Effects of a pre-flower phloem girdle on root pressure and partitioning of carbohydrate in *Actinidia chinensis* var.*deliciosa* 'Hayward'

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Abstract

The objective of this research is to understand the impacts of applying a pre-flower trunk girdle to the kiwifruit cultivar 'Hayward' (*Actinidia chinensis* var. *deliciosa*). The pre-flower girdle is a recent addition to the management of 'Hayward' kiwifruit orchards since it was discovered that it can reduce flower budrot. However, the effects of a trunk girdle in early spring on the partitioning of resources through the vine and the impacts on vine growth are unknown.

Two approaches were used to address the objective. The first approach was to use mature 'Hayward' vines at the Plant and Food Research Orchard, Te Puke in the Bay of Plenty. The aims were to determine if xylem mobile sugars are important in the development of spring root pressure, and to define the impacts of a pre-flower girdle on root pressure, carbohydrate partitioning and storage, and vine phenology within the vine. The second section of research used the detection of Bremmstrahlung radiation to trace the *in vivo* movement of radiolabelled photosynthate through 'Hayward' scions recently grafted onto Bruno rootstocks. The aim was to understand how stem girdling effects the movement of carbohydrate between source and sink tissues of young developing vines.

Key results of this research were that the refilling of xylem vessels and the establishment of hydrostatic pressure in early spring occurs gradually from the roots up the trunk of the vine. Fructose in the fine roots was identified as a key carbohydrate in the generation of the hydrostatic pressure required to refill xylem conduits after winter dormancy. After a pre-flower trunk phloem girdle was applied there was a loss of xylem pressure that lasted for two weeks. The cause has not been resolved though results from this study suggest a relationship with loss of phloem flow, or cavitation induced air embolisms in the xylem preventing xylem pressure recovery.

Leaves from girdled vines exported less carbohydrate than non-girdled vines which suggests a delayed transition to an autotrophic status. Root and trunk

carbohydrates showed little short-term effect of the pre-flower girdle. However, there was a location effect seen with the trunk bark carbohydrates that suggest the utilisation of 'local' reserves is exploited in preference to long-distant transport of reserves. There was no detectable short-term impacts of the pre-flower girdle on phenology.

Further research is proposed to understand the long-term impacts of a pre-flower girdle on root pressure, carbohydrate storage and the development of seasonal organs.

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1.1 Introduction

The kiwifruit industry is of significant economic value to New Zealand, present inputs into the national economy are in the range of 5.5b dollars with the industry accounting for 40% of all horticultural exports. Recent reports predict a 135% increase in Gross Domestic Product by 2030 (Scrimgeour, 2017). The industry not only benefits the wider New Zealand economy, it also directly impacts and benefits regional communities. For example, many jobs are created within the orchards, packing sheds and other industry areas. With the Bay Of Plenty and Northland regions being the main contributors to the high growth rate of the kiwifruit industry, the forecast increases in kiwifruit production could lead to a further 8000 jobs being created in these regions by 2030 (Scrimgeour, 2017).

Central to the success of the kiwifruit industry has been investment into research and breeding programmes. The breeding of more tolerant cultivars has enabled the industry to respond and move forward from major threats, like the *Pseudomonas syringae pv. actinidiae* (Psa bacteria). When Psa-V hit kiwifruit orchards in 2010, research and development played a huge part in the recovery of the industry (Currie *et al.*, 2011a; Parkes & Gea, 2011). Plant and Food Research provided an alternative cultivar with lower susceptibility to the bacteria, as well as scientific research into orchard management and disease control options, for example: vine girdling, harvest timing, protectants. These inputs into the industry have meant productivity increases and premium quality fruit for consumer markets (Currie *et al.*, 2011a; Scrimgeour, 2017).

Kiwifruit vines grow vigorously. Their growth is a dynamic competition between the vegetative plant tissues and reproductive components. During the transition from winter dormancy to active spring growth there is high demand for carbohydrates (CHO) in spring between developing shoots, leaves, flowers and

fruit (Cieslak *et al*., 2011). Girdling has developed as an important tool to promote canopy and fruit growth in horticultural crops since its early use to in the 1960's for propogation (Noel, 1970). In effect a girdle alters the CHO partitioning in the plant (Noel, 1970; Roper & Williams, 1989; Williams *et al.*, 2000). A pre-flower girdle is becoming more common as it appears to reduce the loss of flower buds through rots (Richardson *et al.*, 2016). In addition it is possible that the isolation of the roots from the supply of recently fixed CHO makes more photo-assimilates available to newly developing buds. Additional girdles in late spring and summer have been proven to increase fruit quality (fruit size and dry matter) through the increased partitioning of carbohydrates to the fruit in preference to the roots (Currie *et al.*, 2005; Patterson & Currie, 2011b).

It is well known that CHO supply is paramount in growing the high quality fruit required for export markets. The aim of this review is to look at research into the role of CHO storage and remobilisation during spring growth of kiwifruit vines as they come out of winter dormancy, and review current understanding of the consequences of pre-flower trunk girdling on the supply and availability of CHO to the developing floral and vegetative organs.

1.2 Carbohydrate storage and mobilisation

In deciduous species nutrient reserves play an important part in early spring growth. Reserves of minerals and photosynthates as starch are stored within the root systems and stem tissues (Bazot *et al.*, 2013; Loescher *et al.*, 1990; Tromp, 1983). Nutrient reserves are predominantly remobilised throughout the plant via the phloem sieve tube network. Loading into the phloem transport system occurs at source areas, for example - storage tissues and autotrophic leaves. From these source regions photosynthates and nutrients are mobilised, they are then unloaded at sink areas like growing flower buds, developing shoots and leaves, or they can be accumulated for later use in storage tissues (Boldingh *et al.*, 2015; De Schepper *et al.*, 2010; Minchin & Thorpe, 1987). The process of

phloem loading and unloading complies with the Munch theory developed in 1930 (De Schepper *et al.*, 2013). The Munch theory states that carbon transported via the phloem is the result of carbohydrate (CHO) concentration differences between loading and unloading regions of the plant, an area with a low concentration results in a turgor pressure gradient into a high concentration region (De Schepper *et al.*, 2013). When supply of recently assimilated carbon exceeds the requirements of the plant for plant function and growth, CHOs are accumulated in roots and stem tissue, these are important regions of CHO storage. The reserves of CHO must supply respirational demands during winter dormancy and the same tissues must also provide a wide range of mineral nutrients for spring growth before young organs become autotrophic (Tixier *et al*., 2017). An example is seen with alternate bearing trees where it is normal behaviour to have a heavy crop one year, followed by an 'off' year with little, if any fruit. In research on alternate bearing citrus trees, trees in their 'off, low fruiting year responded to a girdle by accumulating recently assimilated photosynthate in the leaves and shoots above the girdle (Li *et al.*, 2003). However, trees in their heavy fruit load year did not accumulate CHO, this was assumed to be because the demand of the developing fruit for sugars was high, leaving very little CHO available for storage for the following season (Li *et al.*, 2003).

There have been many studies attempting to elucidate the pathway of phloem movement through a plant. Isotope labelling (Minchin 1987), is one method that has successfully traced phloem flow in living plants. The technique utilises the decay of a ^{11}C tracer to detect gamma rays that are produced, this allows recently fixed carbon to be traced as is moves within the phloem. The method has successfully demonstrated the movement of labelled carbon from a photosynthesizing source leaf, to sink tissues like roots and shoots in several species - including: kiwifruit (Black *et al.*, 2012b), lupins (Minchin & McNaughton, 1987), beans (Minchin & Thorpe, 1987) and sugar beets (Geiger & Batey, 1967). The trials using this technique observe a single initial rise in labelled carbon export from the source leaf – the carbon is later observed in sink tissues as phloem delivers the assimilated carbon to these regions (Black *et al.*,

2012a; Minchin & Thorpe, 1987). However, contrary to this pattern of a single pulse of label arriving in the sink tissues, a recent study with *Actinidia arguta* using the Bremsstrahlung technique (similar to previous research but using ^{14}C as an isotope) reported a second phase of transfer of label to a second sink (Boldingh *et al.*, 2015). The authors suggested this result may indicate radiolabelled tracer was being exported from the roots via the xylem sap (Boldingh *et al.*, 2015). The possibility of return export via the xylem is plausible when previous work in the area of phloem transport is considered. There have been studies showing a small percentage of phloem volume is lost along the transport pathway, this indicates 'phloem leakage' where small amounts of photosynthates are unloaded along the phloem pathway into the apoplast. From the apoplast it is possible for photosynthates to move by diffusion into the xylem sap (Minchin & McNaughton 1987; Minchin & Thorpe 1987; Daudet et al. 2005). More recent work also supports these findings, in response to phloem damage or blockage, adaptability is shown with the phloem able to transport laterally across sieve tubes (Asao & Ryan, 2015; De Schepper *et al.*, 2013).

1.3 Carbohydrate remobilisation as a driver of root pressure

Deciduous species lose their foliage and become dormant through the winter season. The spring growth that begins with development of leaf and flower buds must initially be supported by carbohydrate (CHO) and nutrients that have been reserved in storage tissues over winter (Clark & Boldingh, 1992; Clark & Smith, 1988; Loescher *et al.*, 1990).

A common indication that plants are transitioning out of dormancy into an active growth period is the development of root pressure. Root pressure is described as a positive pressure in the xylem, originating from the root system The pressure increases as overnight temperatures become warmer and humid conditions develop, lowering the rate of transpiration as the air becomes more

saturater with water vapor (Clearwater *et al.*, 2007; Pickard, 2003). Root pressure has been observed in many species, including walnut trees (Ewers *et al.*, 2001), grape vines (Sperry *et al.*, 1996), sugar maple trees (Cortes & Sinclair, 1985), kiwifruit vines (Clearwater, Blattmann, et al., 2007) and maize (Enns *et al.*, 2000). In kiwifruit, root pressure often begins early in spring, before budburst (Clark *et al.*, 1986; Clearwater *et al.*, 2007). During this period, CHO remobilisation and / or mineral ion accumulation, in species including strawberries (Takeda F, 1991) and kiwifruit, drives a positive pressure build up in the xylem vessels that becomes high enough to cause guttation of xylem sap from the hydathodes. This can be observed on the sepal margins of developing buds, and on margins of both developing and fully expanded leaves. The presence of guttation is widely accepted as an indication of positive xylem pressure originating from within the roots (Enns *et al.*, 1998; Taiz & Zeiger, 2002). The guttation fluid has been used to confirm the occurrence of high root pressure (Enns *et al.*, 1998).

The mechanism driving root pressure is not altogether agreed upon, with results of research not always being definitive. Generally root pressure is believed to be the result of osmotic processes that occur as dormant plants begin to move into an active growth stage, and throughout growth periods when the roots are actively accumulating solutes in the xylem (Enns *et al.*, 1998; Taiz & Zeiger, 2002). Early development requires conversion of stored insoluble starches into mobile disaccharide and monosaccharide sugars originating from starch hydrolysis (Loescher *et al.*, 1990). While starch conversion sounds plausible, the relationship between osmolality and root pressure continues to be questioned as some studies report no definitive link (Enns *et al.*, 2000; Zholkevich *et al.*, 1979). An example can be found in a study conducted with hydroponically grown *Zea maize*. In this trial the water potential of the hydroponic growing solution was adjusted to test the relationship between xylem osmolality and changes in root pressure. The results failed to find a relationship – in fact the osmolality of the sap was more negative than the osmolality of the hydroponic solution (Enns *et al.*, 2000). Using stem sap as a measure of osmolality assumes a close similarity between the sap within roots and stems (Clearwater *et al.*, 2007; Ewers *et al.*,

2001). Whether this is always true remains to be proven as there is some evidence that the concentration of solutes in stems can be significantly different than concentrations of solutes within the roots (Zholkevich *et al.*, 1979). Due to the difficulty posed in accessing root sap, research has often used stem sap as a surrogate and relied on the assumption of similarity between root and stem sap.

1.4 Phloem girdling

The process of phloem girdling involves the removal of a narrow section of bark tissue from the circumference of the trunk (Currie *et al.*, 2011b). This process isolates the roots from recently assimilated CHO supplies that originate from above the girdle. During dormancy and spring growth the roots function as important storage organs, supplying CHO reserves when photosynthesis and assimilation of photosynthates is negligible (Tromp, 1983). At other times the roots are a major sink for CHO and nutrients. The temporary exclusion of the roots as a carbon sink has the potential to increase the available supply of photosynthates to actively growing reproductive organs and shoots (De Schepper *et al.*, 2010). Phloem girdling can be used to manipulate the sink/source ratio, and can be a valuable tool and when applied at critical times to increase fruit quality and overall harvest value (Currie *et al.*, 2005). Examples can be found in various horticultural crops, for example, girdles applied to grape vines can improve the harvested fruit size, as well as improve the water potential of the overall vine (Williams *et al.*, 2000). In nectarine trees it has been shown that girdling can increase not only fruit quality, but also reduce the time required for ripening and therefore increase the market window (Di Vaio *et al.*, 2001). Phloem girdling has also been incorporated into the fundamental management of kiwifruit orchards in New Zealand since the early 2000s (Harker et al. 2009). Since its introduction, girdling in kiwifruit at optimal fruit growth times has increased the growth and dry matter content of fruit, resulting in improved flavour, size and financial returns (Currie *et al.*, 2005).

The identification, and confirmed presence of Psa-V in New Zealand kiwifruit orchards ignited an initial fear of increasing incidences of Psa-V infection in vines.

There were fears the invasive mechanism of girdling would allow the bacterium easy access through the girdle wound, thereby increasing infection rates and severity. However, research has found that through the use of sound hygiene practice and by avoiding applying girdles during wet weather periods, there is no obvious effect on Psa-V infection levels (Currie *et al.*, 2011b; Max *et al.*, 2011). In contrast, studies have found that a trunk girdle applied before flowering on Hayward vines actually reduces Psa-V-associated blossom blight (bud-rot). Budrot is an infection causing browning and drop of spring flower buds, commonly associated with *Pseudomonas syringae (Currie et al., 2011b)*. However, until recently, neither the mechanism by which *Pseudomonas syringae* colonizes the flower buds, or the reason why phloem girdling reduces bud incidence, are known. Richardson *et al.* (2016) demonstrated that the pre-flowering trunk girdle reduced the level of root pressure in the weeks following the girdle, causing a reduction in the incidence of pre-dawn guttation by the sepals of flower buds. Guttation fluid is a known pathway for the entry of bacterial pathogens into leaves. The reason why the pre-flower trunk girdle reduces root pressure prior to flowering in kiwifruit is not known, although one hypothesis is that the girdle caused a reduction in the supply to the roots of carbohydrates needed for the generation of root pressure (Richardson *et al.,* 2016).

1.5 Physiological response to sink/source manipulations

1.5.1 Sink-source ratio and photosynthesis

Many trials have looked at the effect of sink/source manipulations on plant physiological responses and functioning. These include CHO assimilation and partitioning, phloem transportation, stomatal conductance, photosynthesis rates, water potential and leaf gas exchange (Cheng *et al.*, 2008; Currie *et al.*, 2005; Li *et al.*, 2003; Piller *et al.*, 1998). Reduction of the sink/source ratio has an

effect on photosynthesis rates and the transportation of synthesized products. The ratio can be altered by direct removal of a sink, or by a girdle that interrupts the phloem transport system.

It has been postulated that inhibition of photosynthesis is a direct response to sink reduction (Geiger 1976; Wardlaw 1990), however, studies have indicated that lower stomatal conductance is an initial response to girdling, thereby indirectly inhibiting photosynthesis through inhibiting gas exchange (Geiger 1967, Setter 1980; Williams *et al*., 2000). It is still unclear whether photosynthesis is the driver of stomatal conductance, or stomatal conductance drives photosynthesis, either way it is likely enzymes, hormones and environmental conditions also have a role (Mansfield & McAinsh, 1995).

Stomatal response is known to have key relationships with many aspects of plant functioning, e.g. photosynthesis, CHO accumulation, transpiration, gas exchange. What is still not clear is the mechanism of the response. Some research indicates an association of stomatal control with enzymes and hormone action (Turgeon & Wolf 2009). Research often highlights lowered gas exchange through stomatal closure is an important reaction to stem girdling. For example, stomatal closure has been observed in soybeans (Setter *et al.*, 1980), kiwifruit (Black *et al.*, 2012b), grapes (During, 1978; Roper & Williams, 1989; Williams *et al.*, 2000), apple (Cheng *et al.*, 2008), nectarine (Di Vaio *et al.*, 2001). Conversely, in kiwifruit following the pre-flower girdle, the initial response to a trunk phloem girdle was increased stomatal conductance and photosynthesis (Richardson *et al.*, 2016). An important difference between this and the previously mentioned studies is the observed reduction in stomatal conductance may have been influenced by the early timing of girdle application. At this time the shoots may still have been in transition from being sinks to sources (Richardson *et al.*, 2016).

If stomata are closing before photosynthesis is reduced it would indicate stomata are an important regulator of photosynthesis. Triggers to stomata closure can be variable, stomata will close under water stress and adverse environmental conditions (Patterson & Currie, 2011b). It is well documented that stomata are involved in water regulation - studies focusing on water potentials and drought effects have reported stomatal closing is often a response to conserve water

(Downton et al., 1988; Malcheska et al., 2017; Flexas et al., 2004; Williams et al., 2000). With this is mind, it is reasonable to question whether water potential is impacted by girdle application – potentially causing a stomatal response to conserve water.

There have been a few instances where girdling trials have measured water potential. As an example, the primary response to girdling in trials on grape vines was a reduction in stomatal conductance, this reduced water loss through transpiration leading to an increased whole vine water potential (Williams *et al.*, 2000). In 2009 a trial on kiwifruit, cultivars *Actinidia chinensis* var. *deliciosa* 'Hayward' and *Actinidia chinensis* var. *chinensis* 'Hort 16A' measured hydraulic conductance in response to post-flowering trunk girdling and root pruning treatments. The results indicated hydraulic conductance was unaffected by the trunk girdle application, however both stomatal conductance and photosynthesis were lowered significantly 25 days post girdle. The authors surmised this could be a down-regulation response to decreased demand for carbohydrate (Black *et al.*, 2012b).

1.5.2 Feedback inhibition

A possible response to changes in source to sink ratio that can cause changes in photosynthesis is 'feedback inhibition', whereby the acquisition of photosynthates controls their production (Black *et al.*, 2012b). This phenomenon has been the topic of many studies, however, the exact mechanism by which the photosynthetic inhibition is triggered is still being investigated.

The girdle application and resulting changes in the whole plant sink/source ratio certainly has the ability to alter gas exchange, though mechanisms are still somewhat elusive. Research has found that the excess supply of recent photosynthates that occurs after a girdle results in CHO accumulation, specifically above the girdle site. This is a direct response to the reduction of the sink/source ratio (Currie *et al.*, 2005; De Schepper *et al.*, 2010; Li *et al.*, 2003). In

some instances the trunk girth above the girdle is measurably increased (De Schepper *et al.*, 2010; Geiger, 1976; Setter *et al.*, 1980), CHO can also accumulate directly in the leaves and inhibit further photosynthesis (Iglesias et al., 2002). However a recent paper by Asao and Ryan (2015) found that responses to girdling were varied, they found no evidence of feedback inhibition through CHO accumulation in leaves.

