruit orchards in the Bay of Plenty. *Pseudomonas syringae* pv. *actinideae* enters the plant through natural and manmade wounds (Vanneste et al. In-press), therefore concerns have arisen regarding the application of girdles. Research is required to determine the risk of infection with girdles. In the meantime root pruning may be an alternative means of increasing fruit dry matter content. Root pruning will be less of an infection risk as the bacteria is epiphytic and is not thought to survive in the soil. Care would be required to avoid spread if infected vines were already in the orchard.

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