Some studies have hypothesized that the accumulation of CHO precedes a hormone response within the plant, signalling a down regulation of photosynthesis (During, 1978; Iglesias *et al.*, 2002; Setter *et al.*, 1980). This seems plausible since phloem is known to supply hormones and proteins as well as CHO and nutrients (Taiz & Zeiger, 2002; Turgeon & Wolf, 2009). A prime contender is ABA (abscisic acid), ABA is a hormone that could be affected by alterations of the phloem pathway and therefore affect stomatal behaviour when the sink/source ratio is altered (Downton *et al.*, 1988; Setter *et al.*, 1980).

1.6 Objectives of this research

Clarified during this literature review is the variability of responses to girdling, both across and within species. This variability is clearly demonstrated in a trial by Asao & Ryan (2015) that assessed the impacts of trunk girdling. In this trial, four species of tropical rainforest trees were treated with four levels of girdle intensity. No detrimental effect was found on plant function when the trunk was girdled around 25% of the circumference, 50% the circumference or 75% of the trunk circumference. Only the full girdle, around the complete circumference of the trunk lowered photosynthesis and stomata conductance. In one species of the four species in this study no reductions in gas exchange or stomata conductance was seen at all (Asao & Ryan, 2015).

This research will focus on discerning how the phloem girdle affects carbon partitioning in kiwifruit. Applying a pre-flower girdle and isolating the growing flower buds and shoots from the roots should affect the supply of CHO to or

from these tissues. However, because the shoots are transitioning from being heterotrophic to autotrophic at the time of girdle application, it is unknown exactly how the girdle affects carbohydrate partitioning within the vine. There may also be effects of girdling on root function and the generation of root pressure, that have down-stream effects on carbohydrate concentration in the xylem sap, delivery of carbohydrates to the shoots via the xylem, and the repair of xylem embolism in spring that can occur as a result of xylem refilling after the dormant growth period. This thesis is divided into two experimental chapters. In **Chapter two** I investigate the effect of the pre-flower trunk girdle on shoot development, the generation of root pressure, and carbohydrate concentrations in the xylem sap and tissues of the roots, stems and shoots. In **Chapter three** I use 14C as a tracer to examine effect of the stem phloem girdle on the transport of carbon between shoots and roots, investigating both downward movement from shoots to roots in the phloem, and possible upward movement in the xylem from roots to shoots

2 Chapter Two

The role of stored carbohydrates for spring growth in *Actinidia chinensis* var. *deliciosa* 'Hayward'

2.1 Introduction

As temperature increases after winter dormancy there is an increase in demand for the CHO (carbohydrates) stored in woody tissue like roots and stems. These supplies are paramount in suppling newly emerging plant organs like leaves and flowers during this critical stage in their development (Tromp, 1983). A nutritional shortage early in spring could have effects that flow through to growth and maturation of the fruit (Piller *et al.*, 1998). This reliance of the plant on the stored reserves continues until the developing leaves become autotrophic - converting from being a sink organ, dependant on stored supplies of CHO for growth, into a source organ. Once the leaf is autotrophic it exports any photosynthates that are not required for its own growth and function, and supplies storage organs and growing heterotrophic plant organs (De Schepper *et al.*, 2010).

Kiwifruit is a deciduous vine and is therefore reliant on stored CHO in roots and stems to sustain respirational requirements over winter and to support the flush of spring growth (Currie *et al.*, 2005). While the phloem tissue is the major CHO transport tissue it is likely that the xylem also has a role in the transport of sugars (Boldingh *et al.,* 2015; Minchin & Thorpe, 1987; Sperling *et al*., 2017). Over winter the vines are dormant, there is negligible transpiration and the xylem vessels may become air filled. Previous research has indicated the pressure required to refill xylem vessels and displace air embolisms after dormancy is likely to arise from a positive pressure that develops in the roots in the absence of transpiration driven flow (Clearwater *et al.*, 2007; Pickard, 2003; Sperry *et al.*, 1987; Wegner, 2013). Early in spring buds swell and new leaves are beginning to

develop, however canopy transpiration and the associated xylem tension that drives xylem sap flow remains low due to the lack of canopy. In some plants like strawberries and kiwifruit, positive spring root pressure can result in guttation through the hydathodes, hydathodes are pores found on the margins of the bud sepals, and also at the margins of leaves (Bradfield, 1979; Taiz & Zeiger, 2002). The driving force behind the build-up of positive pressure driving early sap flow may be the result the changing osmotic potential of the xylem sap (Wegner, 2013). This could occur through the active conversion of immobile starch stored in the roots, into mobile hexose sugars like fructose and glucose (Boldingh *et al.,* 2015), by the active uptake of minerals (Ewers *et al.*, 2001), or by falling air temperature in the canopy triggering reactions influencing carbohydrate allocation (Sperling *et al.*, 2017).

However, not all research agrees with this hypothesis. Some studies report a negligible relationship with xylem pressure and the osmotic potential of the sap. For example, in a study on sugar maple trees the change in diurnal pressure patterns was much higher than could be attributed to the smaller changes in osmotic potential, 0.15 Mpa and 0.05 Mpa respectively (Cortes & Sinclair, 1985). A study on hydroponically grown Z*ea maize* L. failed to find a relationship between osmotic potential and xylem pressure. Guttating Z*ea maize* L. had a higher xylem osmotic potential than the surrounding solution and xylem osmolality changed little in response to the osmotic potential of the solution. In contrast, the turgor pressure of the vacuoles showed a stronger response to changing osmotic potential and it was concluded that root pressure was not caused by the osmotic potential of the sap (Enns *et al.*, 2000).

2.1.1 Reduction of sink-source ratio by girdle application

For a kiwifruit orchardist the aim of a phloem girdle applied to the trunk is ultimately to increase the quality of the fruit at harvest (Max *et al.*, 2007). A girdle applied late spring after fruit set has proven to be a useful tool to achieve larger fruit sizes and when applied later in the summer the vine responds by

increasing the dry matter accumulation in the fruit (Currie *et al.*, 2005; Harker *et al.*, 2009; Patterson & Currie, 2011a). Both these attributes are seen as desirable for marketing of kiwifruit, subsequently the application of one or more girdles on kiwifruit vines has become common orchard management practice since 2010 (Currie *et al.*, 2011a; Currie *et al.*, 2005).

A girdle physically removes a section of phloem from around the circumference of a stem, trunk, or branch. In the period preceding the girdle wound healing there is a decrease in the whole plant demand for photosynthates. Any export via the phloem of stored nutrients from roots and other storage tissue below the girdle site and any possible import of carbohydrate (CHO) from tissues above the girdle to the roots is disrupted. By temporarily alienating the roots from the canopy there is an increase in availability of CHO to other growing regions of the vine, e.g. fruits and shoots (Noel, 1970).

However, spring heralds a critical growth period when it is possible that the roots, as storage organs, are acting as a phloem source. A trunk girdle has the potential to isolate the developing shoot tissues from critical supplies of nutrients stored below the girdle (Piller *et al.*, 1998). Consequently, before leaves become autotrophic it is possible to negatively impact the development of growing organs. In contrast to post-flowering or summer girdles, a pre-flower girdle is applied to reduce the incidence of bacterial blossom blight ('budrot') (Richardson *et al.*, 2016). Richardson et al. (2016) showed that the pre-flower girdle reduced root pressure and the incidence of sepal guttation for a period of around two weeks, starting from approximately 10 days after the girdle was applied. The reduction in guttation possibly explains the reduced incidence of blossom blight. Potentially a pre-flower girdle could result in decreased xylem sugar concentrations, reduced root pressure, and reduced transport of mobile sugars in the xylem to the shoots and buds. This is supported by evidence of carbohydrate supply via the xylem, particularly in spring when transpiration rates are lower and xylem CHO concentrations may be higher (Ferguson *et al.*, 1983; Minchin & McNaughton, 1987; Tixier *et al.*, 2017b).

2.1.2 Aims

The aim of this project was to determine the impacts of applying a pre-flower trunk girdle on carbon partitioning in kiwifruit vines *Actinidia chinensis var*. *deliciosa*, 'Hayward' as they move into active spring growth after winter dormancy. Some questions this project will explore include whether the pre-flower girdle has an impact on positive root pressure development by decreasing osmotic potential. This project may also determine whether the movement of stored CHO resources between the roots and shoots are impacted by the girdle, or are the partitioning of newly assimilated photosynthates between competing kiwifruit vine organs altered.

Without autotrophic leaves supplying carbohydrates the vines must be reliant on CHO reserves in storage tissue to support spring growth. Development of root pressure is likely to be correlated with increased transport of soluble CHO originating from the roots.

If this is the case, the hypothesis is that a pre-flower phloem trunk girdle should affect carbon allocation across the competing sink tissues. The trunk phloem girdle should also initiate a decline in stored CHO in storage tissues (roots and below girdle wood and bark tissue) in parallel with altering concentration of soluble CHO in xylem sap.

2.2 Methods

This research project was completed at the Plant & Food Research Orchard, Te Puke, located in the Bay of Plenty of New Zealand. The soils are well drained with a volcanic ash base (Rijkse & Guinto, 2010). Average daily temperatures for 2017 ranged from 4.8°C to 14.6°C in the winter, and 5.6°C to 15.3°C to in the summer. Over the period of this study the minimum daily temperature ranged from 3.5°C to 16°C, the daily maximum temperature ranged from 13.5°C to 27°C.

Twenty mature (approximately 40 years old) female *Actinidia chinensis var*. *deliciosa* 'Hayward' vines growing on a pergola structure were selected from inside rows of an orchard block to avoid edge effects. Hi-cane was not used in the block to control timing of bud break.

From the twenty vines available ten were control vines (no girdle), and ten were treatment vines (pre-flower girdled). The wounding required for the destructive tissue sampling such as root and trunk samples can affect phloem and xylem transport for short periods thus the vines were separated into two sets of five vines per treatment. One set was for destructive analysis (tissue and xylem samples), the other five vines were for non-destructive analysis, (xylem pressure, phenology). The layout of the vines is shown in (Figure 2.1).

2.2.1 Experimental design

Te Puke Research Orchard – Block 4

M	X	M	X	M	
X	X	X	M	X	
M	X	X	X	X	
X	M	X	X	X	
X	Control 5	Treatment 5	M	X	
M	Treatment 4	Control 4	X	X	Destructively
X	Control 3	Treatment 3	X	M	sampled vines
X	Treatment 2	Control 2	M	X	
M	Control 1	Treatment 1	X	X	
X	M	X	X	X	
X	Control 5	Treatment 5	M	X	
M	Treatment 4	Control 4	X	X	
X	Control 3	Treatment 3	X		Non destructive
X	Treatment 2	Control 2	M	X	vines
M	Control 1	Treatment 1	X	X	
X	M	X	M	X	

Figure 2.1: Layout of 'Hayward' vines used in this research trial. Each cell represents a single vine, the yellow cells labelled 'M' represent male vines, and white cells marked 'X represent female Hayward' vines that were not included in this trial. Green cells show location of treated and non-treated vines that had tissue samples removed from them (destructive sampling) and the blue cells show the location of vines from which non-destructive data were collected.

2.2.2 Pressure transducers

Pressure transducers were inserted into the trunk of five control and five treatment vines on 8th September, 2017, before xylem sap began to flow, and before the girdle was applied to the treatment vines. The transducers were constructed based on previous root pressure research in kiwifruit vines, (Clearwater *et al.*, 2007). Two xylem pressure transducers were used per vine to measure the pressure gradient (relative to atmospheric pressure) between the

Figure 2.2: Xylem pressure transducers set up showing the two pressure transducers attached to the stem of the 'Hayward' vine (A). The transducers were enclosed inside plastic jars to provide protection from the weather. A needle with a pressure transducer attached was inserted into a pre-drilled hole with a rubber gasket providing a pressure seal. A 3-way tap allowed the unit to be opened to atmospheric pressure and for sap to be sampled. The transducers were connected to a multiplexor data logger (B).

(A), above girdle pressure transducer and the (B) below girdle pressure transducer. The first xylem pressure transducer assembly was inserted 10-15 cm above the girdle site and the second was 10-15 cm below the girdle site, (Figure 2.2 A). In the non-girdled control vines the two xylem pressure transducers were

inserted in the truck at the appropriate locations above and below where a girdle would normally have been placed.

To install each pressure transducer the trunk of treatment and control vines were drilled with a 4 mm drill to a depth of 4 cm into the woody tissue, woody debris was flushed from the hole using a water filled syringe before a custom-made compression fitting (hypodermic needle with a silicone gasket and a three-way tap) was inserted and secured to the trunk using cable ties. The pressure transducers were connected to a data logger (Campbell Scientific CR1000M) and relay multiplexor (Campbell Scientific AM416). The data were read every minute with an average recorded every 30 minutes, (Figure 2.2 B). The pressure transducer ports were backfilled with silicone oil, the compression fitting and three-way taps were back filled with water to eliminate air bubbles that could introduce embolisms to the xylem. These were left in place until 18/12/17. On the 15/11/17 treatment vine '2' was redrilled as neither of the pressure transducers were recording any pressure. The taps were reinserted as previously. Results are averages of the xylem pressure recorded for every 30 minute interval over a 24 hour period.

2.2.3 Phenological stages

To monitor bud break and phenological development as the vines came out of winter dormancy, three canes were selected from each vine prior to budbreak. Non-fruiting canes were chosen of a similar length and thickness. It was important to monitor bud break so that the timing of girdle application could be accurately estimated to coincide with 30 days before flowering (DBF).

Budbreak began on the 25th of September and continued to the 23rd of October. Monitoring was carried out twice each week during this time, the stage of budbreak for each bud was determined and recorded as either dormant or burst as defined by (Brundell, 1975). The timing of 50% budbreak was used to predict

the timing of 50% flowering. The time between the previous season's budbreak and flowering was used to estimate the probable flowering date. A pre-flower girdle was applied on the date estimated to be 30 days before mid-flowering (30 DBF). Shoot and flower development was tracked by visual assessment using the BBCH scale, this scale provides an inclusive description of growth stages across *Actinidia* species (Salinero *et al.*, 2009). Once the shoots in the canopy had extended beyond 90 cm in length, they became difficult to monitor and leaf area index (LAI) measurements using fish-eye lens photography were used to monitor leaf canopy development.

2.2.4 Canopy cover

To estimate LAI, overcast days were chosen to reduce the chance of reflection or bright spots in the images. A Nikon D5200 digital camera, fitted with a Sigma 4.5 mm fish-eye lens was used. The camera was positioned directly under the leader zone, at a height of 1 m below the canopy for each series of photographs. To avoid a canopy density bias, the exposure was determined by pointing the camera at the sky and using the auto exposure setting with a compensation value of +1. This exposure was then used to manually capture images. Two images were taken for each vine, one from the east-side and one from the west-side. 'Image J' software (v1.49), using a custom Java plugin was used to calculate the LAI by extracting the light transmission within the five rings spanning 180° (Figure 2.8). This procedure gave the ability to block outs sections of the image that were occupied by vine trunks or posts. The images were converted to black and white using the thresholds function, the light transmission was used to calculate leaf area index. The inner four rings were used in the analyses so that only the target vine was included.

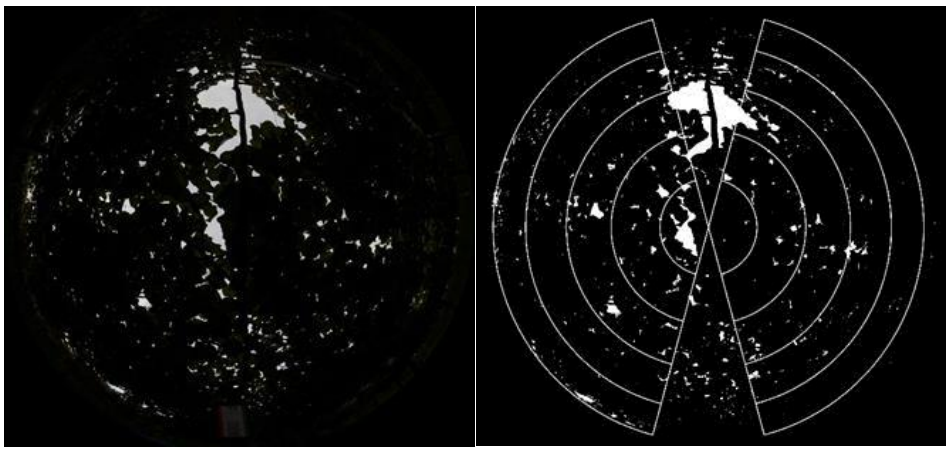


Figure 2.3: An example of images obtained to calculate LAI using a fish-eye lens. An original (left) and processed canopy photograph (right).

2.2.5 Xylem sap collection

Xylem sap was collected weekly between the 19/09/17 to the 8/12/17 for sugar and starch analysis. During this period the root pressure was high enough to enable xylem sap collection under positive pressure from a small hole in the trunk. However, once canopy transpiration began to increase it resulted in consistently lower xylem pressure in all vines and further sap collection was not possible.

To collect the sap a drill was used to make two 4 mm wide holes approximately 8-10 cm deep into the trunks of the 10 destructive vines (five control, five treatment). The holes were at a distance of 10 -15 cm above and below the girdle position of each plant (i.e. consistent with the placement of pressure transducers on the equivalent 'non-destructive' vines). Once xylem exudate was observed a hypodermic needle compression unit, attached to a three-way tap (Clearwater *et al.*, 2007) was inserted into the exuding hole securely, (Figure 2.4). These were left in place for the duration of the trial unless they become blocked with debris and needed replacing. Sap collection was carried out between 6.30am and 8.30am, any later in the day was very difficult to collect sap as the xylem pressure reduced throughout the day with increasing temperature and transpiration. To collect the sap, the taps were opened and first approximately

0.5mls of sap were discarded, this was to ensure any debris and contaminants were flushed out prior to sample collection. Exudate samples were collected in 1.5 mL Eppendorf tubes and diluted soon afterward at a ratio of 1:4 with 80% ethanol, then stored at -20°C for sugar analysis.

Figure 2.4: Vine with a compression fitting and attached three-way tap inserted into the stem used to collect xylem sap. A small droplet of xylem sap can be seen from the port on the right hand side of the open tap.

2.2.6 Tissue sampling for carbohydrate analysis

Tissue samples were collected before budbreak, four weeks before the girdle was applied, one week post girdle, and 4 weeks post girdle. Tissue samples were taken from the roots, trunk, bark and leaves of the 10 'destructive' vines. Due to the timing of leaf growth and development, leaf sampling began later than the other tissue samples, the first leaf sample was one week post girdle, followed by a sample at three weeks post girdle and a final sample collected 4 weeks post girdle. All samples were snap frozen in liquid nitrogen and subsequently stored at -80°C prior to analysis. Fresh weight of individual tissue samples (except for roots) was recorded with the samples being briefly removed from a chilly bin containing liquid nitrogen. Fresh weight of roots was not measured due to the variability introduced by cleaning immediately after collection and any remaining

soil particles. After obtaining fresh weights the samples were transferred to the Labconco Freeze Dryer to be dried over a period of 4-5 days. Once completely dried they were reweighed to obtain a dry weight. Samples were stored at -20°C until they were prepared for CHO analysis. A freeze dryer malfunction meant the root samples from 27/11/17 had to be discarded.

Wood: A 4 mm drill was used to obtain a wood sample an equal distance from above, and below the girdle (10-15 cm), on the five destructive treatment vines, (Figure 2.5). As the control vines had no girdle the wood sample was taken from the same height as the (A) and (B) samples on the treatment vines.

Figure 2.5: (A) Scraping bark off the trunk to prepare for drilling; (B) Drilling a wood sample.

Roots: As kiwifruit vines have root systems that are radially symmetrical around the vine (Gandar & Hughes, 1988), coarse and fine roots were excavated a distance of 0.5 m – 1 m out from the stem from the vine and gathered at an approximate depth of 15 - 30 cm. The sampling of roots reduced the root pressure for up to 10 days post sampling, thus root sampling was limited to only three samples spread over 8 weeks. Random sampling locations were chosen, care was taken on subsequent samplings to avoid areas that had already been disturbed on previous sampling dates. Fine roots were less than 5 mm in diameter and coarse roots were between 10 mm and 15 mm in diameter. The roots were washed in fresh water to remove excess debris and patted dry with

paper towels. The coarse roots were de-barked and the bark was stored separately from the inner root wood. Roots were snap-frozen, stored and dried as described for wood samples, (Figure 2.6).

Figure 2.6: Fine root sample (left); coarse root and bark samples (right), ready to be snap frozen into liquid nitrogen.

Stem bark: Bark was collected from the vines within 10 mm -15 mm from the girdle site. 10 mm cork-borers were used to obtain three bark disks of inner bark tissue from above and below the girdle placement (Figure 2.7). The samples were snap frozen in liquid nitrogen and went through the same post–collection procedure as the root samples.

Leaves: Two leaves were sampled from all vines at each of the three leaf sampling dates (1st, 15th, 27th November). The shoots chosen across vines on a sampling date were from the middle of the cane and of similar length and thickness. The first leaf sampled was the first mature leaf on the cane end on the shoot, a leaf similar in size, age and location on the cane and shoot but from a different shoot was used for each of the subsequent sampling dates. This leaf is referred to as the 'basal leaf'. The second leaf sampled was the first fully expanded leaf at the growing end of the shoot, this leaf is referred to as the 'apical leaf', (Figure 2.8). On each subsequent sampling date the equivalent aged leaf was used, the leaf chosen was from the same position on each of the shoots.

After leaf removal the centre vein was cut out and removed. The samples were snap frozen and underwent the same post collection procedure as described for roots.

Figure 2.7: Once the loose bark was scraped off a 10 mm cork-borer was used to obtain three 'plugs' of cortex tissue (A). The samples were chopped into smaller pieces and snap frozen in liquid nitrogen prior to analysis for carbohydrate content (B).

Figure 2.8: Preparation of basal leaf (right) and apical leaf (left) for carbohydrate analysis.

2.2.7 Compositional analyses

The dried samples were ground and homogenised. Subsamples of the homogenised plant tissue, approximately 0.5 gm (± 0.05 gm) were weighed into 80% ethanol for extraction using fucose (20µl of 10 mg/ml (10% iso-propanol)) as an internal standard. They were extracted for 60 minutes at 60^{o}C, the samples were then centrifuged and the supernatant decanted off the residue pallet. Two further cycles of washing in 80% ethanol (5ml, 2.5ml) and decanting were carried out with the supernatants being combined at the end of the third cycle and used for sugar analysis. The insoluble pellet that remained was washed into an Erlenmeyer flask with MilliQ water and autoclaved at full pressure for 1 hour. The residue was then incubated with amyloglucosidase in a 20% acetate buffer (50µl of sample to 200µl of buffer) for 1 hour at 60^{o}C. Duplicates were also run in micro-Cuvettes with Trinders (750µl), and Phenol (25µl), along with colour blanks that had the phenol omitted and water added. A Shimadzu, UV-16501A Spectrophotometer was used to calculate the starch concentrations at 510 nm.

The supernatant was subsampled into QB well plates in sufficient quantity calculated to contain 3µg of internal standard. These were dried in a centrifuge evaporator before being dissolved in 300 µl of MilliQ water. The samples were run through a Thermofisher Dionex ICS 5000 (Dionex Corp., Sunnyvale, CA, USA). The sugars being analysed had known standard retention times and associated chromatographic peaks, these were used to identify the sugars present in the samples. For the analysis, the sugars included in the 'total sugars' category were: myoinositol, galactose, glucose, fructose, sucrose, planteose, raffinose, stachyose and any significant unidentified sugars. The hexose sugars are the combined total of fructose and glucose. The concentrations of sugars are expressed as mg g^{-1} of dry weight.

2.2.8 Girdle application

When the pressure transducers were secured to the vines in early spring, the vine trunks were 'taped' to mark the approximate location of the girdle. .The 'tape' and subsequent girdles were positioned 30 – 45 cm from the ground, depending on any irregularities in the trunk uniformity (Figure 2.9 A). Timing of budbreak was used to work out when to apply the trunk girdles as described in the phenological section of the methods. The girdles were applied on the 24/10/17, this was the predicted date of 30 DBF (days before flowering) as determined by budbreak. A standard trunk girdling tool was used to remove the outer layer of cortical and phloem tissue. The girdles remained open for approximately six weeks and were fully closed by 6/12/17 (Figure 2.9 C).

Figure 2.9: Girdle on a treatment vine at four stages of healing**:**

A. Freshly girdled trunk on the 24/10/17 (day 0).
B. Open girdle, 9/11/17 (day 15)
C. Partial healing of girdle, 20/11/17 (day 27).
D. Closed girdle, 6/12/17 (day 43).

2.2.9 Data analysis

Analysis of variance (ANOVA) was used to test for treatment and sample location effects at the 5 % level. Where significant differences were found Fisher's LSD was calculated to compare pairwise means. All results are averages ± standard error of the mean. Percentage data was log transformed prior to analysis.

2.3 Results

2.3.1 Root pressure

Xylem pressure transduces were fixed to the vine stem above the girdle (A), and below the girdle (B). In seven out of ten vines the xylem pressure transducer located at (B) started to record positive pressure 1 – 6 days before the (A) positioned pressure transducer, the remaining three vines recorded positive pressure in both xylem pressure transducers simultaneously. Once both transducers were recording, the two pressure transducers in each vine generally measured similar fluctuations in pressure, (Figure 2.10). The lower (B) transducer usually had a slightly higher xylem pressure than the pressure transducer located higher in the stem (A), however there were times when this trend was reversed, for example control vine 5 (C5) had periods where the pressure was more positive in the (A) xylem pressure transducer than the (B) pressure transducer, (Figure 2.11).

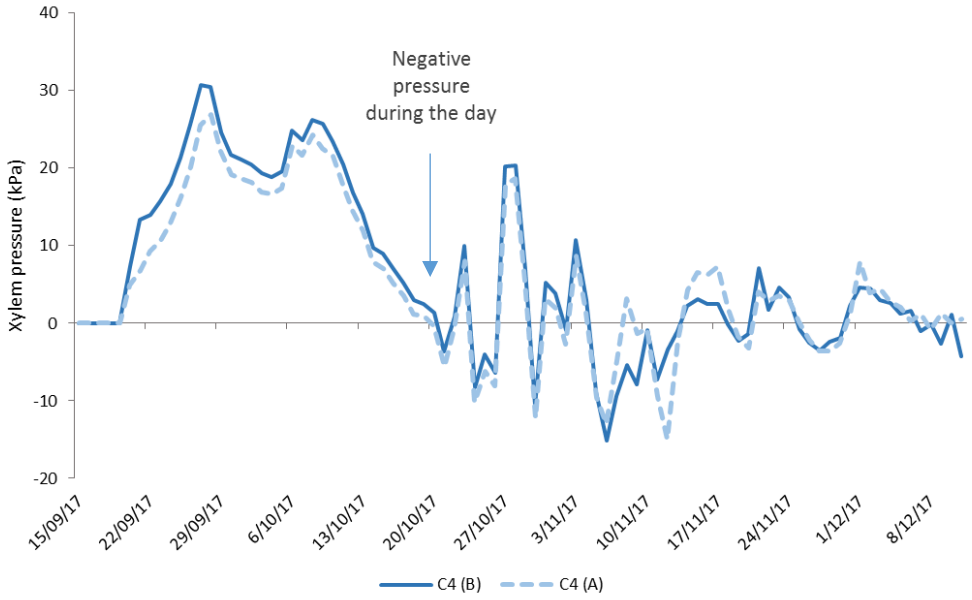


Figure 2.10: An example of mean daily xylem pressure (kPa) measured from the (A) above girdle, and (B) below girdle xylem pressure transducers on the trunk of a non-girdled control vine (vine C4). Both transducers showed the same pattern of pressure with the (B) pressure transducer often with slightly higher pressure than the (A) pressure transducer.

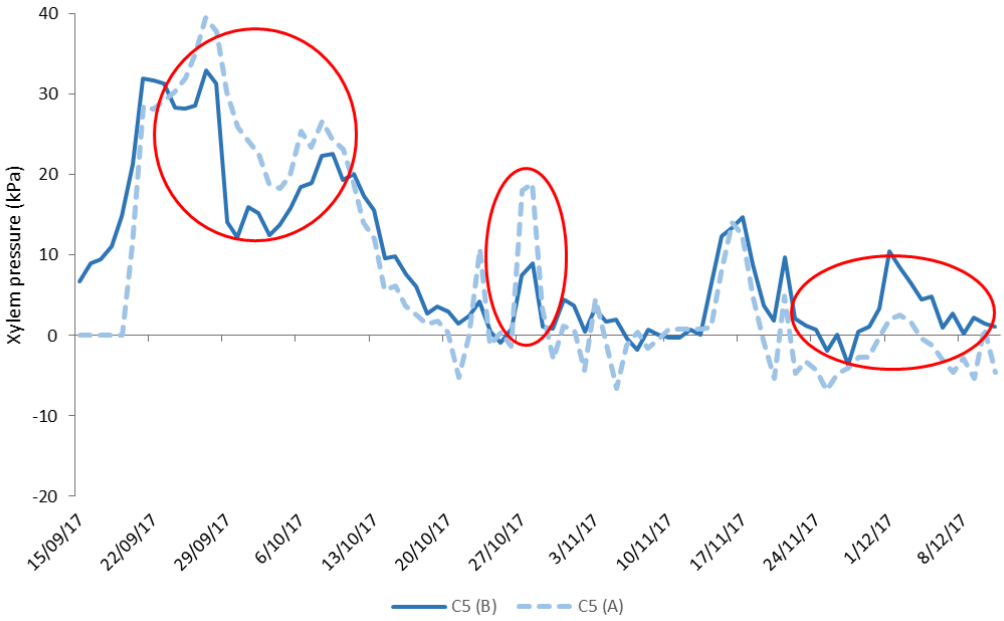


Figure 2.11: Mean daily pressure (kPa) measured from two xylem pressure transducer in the trunk of control vine five (C5). The red circles indicate times when the higher placed pressure transducer was recording a more positive or negative pressure than its lower counterpart.

From 20th October 2017 negative pressure readings were recorded during the day and positive readings were being recorded at night, in the figure below the diurnal pattern can be seen, (Figure 2.12). Rainfall events usually coincided with a higher positive pressure than periods with no rainfall. The cessation of a rainy period was followed closely by a fall in pressure in all vines across both xylem pressure transducers, (Figure 2.13).

Immediately after girdling, the xylem pressure in the girdled vines was reduced. After two to threee days the girdled (treatment; T) vines recovered pressure to levels comparable to the pressure in the non-girdled (control; C) vines. Between 16 and 21 days after girdling, the mean maximum positive pressure was lower in the T vines than the non-girdled C vines, (Figure 2.13). Pressure declined in both treatments during a period without rainfall. However, the T vines remained at a low pressure between the 10/11/17 and 1/12/17 while the C vines recovered (Figure 2.13). After a 1/12/2017 the pressure in the T vines increased to similar levels to that of the C vines.

During the four week period post-girdle, the fall in pressure in the T vines was coupled with a seperation of the pressure between the below girdle (B) and above girdle (A) pressure probes (Figure 2.14; B, D), with the (A) pressure measurement becoming clearly lower or more negative than the (B) pressure measurement. This pattern was not consistent over all T vines, there was some variation in the size of pressure difference between (A) and (B) measurements, and in the length of time the measurements were seperated. This is different from the same period in the control vines. While the pressure did fall in the C vines, the overall difference between the (A) and (B) pressure probes was negligible (Figure 2.14; A, C).

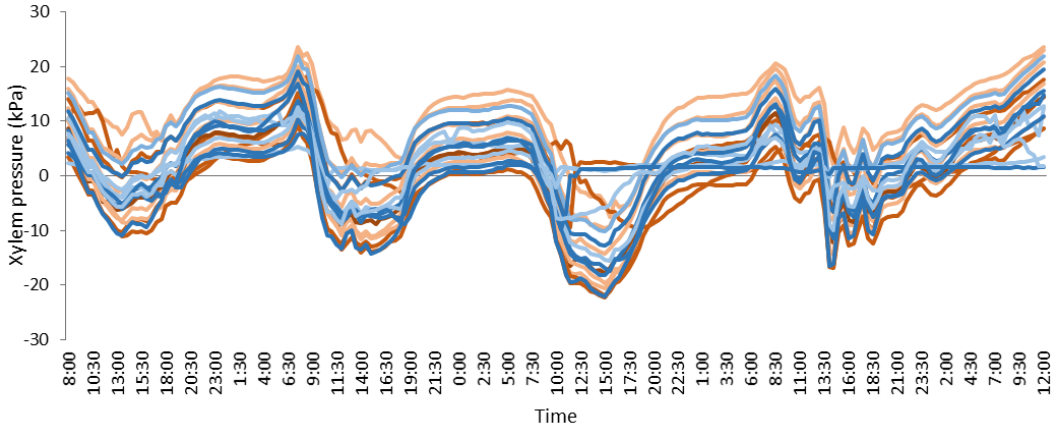


Figure 2.12: Xylem pressure in the higher transducers in the trunk of the vines, recorded over a three day period. Each line represents a single xylem pressure transducer on a vine. The blue lines are non-girdled (control) vines, the tan lines are girdled (treatment) vines. The x-axis shows time (24 hour clock) from 8:00 am on the 20/10/17 to 12:00 am (midday) 24/10/17. Results are averages of the xylem pressure recorded over 2 minute intervals.

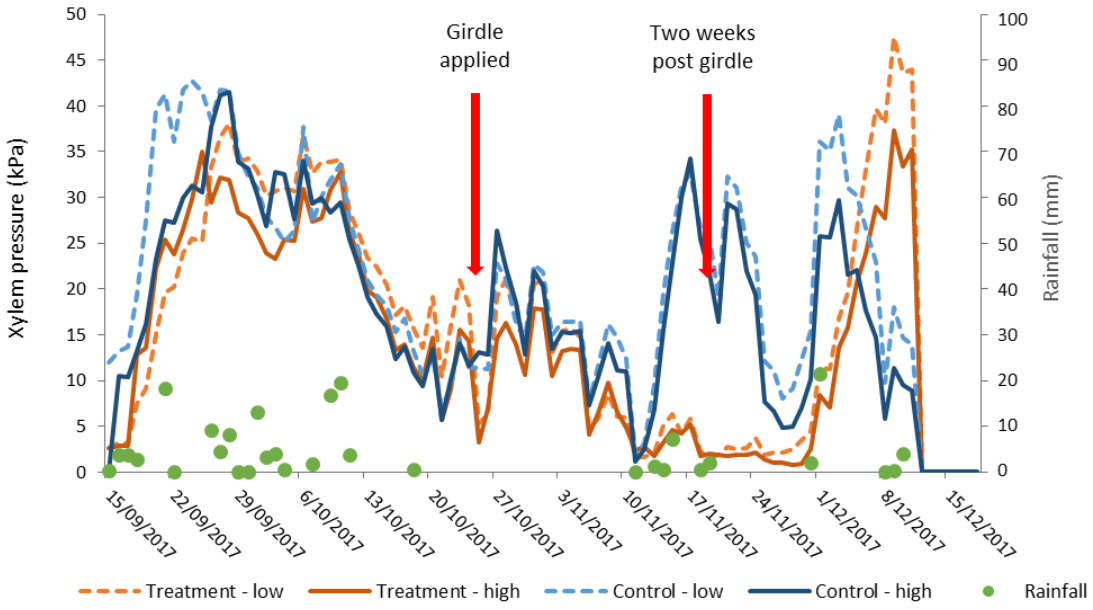


Figure 2.13: Mean maximum, positive xylem pressure of the five girdled (treatment; T) vines and five non-girdled (control; C) vines. Solid lines represent the xylem pressure transducers above the girdle, dashed lines represent pressure placed below the girdle. Green symbols show the daily rainfall (mm); n=5 vines for each treatment.

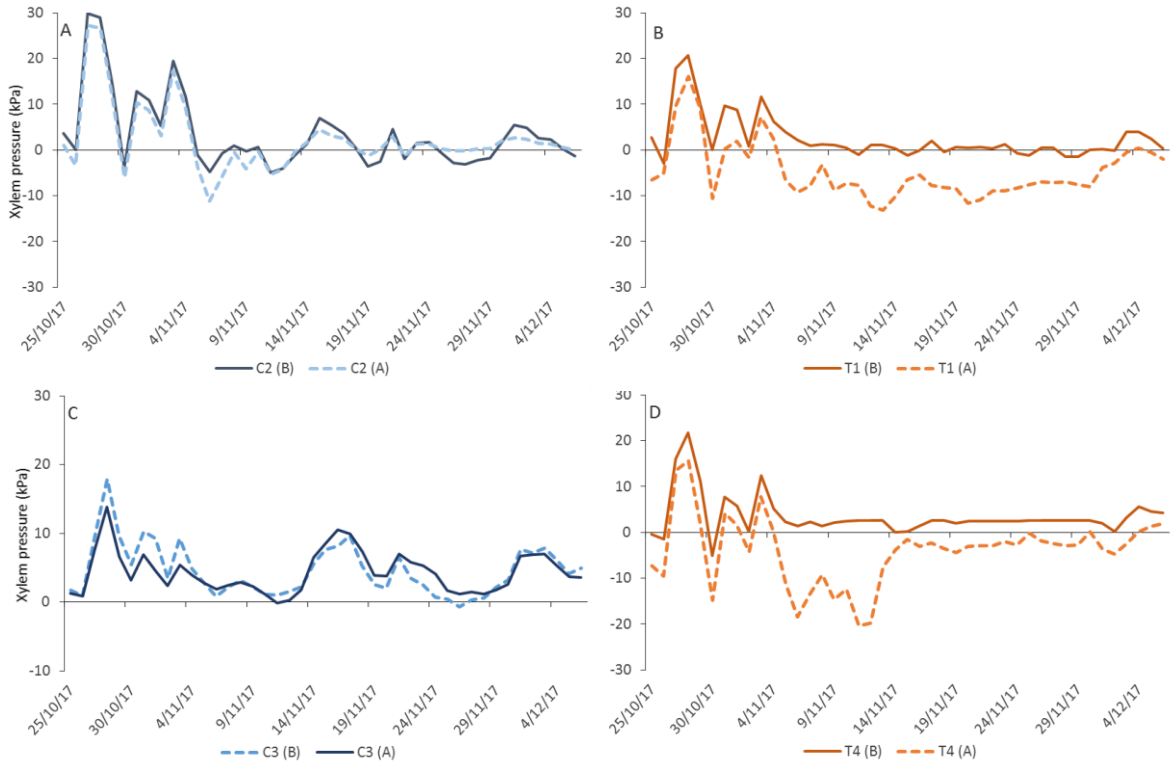


Figure 2.14: Xylem pressure (kPa) is shown from girdle application to six weeks post-girdle in two non-girdled (control; C) vines, A and C; and two girdled (treatment; T) vines, B and D. Readings from both xylem pressure transducers, above girdle (dotted lines) and below girdle (solid) lines are shown. Results are averages over 24 hours of the pressure recorded at 2 minute intervals; n=5 vines for each treatment.

2.3.2 Phenological development

Budbreak occurred over a period of 4 weeks starting from 25/09/17 and finishing on 23/10/17. The percentage of final budbreak between vines selected for the C and T groups was not significantly different (ANOVA; p=0.0501), 29.3% of buds on C vines burst and 30.8 % of buds on the T vines burst. Midpoint of budbreak was the 2/10/17 with the predicted date of 30 DBF (days before flowering) being 25/10/18, this was one day different from the estimated date of 30 DBF that was used as the day of girdle application.

There were no significant differences in shoot development, LAI (leaf area index), terminal flower numbers, or fruit volume (12/1/18 – 13/4/18), between the C

and T groups (ANOVA; $p>0.05$). Four days after the girdle was applied (30/10/17), 50 % of the C vine shoots and 60% of T vine shoots were beginning to extend in length and had young leaves expanding. Three weeks post girdle there was no difference in shoot development between C and T vines, both had 95% of the shoots continuing to lengthen and mature leaves present.

2.3.3 Xylem sap composition

Significant changes in xylem sap CHO were seen six weeks post girdle (8/12/17). Sucrose concentrations increased significantly in the T vines (ANOVA; $p<0.05$), (Figure 2.15). Glucose, undetectable in earlier samples was identified for the first time. Glucose and fructose concentrations were higher in the T vines compared to the C vines (ANOVA; $p=0.02$), (Figures 2.17; Figure 2.18). However, fructose concentrations continued to decline in C vines (ANOVA; $p=0.15$), (Figure 2.18). There was a significant difference between above and below girdle sites in fructose and total sugar concentrations in both C and T vines, (ANOVA; $p<0.05$).

Before budbreak, (19/09/17) there were negligible soluble CHO detected in the xylem (<2 μg/ml of xylem sap). After budbreak (26/10/17) sugars were detected in xylem samples from all vines, but there were no differences between total soluble CHO concentrations or individual sugars between treatments or sample locations (ANOVA; $p>0.05$).

A trend in all vines was the higher concentrations of all xylem sap sugars analysed above girdle site, in comparison to the below girdle site (Figure 2.15-2.18). As xylem pressure was low on 14/11/17, no sap was able to be collected from the above girdle collection sites from the treatment vines on that date.

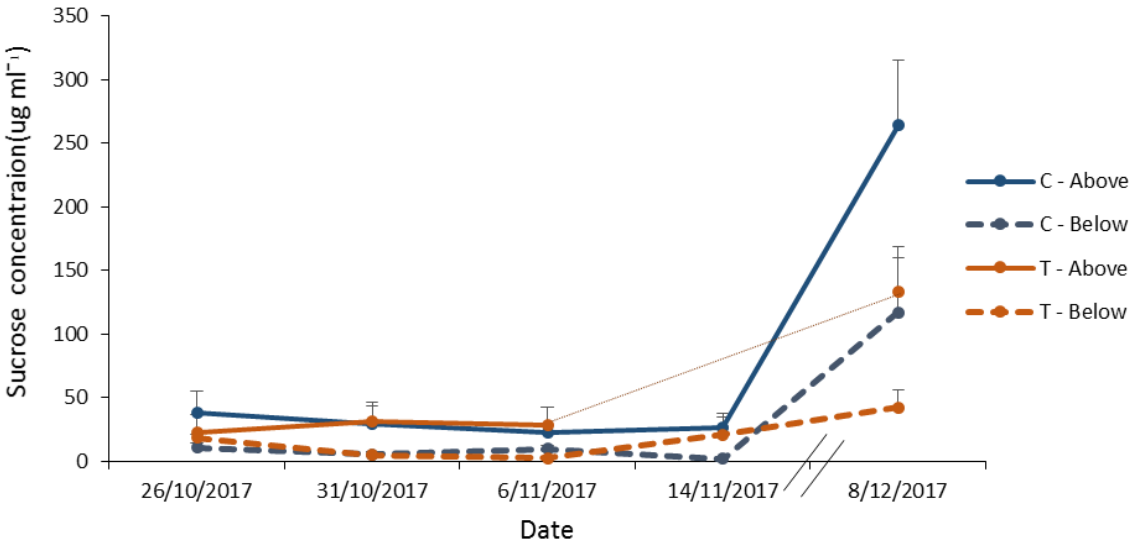


Figure 2.15: Mean xylem sap sucrose concentrations of non-girdled (control; C) and girdled (treatment; T) vines. Error bars show the SE of the mean; n=4-5 vines for each treatment. The x-axis is non-linear.

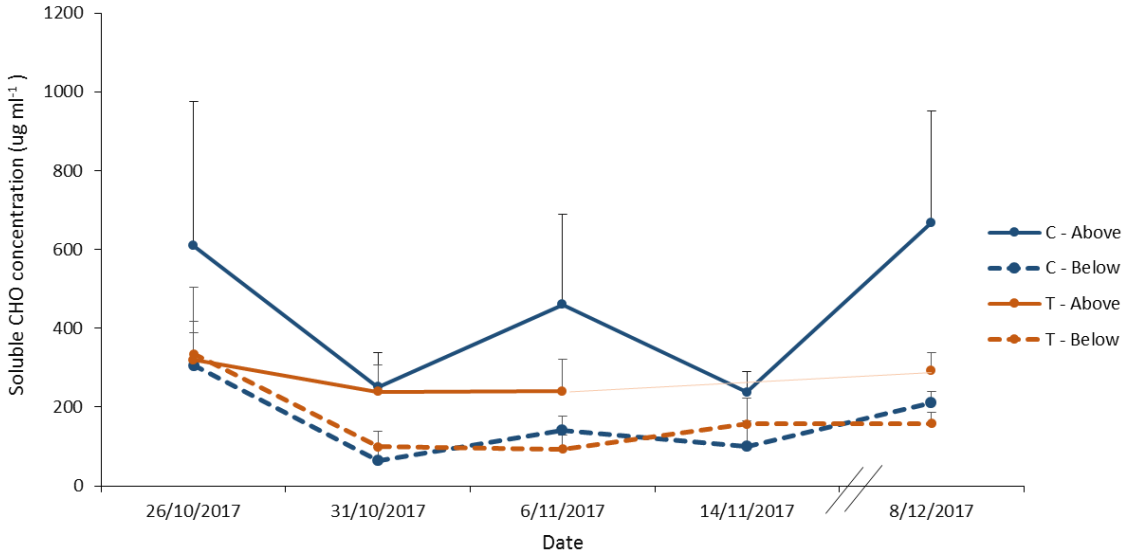


Figure 2.16: Mean xylem sap soluble carbohydrate (CHO) of non-girdled (control; C) and girdled (treatment; T) vines. Error bars show the SE of the mean; n=4-5 vines for each treatment. The x-axis is non-linear.

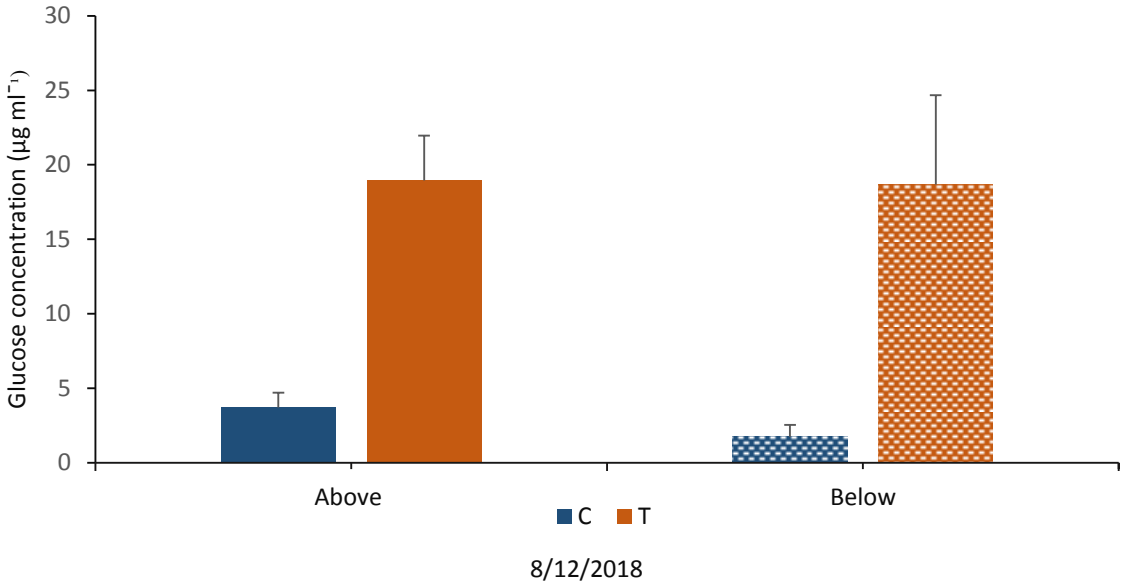


Figure 2.17: Mean xylem sap glucose concentration of non-girdled (control; C) and girdled (treatment; T) vines sampled on 8/12/2017. Error bars show the SE of the mean; n=4-5 vines for each treatment.

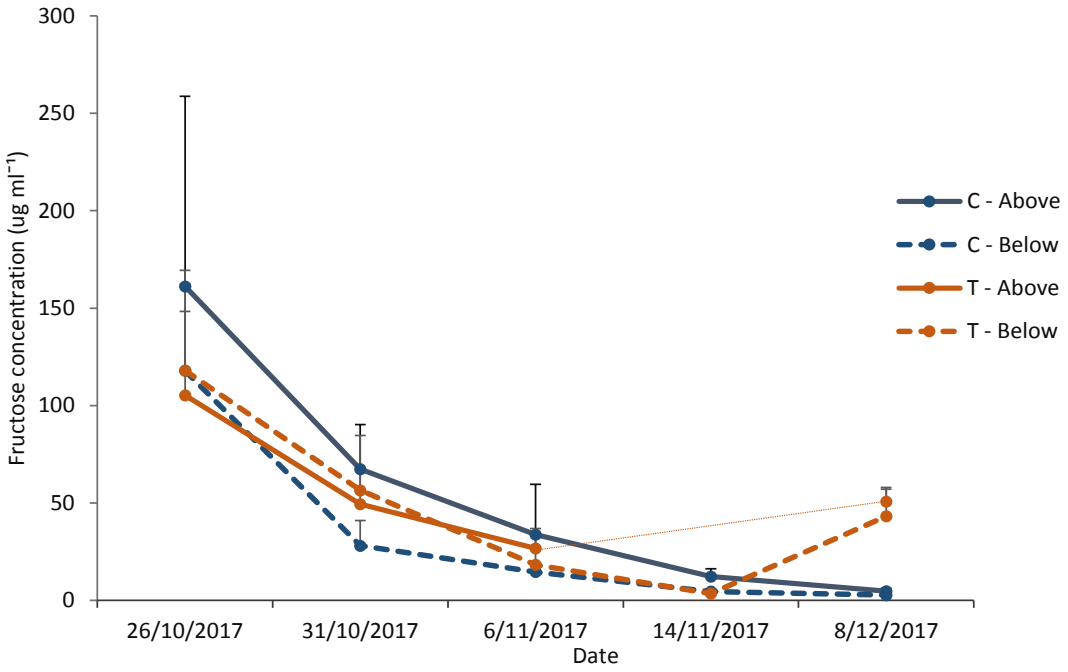


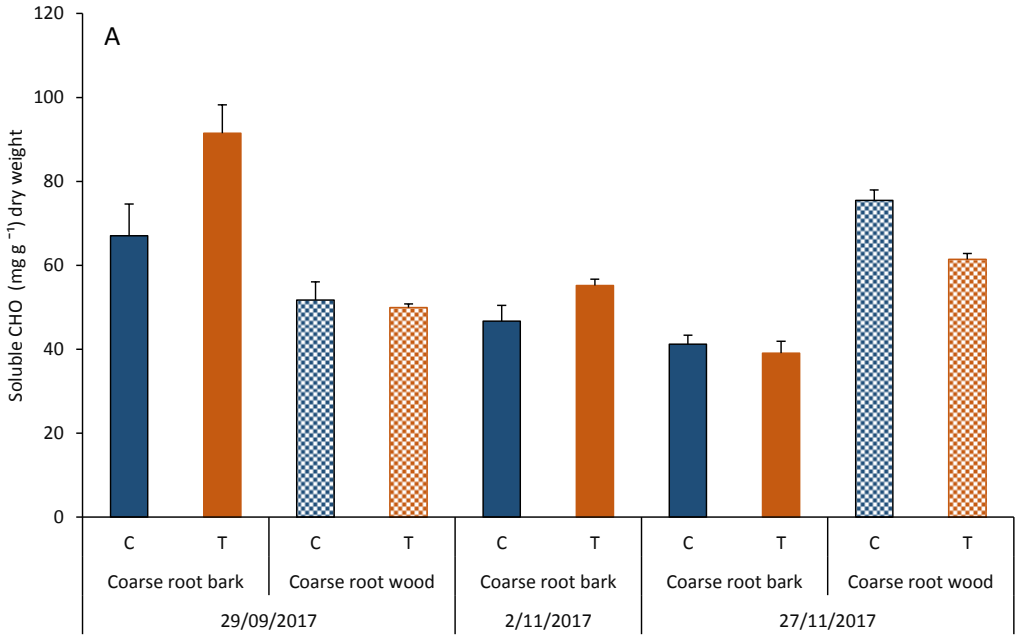
Figure 2.18: Mean xylem sap fructose concentrations of non-girdled (control; C) and girdled (treatment; T) vines. Error bars show the SE of the mean; n=4-5 vines for each treatment. The x-axis is non-linear.

2.3.4 Tissue carbohydrates

Roots

There were no treatment effects in the concentration of total soluble CHO between the T or C vines in coarse root wood measured in either September or November (ANOVA; $p>0.05$; Figure 2.19 A). However, there was an effect of date of sampling on total soluble CHO concentrations. Coarse root wood CHO concentration significantly increased in samples collected 27th November compared to those collected on 29th September (ANOVA; $p<0.01$). Sucrose was the dominant sugar while hexoses were negligible (Figure 2.19 A; Figure 2.20 A). The changes in concentration of sucrose between dates followed the same pattern as the changes in total soluble CHO concentration (Figure 2.19 B). The increase of sucrose in coarse roots was significant in C and T vines (ANOVA; $p<0.001$). Starch concentrations also increased, however there were no significant differences between treatments or date of sampling (ANOVA; $p>0.05$; Figure 2.20 B).

There were no differences in the concentration of total soluble CHO between the T or C vines in coarse root bark measured in either September or November (ANOVA; $p>0.05$; Figure 2.19 A). Coarse root bark declined in total soluble CHO between September and November, (ANOVA; $p=0.01$; Figure 2.19 A). Sucrose was the dominant sugar while hexoses were negligible. The changes in concentration of sucrose with date followed the same pattern as the change in total soluble CHO concentration (ANOVA; $p<0.005$; Figure 2.19 B). There was also a large decline in coarse root bark hexose concentration from the first to last sampling date, significant in both T and C (ANOVA $p<0.0005$; Figure 2.20 A), although hexoses were a minor component of the total soluble CHO pool. In contrast to soluble CHO, starch concentrations increased in the coarse root bark from the first to last sampling date (ANOVA; $p=0.01$; Figure 2.20 B). There was no significant effect of treatment on starch concentration (ANOVA; $p>0.05$).



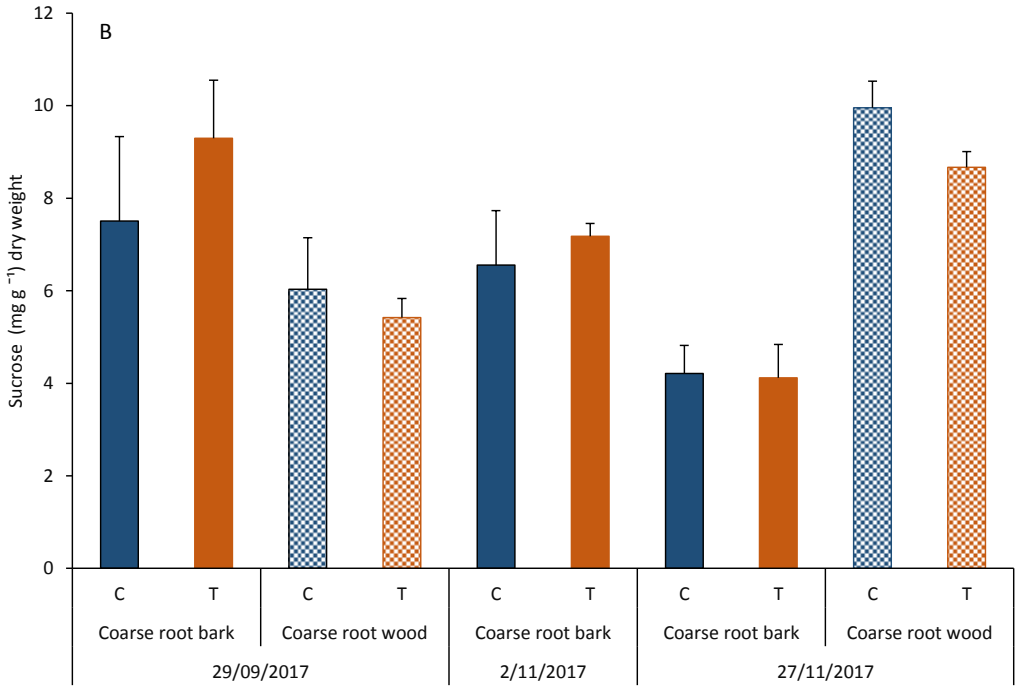


Figure 2.19: Mean concentrations of total soluble carbohydrates (A) and sucrose (B) in the coarse root wood and coarse root bark (CR bark) of the non-girdled (control; C) vines and the girdled (treatment; T) vines. LSD in coarse roots is 3.52 total soluble carbohydrates and 0.69 sucrose. LSD in CR bark is total soluble carbohydrates, 6.11; sucrose 3.21. Error bars show the SE of the mean; n=5 vines for each treatment and sample date.

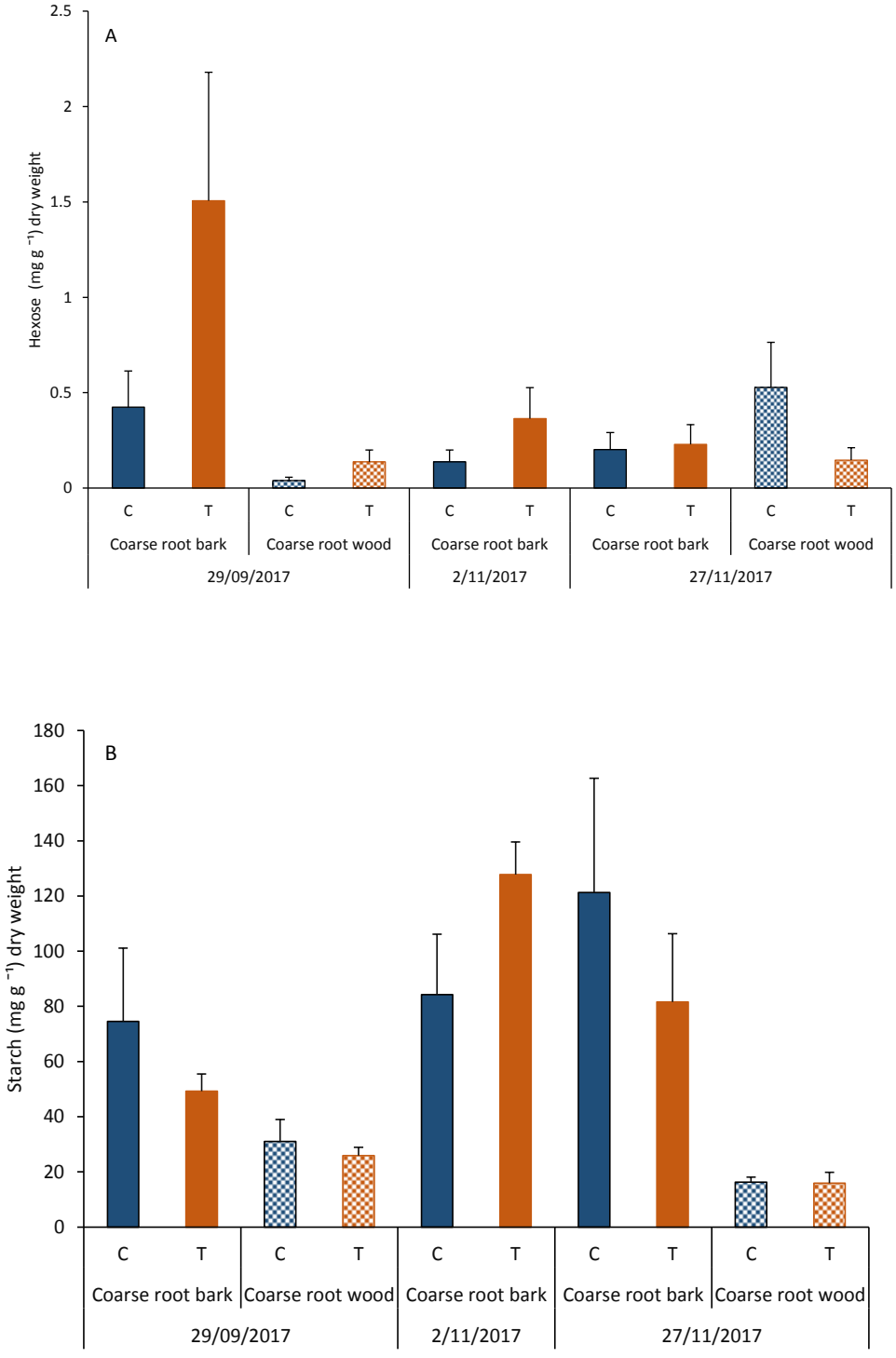


Figure 2.20: Mean concentrations hexose sugars (A) and starch (B) in the coarse root wood and coarse root bark (CR bark) of the non-girdled (control; C) vines and the girdled (treatment; T) vines. LSD in coarse roots is 0.46 hexose and 3.6 starch. LSD in CR bark is 0.87 hexose and 14.7 starch. Error bars show the SE of the mean; n=5 vines for each treatment and sample date.

The fine roots showed no significant difference in the concentration of total soluble CHO between the T or C vines (ANOVA; $p>0.05$; Figure 2.21). However, there was an effect of date of sampling on total soluble sugar concentration. The fine root soluble CHO concentration reduced in samples collected on 2nd November compared to those collected on 29th September (ANOVA; $p<0.05$). As found in coarse roots, sucrose was the dominant sugar and hexoses were negligible (data not shown). The changes in concentration of sucrose with date followed the same pattern as the changes in total soluble CHO, (Figure 2.22 A). The decrease in sucrose in fine roots with date was significant in all vines (ANOVA; $p<0.01$). Starch concentrations also decreased, however there was no significant effect of treatment or date of sampling (ANOVA; $p>0.05$; Figure 2.22 B).

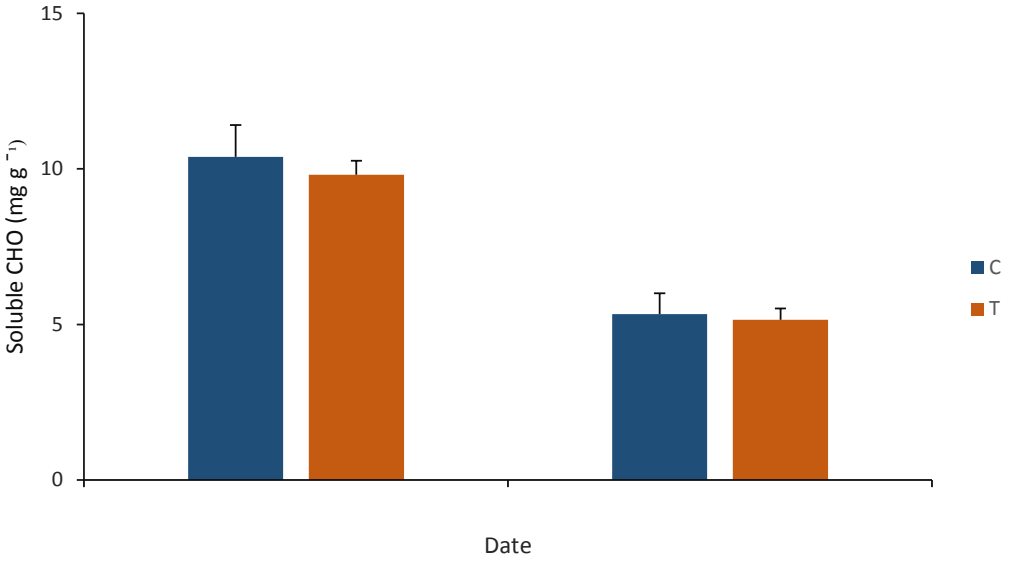


Figure 2.21: Concentrations of total soluble carbohydrates in the fine roots of the non-girdled (control; C) vines and the girdled (treatment; T) vines. LSD is 0.97 total soluble sugars, and 2.03 sucrose. Error bars show the SE of the mean, n=5 vines for each treatment and sample date.

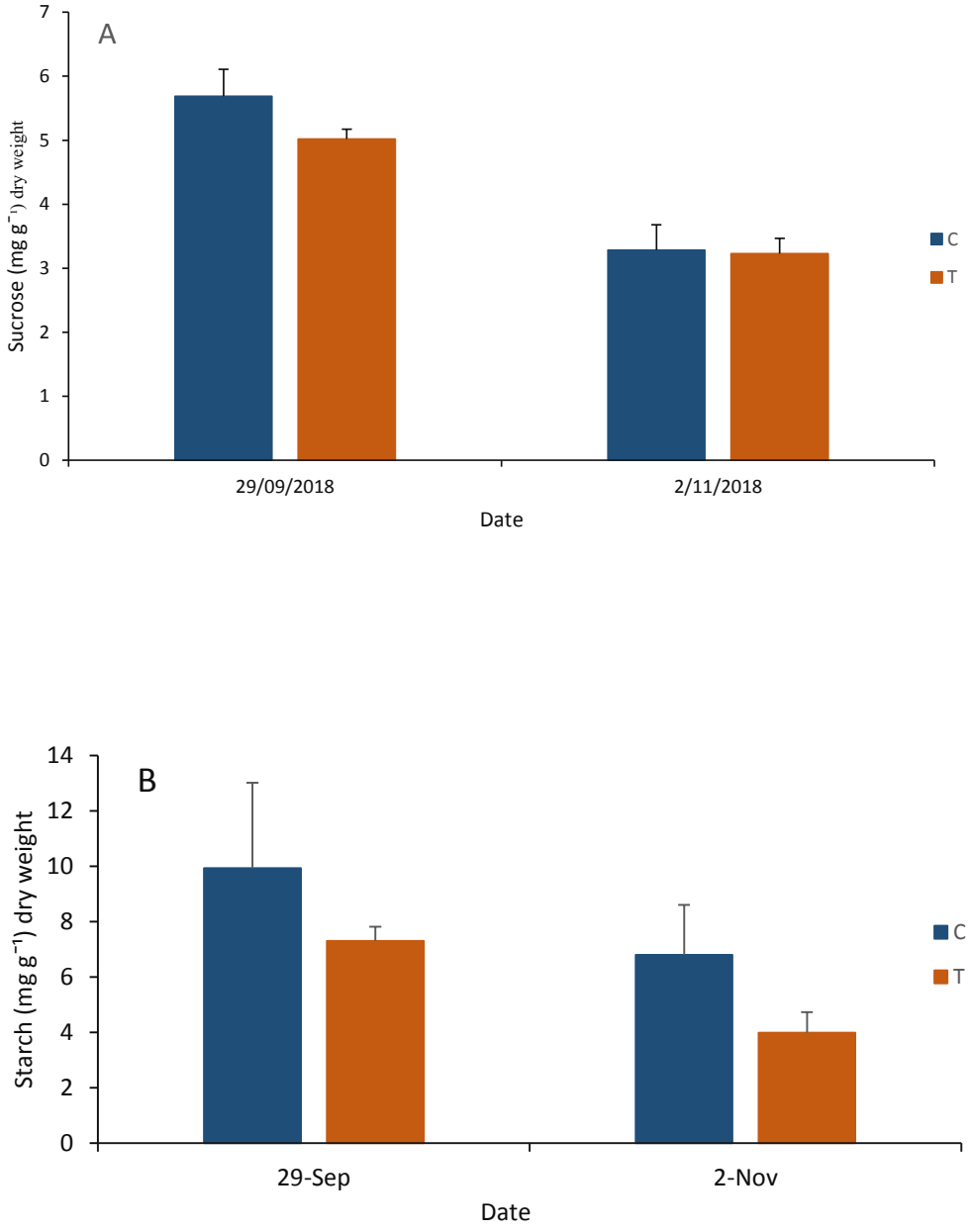


Figure 2.22: Concentrations of sucrose (A) and starch (B) in the fine roots of the non-girdled (control; C) vines and the girdled (treatment; T) vines. LSD is 2.03 for hexose, and 5.16 starch. Error bars show the SE of the mean, n=5 vines for each treatment and sample date.

Trunk bark

There was no significant difference between treatments in total soluble CHO, sucrose or hexose sugars in trunk bark sampled in September before girdles were applied (Figure 2.23 A, 2.23 B, 2.24 A). All soluble CHO in trunk bark samples were at similar concentration levels. There were no significant differences between C and T vines, or between (A), above girdle and (B), below girdle sites (ANOVA; $p>0.05$). Sucrose was the dominant sugar. On the second sampling date, one week post girdle, sucrose was higher in concentration in the (A) site compared to the (B) site, (ANOVA; $p=0.002$; Figure 2.23 B). A month after the girdle was applied on 24/10/2017, the (B) girdle samples increased in sucrose concentration while the (A) girdle sites remained stable, 27/11/2017 (Figure 2.23 A). In T vines this was a significant increase in bark sucrose with date of sampling (ANOVA; $p<0.001$), the difference between the T and C (A) girdle site was also statistically significant (ANOVA; $p=0.004$). (Anova pairwise comparisons)

There were no treatment effects on trunk bark total sugars, hexose sugars, starch or dry matter (Figure 2.23 A, 2.24 A, 2.24 B). However there were location differences between (A) and (B) sampling sites The (B) girdle sample sites in both C and T vines on 1/11/17 had lower concentrations of hexose than samples taken from the (A) sites (ANOVA; $p<0.001$; Figure 2.24 A). Starch was the opposite of hexose with concentrations from the (B) girdle site consistently higher than starch from (A) girdle sites in both C and T vines (ANOVA; $p<0.05$; Figure 2.24 B). All vines decreased in starch a month after the girdle, 27/11/17 from the (B) girdle sites, this decrease was only significant in the C vines (ANOVA $p=0.008$). There was no effect on bark dry matter between treatments or (A) and (B) location (ANOVA; $p>0.05$).

Dry matter decreased between 29/9/17 and 27/11/17 from 60% to just under 40% (ANOVA; $p<0.001$) in all vines (data not shown).

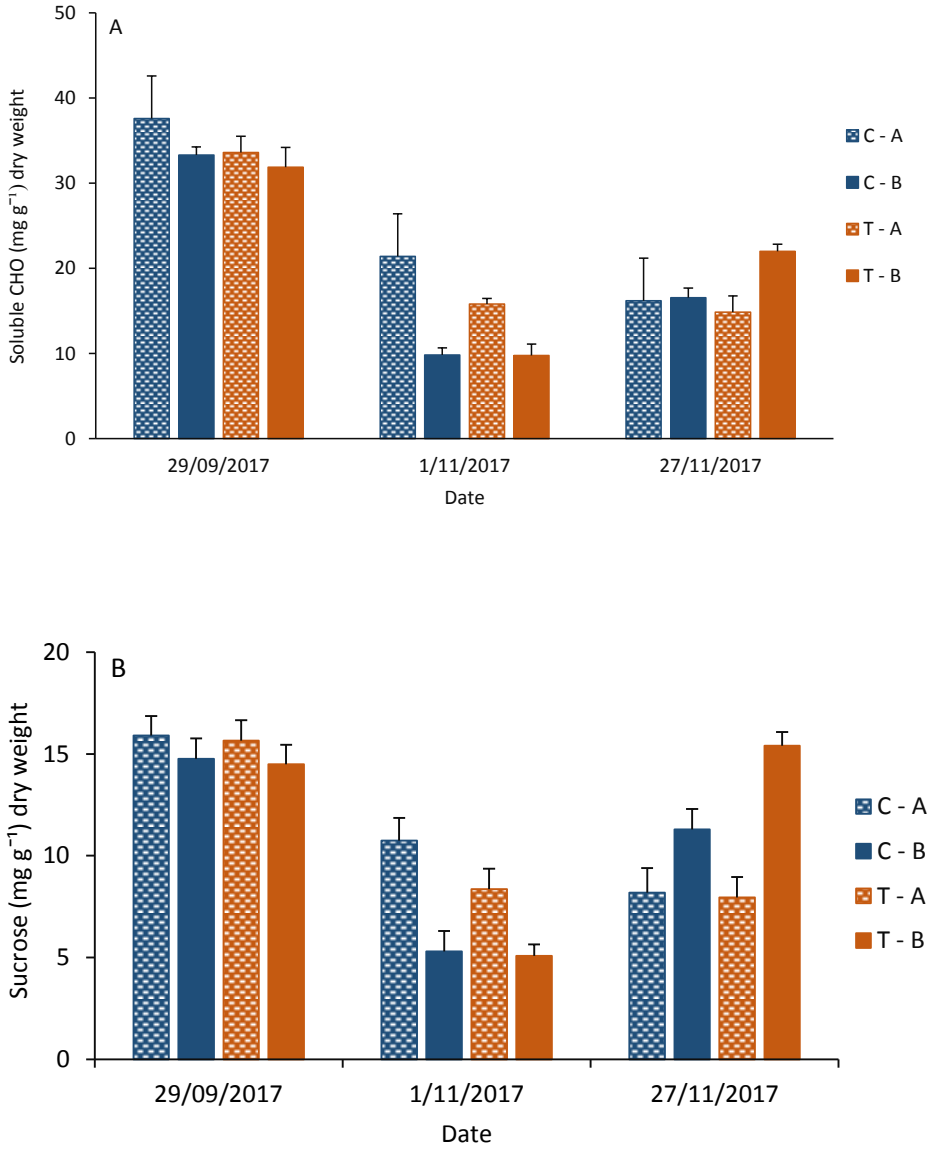


Figure 2.23: Mean concentrations of total soluble carbohydrates (A) and sucrose (B) in the trunk bark of the non-girdled (control; C) vines and girdled (treatment; T) vines. Solid coloured bars are 'below' girdle sample means from girdled vines and a comparative position on non-girdled vines; patterned bars are 'above' girdle sample means from girdled vines and a comparative position on non-girdled vines. LSD is 4.81 total soluble carbohydrates, and 2.3 sucrose. Error bars show the SE of the mean; n=5 vines for each treatment and sample date.

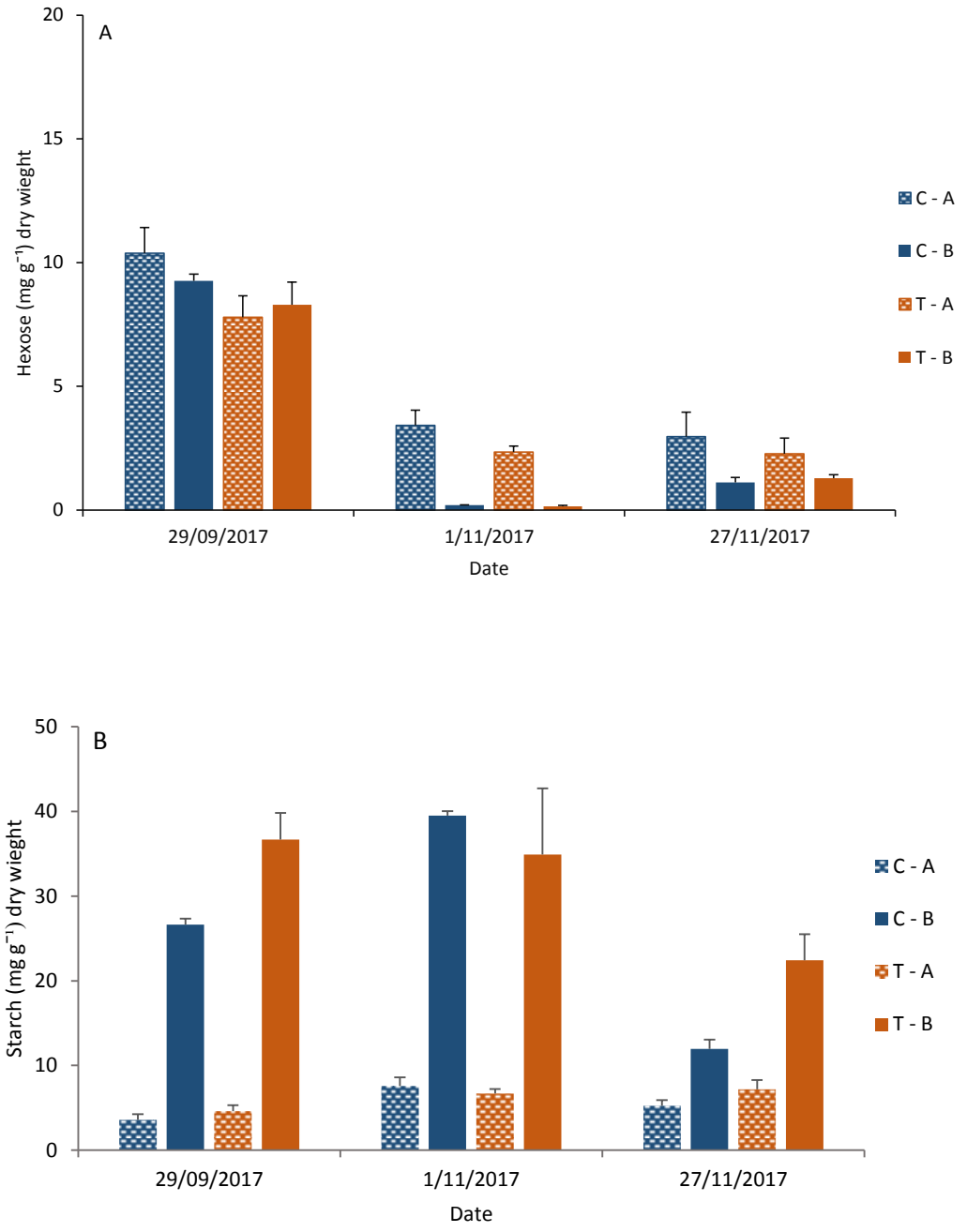


Figure 2.24: Mean concentrations of hexose (A) and starch (B) in the trunk bark of the non-girdled (control; C) vines and girdled (treatment; T) vines. Solid coloured bars are 'below' girdle sample means from girdled vines and a comparative position on non-girdled vines; patterned bars are 'above' girdle sample means from girdled vines and a comparative position on non-girdled vines. LSD is 1.75 hexose, and 12 starch. Error bars show the SE of the mean; n=5 vines for each treatment and sample date.

Wood

The main effect of the girdle on CHO in the wood was an increase in the concentration of hexose sugars in samples from above girdle sites compared to samples from below girdle sites, significant in T vines (ANOVA; $p<0.01$), but not in C vines (ANOVA; $p>0.05$; Figure 2.26 A). Starch shows a sudden increase in the below girdle samples in T vines on 1/11/17, (ANOVA; $p=0.04$). This returned to levels similar to the other samples at the end of November (ANOVA; $p>0.05$; Figure 2.26 B).

Hexose was the only CHO that changed in concentration in the stem wood over time, decreasing significantly between pre-budbreak and leaf maturity in all vines (ANOVA; $p<0.05$; Figure 2.26 B). Dry matter increased in all vines from pre-budbreak to leaf maturity (1/11/18), ANOVA; $p<0.001$, dry matter then remained consistent to the last sample collected on the 27/11/17 (data not shown).

Prior to girdling, all vines had similar concentrations of all soluble CHO in the wood samples, there was no obvious location effect (ANOVA; $p>0.05$; Figure 2.25 and 2.26 A). Concentration of total soluble CHO and sucrose concentrations showed no obvious trends or treatment effects over the sampling period ($p>0.05$).

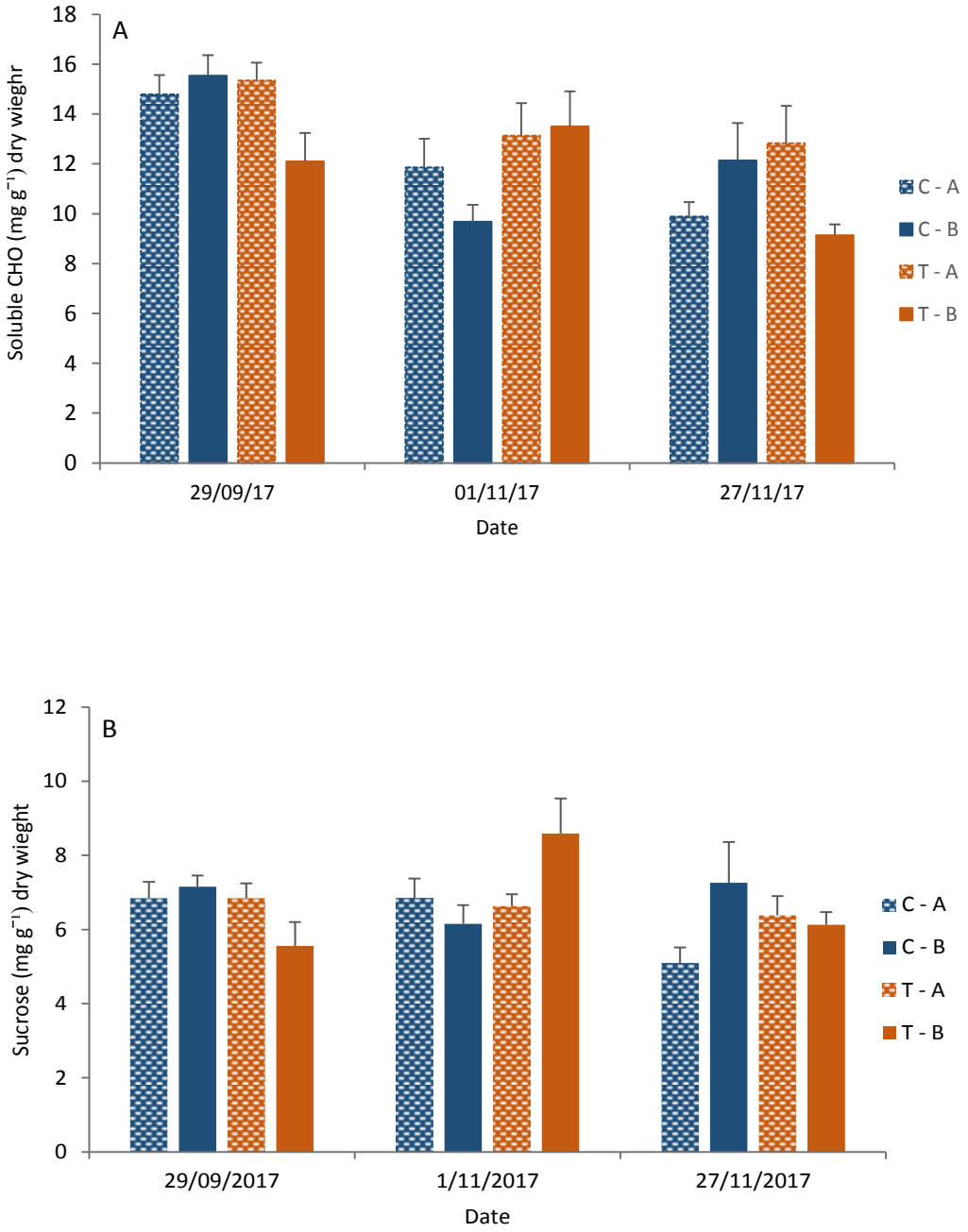


Figure 2.25: Mean concentrations of total soluble carbohydrates (A) and sucrose (B) in the wood of the non-girdled (control; C) vines and the girdled (treatment; T) vines. Solid coloured bars are 'below' girdle sample means from girdled vines and a comparative position on non-girdled vines; patterned bars are 'above' girdle sample means from girdled vines and a comparative position on non-girdled vines. LSD is 1.67 total sugars, and 3.02 sucrose. Error bars show the SE of the mean; n=5 vines for each treatment and sample date.

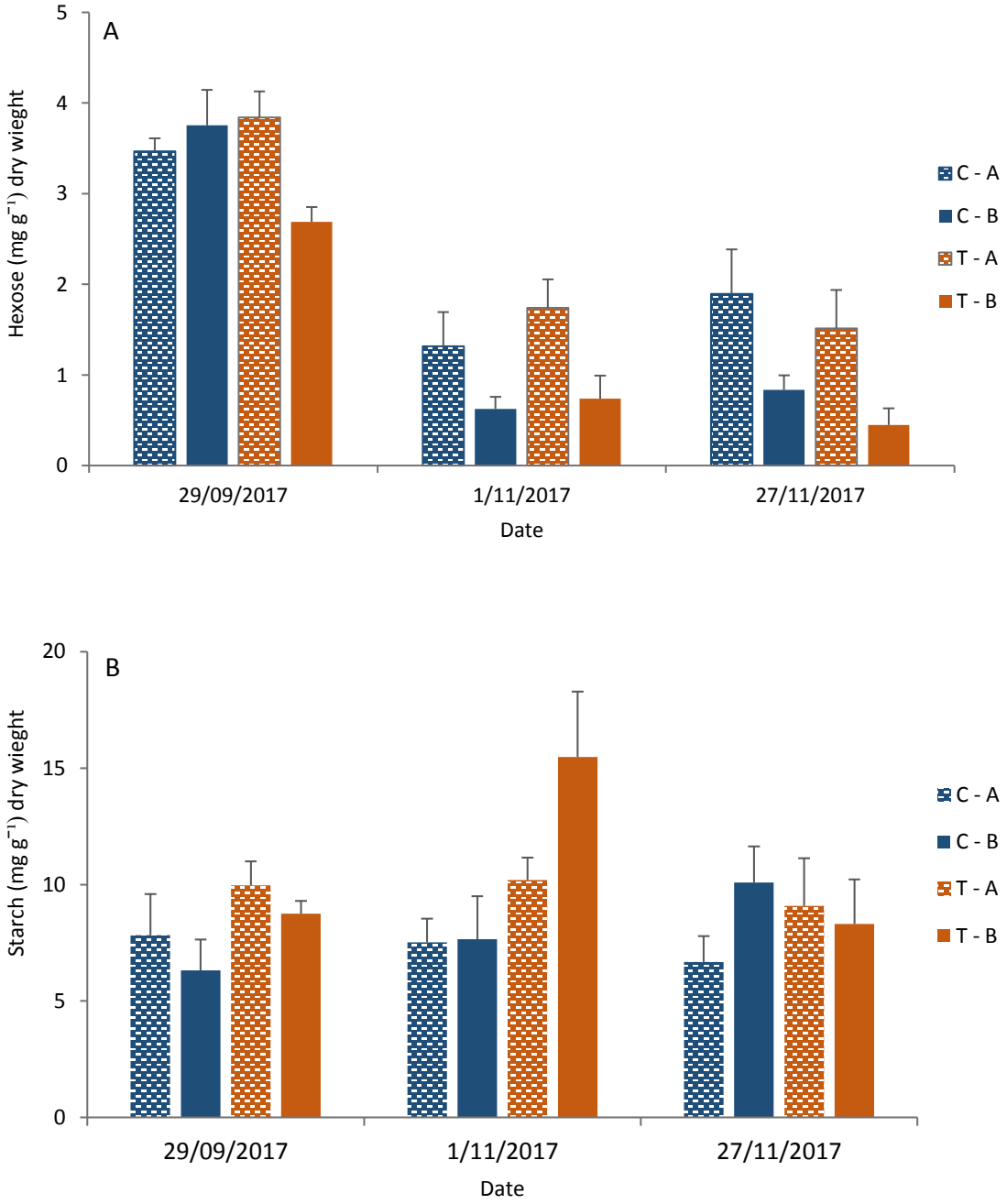


Figure 2.26: Mean concentrations of hexose (A) and starch (B) in the wood of the non-girdled (control; C) vines and the girdled (treatment; T) vines. Solid coloured bars are 'below' girdle sample means from girdled vines and a comparative position on non-girdled vines; patterned bars are 'above' girdle sample means from girdled vines and a comparative position on non-girdled vines. LSD is 0.73 hexose, and 4.57 starch. Error bars show the SE of the mean; n=5 vines for each treatment and sample date.

Leaves

The basal leaves had higher concentrations of all CHO analysed when compared with the apical leaves, for all sample dates and in both treatments. In both basal and apical leaves there were no significant differences in soluble CHO concentrations (total sugars, hexose, sucrose) between C and T vines ($p>0.05$) up to 15/11/17, (Figure 2.27 A-C). However, from samples collected a month post girdle a treatment effect was seen in total sugars. The basal leaves had higher concentrations in the T vines compared to C vines ($p<0.005$), this was also observed for apical leaves (excluding sucrose), although non-significant (ANOVA; $p=0.07$).

Starch in the basal leaves of C vines was consistently higher than in leaves from T vines, $p=0.008$ (Figure 2.22 D), the decrease in starch seen a month post girdle (27/11/17) was also significant (ANOVA; $p=0.008$). In the apical leaves starch was not significantly different over time of between treatments (ANOVA; $p>0.05$). Leaf dry matter was similar over the first two sampling dates (ANOVA $p>0.05$). By the 27/11/17, one month after the girdle was applied, the leaves from the T vines were higher in dry matter content than the equivalent leaves from C vines (ANOVA; apical, $p=0.014$; basal $p=0.018$; Figure 2.23).

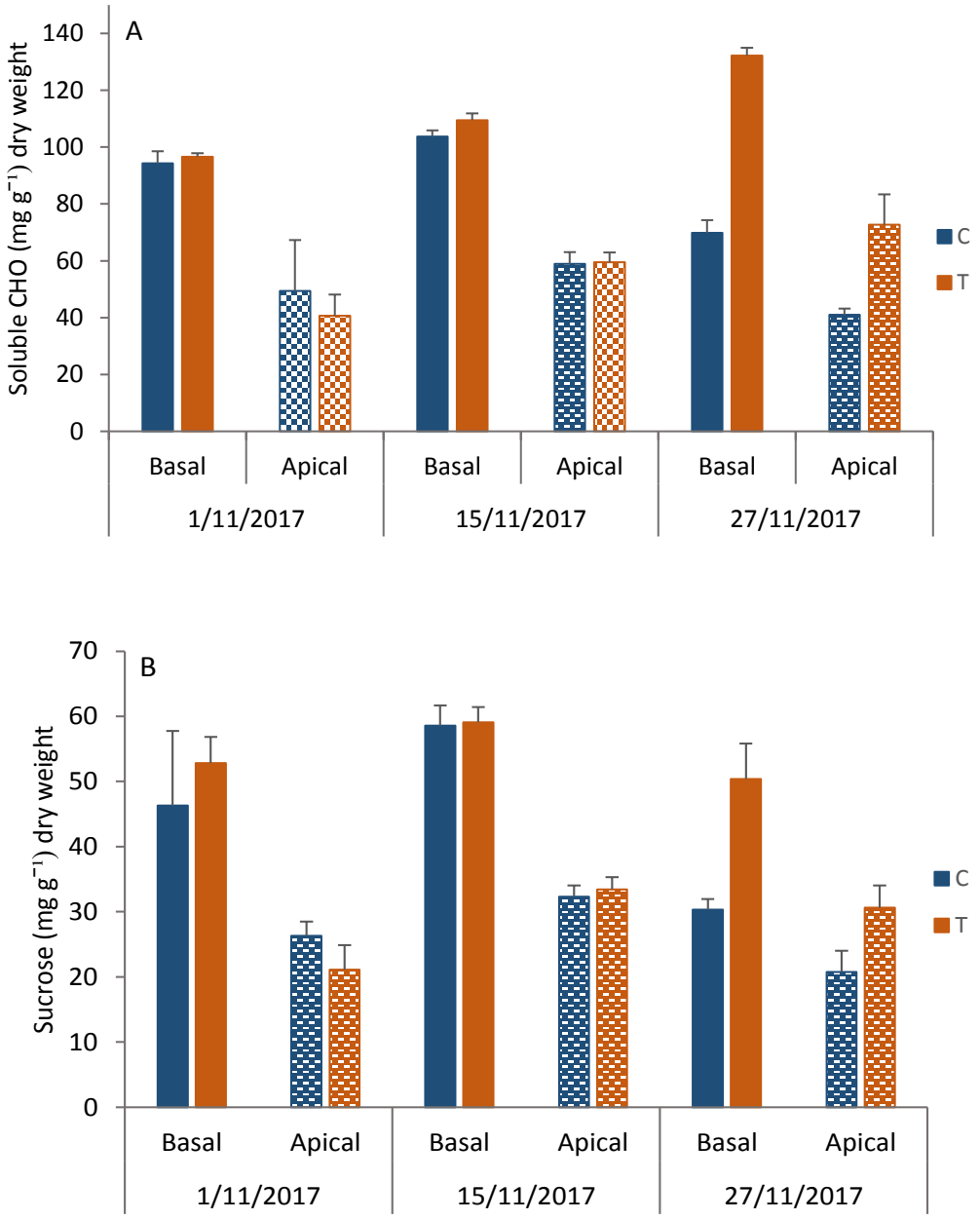


Figure 2.27: Mean concentration of (A) soluble carbohydrates, (B) sucrose in the basal and apical leaves of the non-girdled (control; C) vines and the girdled (treatment; T) vines. LSD for basal leaves is 2.42 (soluble carbohydrates) and 16.9 (sucrose), LSD for apical leaves is 17 (soluble carbohydrates) and 8.2 (sucrose). Error bars show the SE of the mean; n=5 vines for each treatment and sample date.

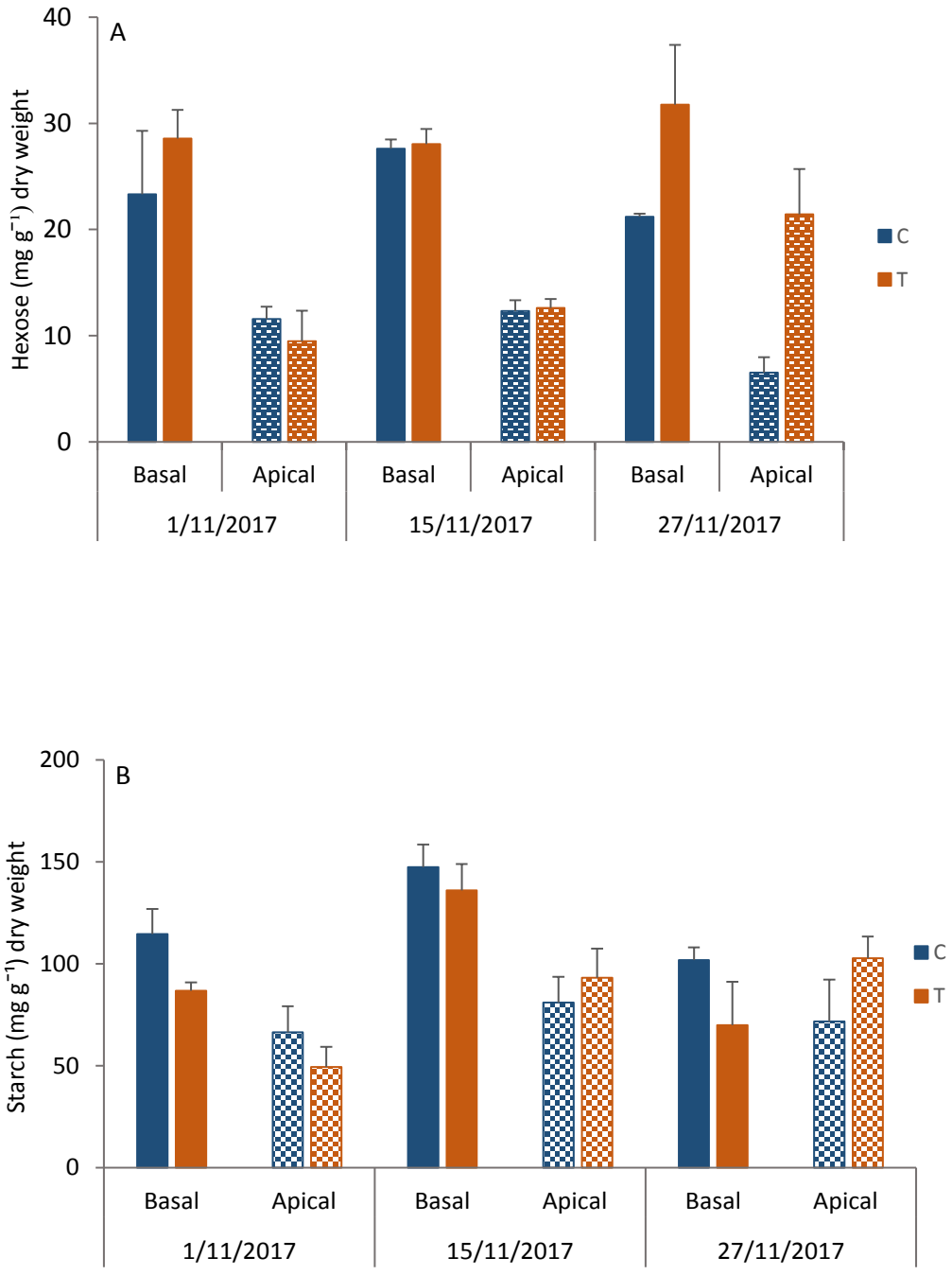


Figure 2.28: Mean concentration of hexose (A), and starch (B) in the basal and apical leaves of the non-girdled (control; C) vines and the girdled (treatment; T) vines. LSD for basal leaves is 1.7 (hexose) and 4.9 (starch). LSD for apical leaves is 17 (hexose) and 36.7 (starch). Error bars show the SE of the mean; n=5 vines for each treatment and sample date.

2.4 Discussion

2.4.1 Carbohydrate remobilisation as a driver of positive root pressure

The hypothesis that increasing mobile xylem sugars initiates spring root pressure is supported in this study. The timing of soluble carbohydrate (CHO) presence in the xylem sap and increases in root pressure suggests that remobilisation of sugars are involved in the generation of root pressure (Figure 2.10). Prior research on seasonal nutrient changes also confirm high concentrations of CHO in sap at this time (Ferguson *et al.*, 1983). In this study negligible sucrose was detected in the xylem sap prior to budbreak (Figure 2.15). As pressure increased in the xylem and as the canopy was starting to develop there was evidence of mobile sugars in the sap, in particular fructose (Figure 2.18) was present at a 6:1 ratio compared to sucrose (Figure 2.15). This supports past evidence for sucrose as a ubiquitous phloem transport sugar for metabolism in sink tissues (Liu *et al.*, 2012). There is evidence to show fructose, sucrose and glucose are commonly supplied to developing buds from the hydrolysis of starch (Maurel *et al.*, 2004; Tixier *et al.*, 2017a). Fructose is an important xylem-mobile sugar early in spring. It has twice the osmotic value of sucrose on a weight basis (Kameli & Losel, 1995), this enables fructose to create a lower water potential in the xylem, generating a higher hydrostatic pressure to efficiently refill embolised xylem vessels (Enns *et al.*, 2000). Theoretically this osmotic-sap-mechanism would cause root-pressure-driven xylem sap flow in a similar way to the Mass Flow mechanism that facilitates long distance phloem transport (Gould *et al.*, 2005).

There are two possible origins for the increase in xylem solutes. The first is from parenchyma cells within the xylem itself (Sperling *et al.*, 2017; Wegner, 2013), Accumulation of monosaccharide and disaccharide sugars is often found in the xylem sap during spring (Cortes & Sinclair, 1985; Loescher *et al.*, 1990). These

sugars can be loaded from the apoplast into the xylem during budbreak (Salleo *et al.*, 2009; Sperling *et al.*, 2017; Spicer, 2014). However, this is an avenue of research that could not be explored in the current study.

The second possible origin for the xylem solutes is the root reserves. The different locations of the xylem pressure transducers (above and below the girdle placement) did not develop pressure evenly. The lower transducers recorded pressure up to 48 hours before pressure was recorded in the pressure transducers higher in the trunk. This is consistent with the hypothesis that xylem vessels are loaded with CHO originating from root reserves and this process has a key role in driving out air embolisms. The decrease in starch and solutes in the fine roots in this study suggest they were necessary for the development of spring root pressure, alternatively the demand for CHO may have meant the fine roots were receiving less CHO than they were using for survival and growth.

Though the coarse root wood showed no decrease in starch or soluble CHO, it is possible the timing of the tissue sampling period may not have been early enough to capture the decline in coarse root wood starch, in some species this occurs before budbreak (Loescher *et al.*, 1990). However, as coarse roots are not directly involved in soil water up-take they are less likely to be responsible for the generation of spring xylem pressure than the fine roots. The CHO in the fine roots showed evidence of a gradual decline in soluble CHO (mainly sucrose) and starch (Boldingh *et al.*, 2000). The mobilisation of sucrose could have been creating the osmotic gradient necessary for water uptake in the xylem, refilling of xylem vessels and the generation of root pressure.

2.4.2 Effects of a trunk girdle on root pressure

As a consequence of the trunk girdle, leaf gas exchange, transpiration and CHO export would have been interrupted (Clearwater *et al.*, 2007; Richardson *et al.*, 2016). In this study the girdle had two observed effects on root pressure.

The immediate effect was an immediate but short-term reduction in root pressure. The recovery of pressure took three days. This is probably a consequence of xylem damage during the girdling process and has been reported in previous research (Richardson *et al.*, 2016). The second and more prolonged effect occurred twelve days after the girdle was applied. In the girdled vines the mean maximum positive xylem pressure remained low, at around zero kPa. However in the control vines when it rained after a dry period, xylem pressure increased. Root pressure in the girdled vines did not start to increase to levels similar to thoses found in the non-girdled vines until 34 days after the girdle was applied. The difference in positive pressure indicates a major difference in the role and functioning of the xylem at this time between these two treatments. This delayed and prolonged period of reduced root pressure post girdle observed here is consistant with previous observations in 'Hayward' vines (Richardson *et al.*, 2016).

The delayed response to girdling may be related to the time taken for root reserves to become depleted. Once a critical level is reached and there are no replenishing supplies from the canopy (because of the girdle), the ability to generate root pressure is affected (Clearwater *et al.*, 2007; Cortes & Sinclair, 1985). However, the present study did not show a treatment effect in root CHO concentrations that would indicate reserve depletion. It is possible that the timing of sample collection or the type of root tissue collected may not have captured the loss of reserve CHO (Loescher *et al.*, 1990). An alternative explanation is that root pressure is dependent on phloem flow rather than the reserve pool of carbon. Interruption of phloem transport would have interfered with the flux of carbon from the shoots, this may have initiated a signal from the shoots that causes a delayed decline in root pressure (De Schepper *et al.*, 2013). For example, it is known that sucrose has a role as a signalling molecule that could impact on the physiology of the vine (Turgeon & Wolf, 2009; Wind *et al.*, 2010); abscisic acid could be involved through signalling a stomatal response or some other chemical regulation may have been compromised (Downton *et al.*, 1988; Setter *et al.*, 1980).

During the period of low pressure in the girdled vines, separation in pressure between the lower and upper xylem pressure transducer was observed. The upper pressure transducers recorded pressures well below zero while the lower transducers remained at around zero. A possible explanation is that cavitation was occurring in the xylem that caused air embolisms to form during the day when the canopy was transpiring (Sperry *et al.*, 1987; Sperry & Sullivan, 1992). The disruption to vascular function caused by the trunk girdle meant that the vine was unable to repair these embolisms (Sperry *et al.*, 1987; Sperry *et al.*, 1996). The timing of pressure recovery in the xylem coincides with the expected girdle recovery window for the treated vines (Boyd & Barnett, 2011). Partial or full girdle recovery would allow the phloem to resume pre girdle levels of translocation and re-establish xylem pressure and normal plant function.

2.4.3 Effects of a pre-flower girdle on phenological development

There were no differences in the time of budbreak in the two treatment groups before the girdle was applied in this study. The development of shoots, LAI and terminal flowers were not affected by the girdle (data not shown). Fruit volume was also unaffected up until the last measurement 13/04/18 (data not shown). However, in previous work phloem girdling post-flowering and in summer has increased fruit size and dry matter through increased allocation of carbohydrate to the fruit (Boyd & Barnett, 2011; Currie *et al.*, 2011a). Studies have also found increased canopy development (Snelgar *et al.*, 2012) but there is no data to date on the effects of a pre-flower girdle on vine physiology or fruit development.

2.4.4 Utilisation of carbohydrate after a pre-flower girdle

CHO concentrations in the root tissues did not show an effect of the pre-flower girdle between budbreak and flowering in this study. Even with the high demand

for CHO in spring the root CHO reserves were not depleted. The trend seen in root tissue CHO was one of increasing soluble CHO in coarse roots (10-15 mm), and a decrease in fine roots (<5 mm) between budbreak (29/09/17) and early November. The lack of detectable CHO response to the girdling could be related to the phenological stage of the vine (Loescher *et al.*, 1990). Over the period of the study the shoots would have been transitioning from being sinks to sources (Greer *et al.*, 2003). They would not be transporting large concentrations of photo-assimilates to storage tissues and hence phloem girdling did not have an obvious effect on CHO concentrations in the roots.

There was a large difference in bark starch concentrations between above and below trunk sampling sites from 01/11/17 to 27/11/17. This was not an effect of the girdle as it was present prior to girdling. This suggests starch in closer proximity to the sink demand (developing canopy) is mobilised before reserves further from the sink tissues. The decline of starch seen in the trunk bark shows utilisation of 'local' CHO for growth and respiration at a time of high competition between sinks (Buwalda & Hutton, 1988; Cieslak *et al.*, 2011). This use of local reserves has been seen in a previous experiment carried out on excised extension shoots in the 'Hayward' cultivar (Ferguson *et al.*, 1983). The work with 1 year old shoots found changes in nutrient concentrations occurred in proximity to the sink tissue much more readily than within older parts of the vine like the trunk (Ferguson *et al.*, 1983). This use of local reserves has also been seen in more recent research on spring growth in *Juglans regia* where it was found girdling depleted non-structural CHO in the vicinity of the apical bud and had negative impacts on bud growth, indicating the bud was utilizing local reserves (Tixier *et al.*, 2017a).

While there was no effect of the girdle on bark CHO concentrations there was an overall general reduction in total soluble CHO in the bark between the end of September and November. On 1/11/17 the 'above' girdle sample sites were higher in soluble CHO than the 'below' girdle sample sites. By the end of November sucrose concentrations increased in the 'below' sample sites. At the same time there was a decrease in starch from the 'below' sites, this was

significant in the girdled vines. The increase in sucrose, and reduction in starch, occurred in the middle of flowering. This is a time when demand for CHO is high by flowers and developing fruits (Loescher *et al.*, 1990). As the canopy was probably not yet fully autotrophic, presumably starch reserves were being mobilised from lower on the vines trunk into mobile sugars to supply resources for spring growth (Piller & Meekings, 1997).

There was no effect of the girdle, or change over time, seen in the concentrations of soluble CHO or starch in the trunk wood. As with the other vine tissues sucrose was the dominant sugar. The consistency over time of the CHO in the trunk wood suggest that stored CHO was not a significant source of reserve sugars for flowering and spring growth.

Leaf CHO concentrations in both basal and apical leaves showed no effect from the girdle until the last sample date at the end of November. At the end of November, four weeks post girdle, the control vine leaves declined in soluble CHO. The girdled vines retained similar CHO concentrations to previous samples taken at earlier dates. At this stage the canopy would be transitioning to an autotropic status, exporting carbon from photosynthesis (Piller *et al.*, 1998; Salinero *et al.*, 2009). The results suggest CHO are accumulating in the leaves of the girdled vines and less is being exported. At four weeks post girdle this could be due to the recovery of the girdle not yet supporting fully functioning phloem translocation of recently assimilated photosynthates (Snelgar *et al.*, 2016).

2.5 Conclusions

This main objective of this project was to determine the impacts of applying a pre-flower trunk girdle on carbon partitioning in kiwifruit vines *Actinidia chinensis* var. *deliciosa*, 'Hayward' as they move into an active spring growth stage after winter dormancy. It also aimed to gain understanding of the relationship between xylem hydrostatic pressure changes and the composition of carbohydrates in the xylem sap and the use of CHO reserves between developing plant organs.

The main effect of the pre-flower girdle on tissue CHO concentrations was seen in the leaves. As the canopy was becoming autotrophic the leaves from non-girdled vines exported soluble CHO while the leaves from girdled vines exported less CHO. This indicates that the pre-flower girdle was still affecting the phloem's ability to fully resume transport of photosynthates or other compounds. As the girdle was applied when the canopy was just starting to become autotrophic, the reduction in absolute amount of photo-assimilates exported may have been minimal, this would limit the detectable effect of the girdle on the CHO tissue concentrations in the shoots, stems or roots. In a study by Pillar *et al.* (1998), they found the timing of the girdle was important in determining the plants response. An early girdle (just after budbreak) had negative effects, including defoliation and lower fruit set and fruit size in response to the reduced carbon supply. However, a later, pre-flower girdle increased fruit set and fruit size (Piller *et al.*, 1998).

This trial presented new evidence in kiwifruit vines that spring reserves of CHO are first utilised from locations closest to the stronger sink tissues in preference to long distant transport from the roots. The observation of strong gradients in carbohydrate reserves within the trunk bark tissue (lower starch and higher soluble carbohydrates higher in the trunk) supports the conclusion that the shoots at this time of the year have not yet transitioned to being fully heterotrophic, and are still reliant on local reserves.

This study found that soluble CHO started to increase in the xylem sap at the same time that positive root pressure was developing, this supports the hypothesis that an increase in mobile xylem sugars initiates spring root pressure. Fructose was present in high concentrations and plays a key role in the development of xylem root pressure by increasing the osmolality of the sap. Significantly glucose did not play a role in xylem sap solute concentration early in spring. However, by December when the canopy was transitioning from a heterotrophic status to an autotrophic status, sucrose and glucose became the key sugars in the xylem sap. In contrast the role of fructose was reduced. Conversely, in the girdled vines the increase in sucrose concentration was not as pronounced as is was in the non-girdled vines, this appeared to be compensated for by increases in fructose and glucose.

The pre-flower girdle had a delayed affect on the vines ability to build positive pressure in the xylem. This was not due to reduction in tissue CHO concentrations or changes in flow of CHO between roots and shoots. It is more likely related to an unknown signalling mechanism impacting on root xylem loading and subsequent pressure (Turgeon & Wolf, 2009; Wind *et al.*, 2010). The girdle also had a location effect on xylem pressure. It was found that higher on the trunk the pressure became more negative. A possible explanation is that embolisms were occurring in the xylem through during the day when the canopy was transpiring, the disruption to vascular function caused by the trunk girdle meant that the vine was unable to repair these embolisms (Sperry *et al.*, 1996; Sperry & Sullivan, 1992).

3 Chapter Three

The effect of a phloem girdle on the transport of carbon from source leaves to sink tissues in *Actinidia chinensis* var. *deliciosa* 'Hayward'

3.1 Introduction

A trunk phloem girdle isolates the roots from the transport of photo-assimilates from the shoot, ensuring that carbon fixed by the leaves is partitioned to the fruit and growing shoots rather than the roots. It is important to understand how isolating the roots from the supply of recently fixed carbon effects the supply of photosynthate to the remaining shoot sink tissue. In kiwifruit orchards 'Hayward' vines can be trunk girdled up to four times a season. A late spring girdle is applied at the end of December to increase fruit size and two girdles are applied about four weeks apart in summer to increase dry matter partitioning to the fruit. A recent addition to the orchard management practises is a pre-flower girdle applied 30 days before flowering. This forth girdle is applied as it has shown success in reducing the loss of flower buds from rot (Richardson *et al.*, 2016), however very little is understood regarding the impact of this pre-flower girdle on carbon fixation and partitioning between sink tissues. The pre-flower girdle is applied during a period of active growth (both canopy and flower development), the demand for nutrients is high, but as leaves have yet to fully develop and become autotropic the plant must rely on the reserves of carbohydrates (CHO) within the roots and other storage tissues (Tromp, 1983).

The work presented in this chapter uses radiolabelled, recently fixed carbohydrate to trace the source leaf export rate and the partitioning of carbon

to sink tissues. Non-fruiting, young potted vines with a single developing shoot are used to study the diurnal movement of carbon around a vine (between competing sinks), as well as the impact of trunk girdling on the export of carbon from source leaves and the subsequent partitioning to developing shoot tissues.

3.1.1 Transport from sources to sinks

Carbon is fixed in the photosynthetically active tissues of the plant, usually mature leaves. CHO is then stored in the leaf as starch, used for respiration, or transported as soluble sugar in the phloem to sinks such as developing shoots, leaves and roots (De Schepper *et al.*, 2010). Concentration differences in the phloem are created by supply and demand for CHO (carbohydrates) in various tissues, this creates a pressure difference between regions of the plant, in turn resulting in phloem flow and unloading at sink tissues where the demand is highest for CHO (De Schepper *et al.*, 2013). A pre-flower girdle temporarily removes the roots as a potential sink for fixed carbon, but it also has the potential to alter the transport of reserve CHO from storage tissues below the girdle (Black *et al.*, 2012a; Boyd & Barnett, 2011; Currie *et al.*, 2005).

3.1.2 Using radiolabelled tracers for *in vivo* monitoring of carbon transport in plants.

Earlier research into carbon transport using radioactive isotope labels has used ^{11}C , this isotope has a short half-life of 20 minutes, repeat labels are needed to trace the decay of ^{11}C by counting the gamma rays that are emitted (Minchin & Thorpe, 1987). The method was used successfully with lupins (Minchin & McNaughton, 1987), and beans (Minchin & Thorpe, 1987). The method is expensive as it requires a plant laboratory with close access to a cyclotron (Babst *et al.*, 2013). However, an alternative system has been developed using ^{14}C to trace carbohydrate flows utilising the detection of Bremsstrahlung emissions.

The application has been limited to maize seedlings (Sowinski *et al.*, 1990) and more recently on kiwifruit (Black *et al.*, 2012a; Boldingh *et al.*, 2015). The technique allows *in vivo* measurement of a labelled tracer and has the ability to monitor carbon transport before and after a treatment in an individual plant.

The Bremmstrahlung technique measures the decay of a radioactive isotope like ^{14}C which has a long half-life of 5500 years. The ^{14}C is loaded into a mature photosynthesizing leaf and X-ray detectors placed on sink tissues measure the amount of radioactive label visible to the detector at any one time. The technique was used successfully with *Actinidia arguta* in 2012 (Black *et al.*, 2012a). Here it was used to trace carbon transport in the phloem in response to root pruning, increased counts of the ^{14}C label were detected in the fruit suggesting the fruit was a strong sink tissue. Diurnal trends of carbon transport have also been reported using this technique with less export occurring at night (Black *et al.*, 2012b). In another study on *Actinidia arguta* the ^{14}C label was traced from the source leaf to the roots, it was then remobilised from the roots to the shoot apical meristem at night. This could show an export of carbon was occurring from the roots via the xylem at night when no photosynthesis was occuring (Boldingh *et al.*, 2015).

3.1.3 Aim

The alteration of the source-sink ratio with a trunk phloem girdle will remove any CHO import and export, to or from the root system via the phloem. With a pre-flower girdle it is unknown how the girdle will alter the dynamics of carbon supply to sinks at a time when respiration and growth puts CHO in high demand. The aim of this chapter is to use the Bremsstrahlung technique to understand the movement of ^{14}C labelled photo-assimilate as it is partitioned throughout the plant. It will investigate if carbon is cycled between shoots and roots of young Hayward vines. If cycling of carbon does occur what will the effect be of a trunk girdle on this movement of carbon?

The hypothesis is that the trunk girdle will arrest the flow of carbon to the roots. When this occurs there should be an increased the proportion of carbon available for shoot growth. This will be confirmed if the labelled carbon, ^{14}C accumulates in the trunk above the girdle. If there is no trunk girdle, or the girdle has no effect, the ^{14}C will not accumulate above the girdle.

If cycling of carbon is occurring between the shoots and roots via the xylem, this will be seen as a second pulse moving through the stem before the second ^{14}C label is applied.

3.2 Methods

3.2.1 Experimental design

Graft wood was collected from mature 'Hayward' vines in block 4 at the Te Puke Orchard site of Plant & Food Research. The cuttings were approximately 20 cm in length and were selected from canes approximately 1 cm in diameter. These were grafted onto established Bruno rootstocks on the 29th of August 2017 and grown in potting mix. Early in January, the plants were removed from their pots, the majority of the potting mix was removed by rinsing in water, the roots were then submerged into a hydroponics nutrient solution in 5L buckets. The nutrient solution was aerated by air stones connected with rubber tubing to a 4-port aqua air pump. The pump was turned on with all attached air stones fully submerged, this ensured the air stones were all bubbling at a similar intensity in each bucket, (Figure 3.1 A). The plants were then left for a minimum of 2 weeks in the glasshouse to acclimatise to the new liquid growth medium.

The hydroponics solution was a modified half-strength Hoagland N_2 solution (Hoagland, 1950). Half strength was used to reduce iron chlorosis that can occur when growing plants in a hydroponic medium. The amount of $NH_4H_2PO_4$ was reduced and the $FeCl_3$ was replaced with Fe-EDTA (Loupassaki *et al.*, 1997). The solution was replaced weekly and the buckets cleaned of any algae.

The plants were prepared for ^{14}C labelling once they had acclimatised and suitable sink tissue (growing shoot and roots) and source tissue (mature leaves) were present, (Figure 3.1 B). Before being transferred into the laboratory they were pruned of excess foliage and then left for 24 hours in the laboratory to acclimatise. In the laboratory PAR levels of around 150–200 μmol m^{-2} s^{-1} were maintained between the hours of 06:00 to 21:30, coinciding with outdoor daylight hours. The temperature was maintained at a constant 20°C. A total of three plants were used as controls (non-girdled), and four were used as treatments (girdled).

Figure 3.1: (A) Grafted *A*. *chinensis* var*. delicosa* 'Hayward' vines growing in the glasshouse in an aerated hydroponic solution. (B) The growth of new white roots can be seen clearly after seven days in the hydroponic solution.

3.2.2 Radioactive labelling and monitoring

After the acclimatisation period to hydroponics in the glasshouse, over a period of six weeks each plant was prepared for ^{14}C labelling in the laboratory. Each 'Hayward' vine had four Bremsstrahlung scintillation detectors positioned to monitor ^{14}C tracer export from the source leaf and import into the roots and shoot meristem. The first X-ray detector was directly over a mass of fine roots. The roots were semi-contained at the water surface using mesh and plastic (Figure 3.2 A; Figure 3.3), this minimised movement of the roots and protected the detector from the water.

The second X-ray detector was positioned to monitor tracer import into the shoot meristem, (Figure 3.2 B; Figure 3.3). The source leaf that was to be labelled was enclosed in a plastic leaf chamber, an air hose was inserted and connected to a pump to circulate air around the leaf. A third detector was placed immediately above the point of the girdle, and was used to monitor the movement of ^{14}C down the stem towards the roots. A fourth detector was positioned under the source leaf to monitor the export of ^{14}C tracer from the leaf.

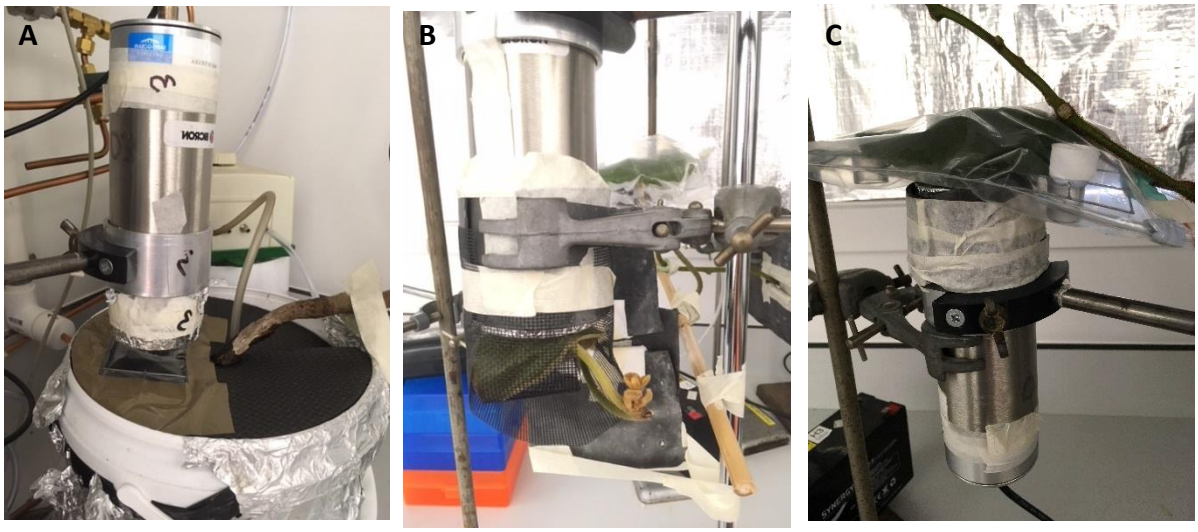


Figure 3.2: From the left, figure (A) shows the X-ray detector set up over the roots, the detector is lowered so it is just touching the plastic shield over the gap cut into the foam cover. In the centre is figure (B), a shoot enclosed in netting and held in place over the head of the X-ray detector using masking tape. To the right, figure (C) shows a source leaf enclosed in a sealed plastic chamber with an X-ray detector placed underneath the leaf.

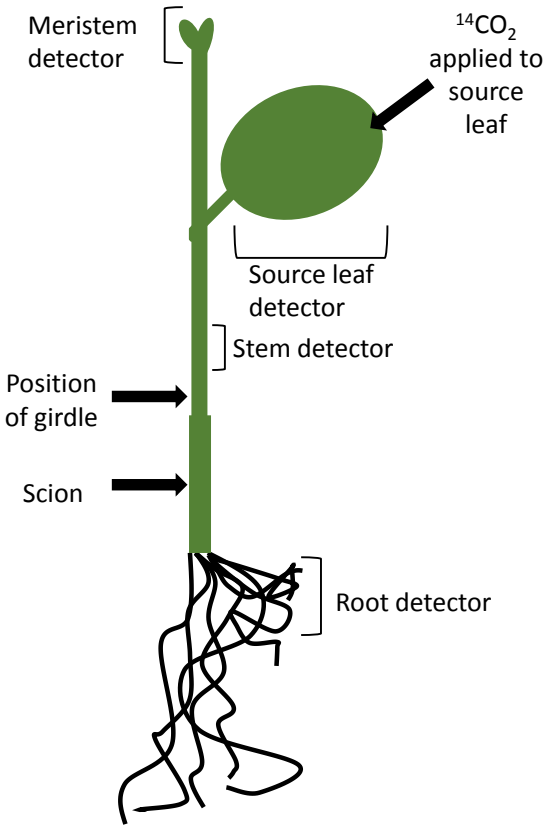


Figure 3.3: A schematic diagram to illustrate the position of the X-ray detectors relative to the vine tissues that were monitored.

Positioning the detector underneath the leaf ensured the leaf would receive maximum light from above without shading, (Figure 3.2 C; Figure 3.3). Lead sheets were positioned around the X-ray detectors to shield the detectors from radiation not coming from the tissue of interest. Once the plant preparation was complete the plant was left to stabilise for at least two hours before labelling with ^{14}C.

During labelling the pump circulating the air into the leaf chamber was turned off and the tubes to and from the chamber were clamped to ensure the leaf chamber was sealed and no ^{14}C labelled CO_2 could escape to the atmosphere. One mL of saturated citric acid solution was injected through the plastic of the leaf chamber and into a small plastic dish located in the leaf chamber. 0.12 mL (12MBq) of ^{14}C bicarbonate (1880 MBq $mmol^{-1}$) was added to the labelling dish, releasing $^{14}CO_2$ into the leaf chamber, before resealing the leaf chamber using Blu-Tack® (Bostik). After 30 minutes the air pump was turned back on and the tubes unclamped to allow any remaining $^{14}CO_2$ not taken up by the leaf to be flushed from the leaf chamber, (Figure 3.2 C). The first labelling pulse was applied between 9.30 and 10.00 am day 1 (load 1). 24 hours after load 1 a second 30-minute pulse label of ^{14}C was applied to the same source leaf in the same manner (load 2).

After the first labelling of tracer, the Bremsstrahlung radiation of X-rays produced via the decay of ^{14}C was monitored for 24 hours using the Bremsstrahlung scintillation detectors. Pulses from each of the separate detectors were counted and recorded as an average count every two minutes by Ludlum 260 data loggers. The counts per minute (CPM) is the amount of labelled carbon that is visible to the detector at one time. When the second load was applied to the source leaf the logger counts were monitored closely. Once a linear pattern of export from the labelled leaf could be seen, as well as import into the sink tissue, the stem girdle was applied. This occurred approximately two hours after labelling. The girdle was applied using a double-bladed commercial girdling tool. A 5 mm section of cortex tissue and phloem was

carefully removed with the tool. Monitoring and data collection continued for a further 24 hours or longer.

Slopes of carbon import and export were compared before and after girdling, and between loading episodes. The section of the slope analysed was determined by the period post loading that showed the linear export or import rate. The period of linear export had a variable time span between plants. Comparisons of slopes were only made within a vine due to each vine having different geometrical dimensions and variable sensitivities of the individual detectors. In all repetitions 'R' statistical software and analysis of variance (ANOVA) at 5% level was used to determine significant differences in export slopes from source leaves or import slopes into the growing shoot meristems, the slopes were compared using least squares regression.

3.3 Results

3.3.1 Export from the source leaf

Each vine had two loads of ^{14}C applied 24 hours apart. The export rate of the radiolabelled isotope from the loaded source leaf did not change significantly between the two loading episodes in any of the vines (ANOVA; $p>0.05$). The up-take of radiolabelled isotope occurred in the source leaf within minutes of loading and continued for 30 minutes until the excess ^{14}C was flushed from the system. Export from the source leaf was detected approximately two hours after loading commenced. This pattern of ^{14}C uptake and subsequent export of the isotope occurred similarly on all vines (treatment and control). Figure 3.4 illustrates a typical example of a loading and subsequent export pattern. Figure 3.5 shows the period 200 minutes after loading to approximately 200 minutes post loading. In this vine that time period was the period of greatest linear export and it was used to calculate the export slope.

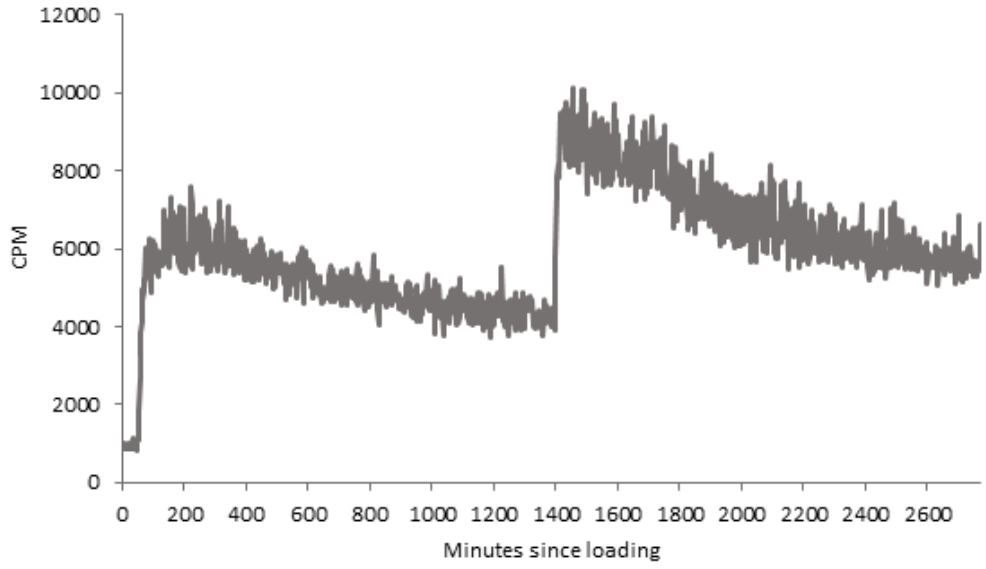


Figure 3.4: CPM (counts per minute) of the ^{14}C radiolabelled isotope is shown from the X-ray detector monitoring the initial uptake and subsequent export from the labelled source leaf on a control vine. The minutes since loading on the x-axis refers to the time since the source leaf was first labelled with ^{14}C.

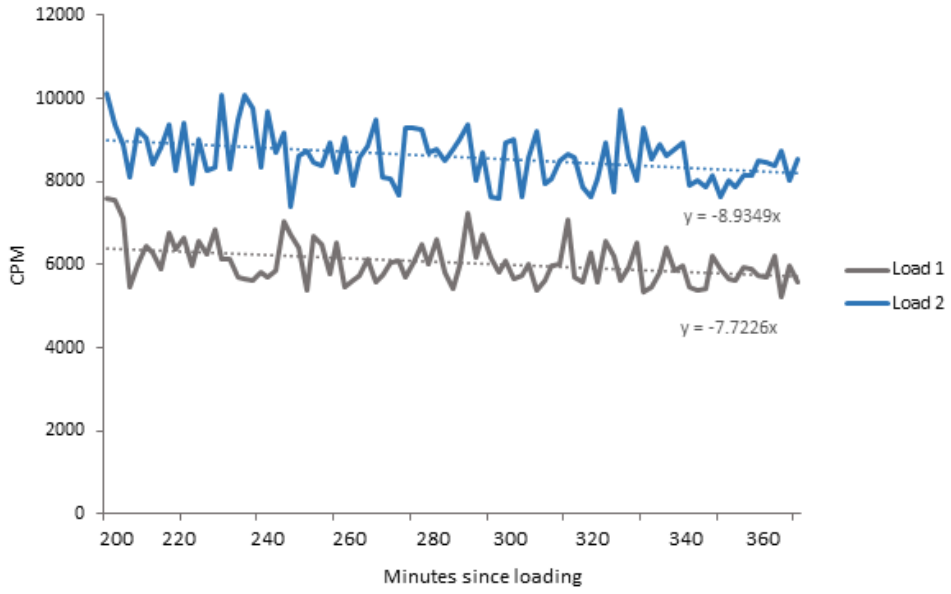


Figure 3.5: CPM (counts per minute) of the ^{14}C radiolabelled isotope is shown for the period with the greatest linear export from the source leaf of the same vine shown in the previous figure. The export slope is shown from 200 minutes after loading to 180 minutes post loading. The minutes since loading with on the x-axis refers to the time since the source leaf was labelled with ^{14}C. The slope of each line is shown.

3.3.2 Import through the stem and into the roots

In both non-girdled (control; C) and girdled (treatment; T) vines the X-ray detector on the stem was positioned directly above the girdle placement. Within two hours of labelling, a pulse of the ^{14}C isotope was moving through the stem, (Figure 3.6). In two girdled vines there was a treatment effect. The first load of isotope resulted in the CPM of ^{14}C either remaining stable or reducing. However, after the stem girdle was applied the pulse of radioactive isotope continued to increase though the import slope was reduced (Figure 3.6 and 3.7). The slopes were calculated from 80 minutes after the load went on until a decrease in import slope was seen (Figure 3.6.). The time of greatest linear export of radiolabelled ^{14}C used for load 1, for load 2 slope was calculated before and after the girdle was applied. In the remining two T vines there was no evidence of the girdle having any affect on the CPM of the ^{14}C isotope moving through the stem above the girdle, the difference in import slope post-girdle was not significant (ANOVA; $p>0.05$), data not shown.

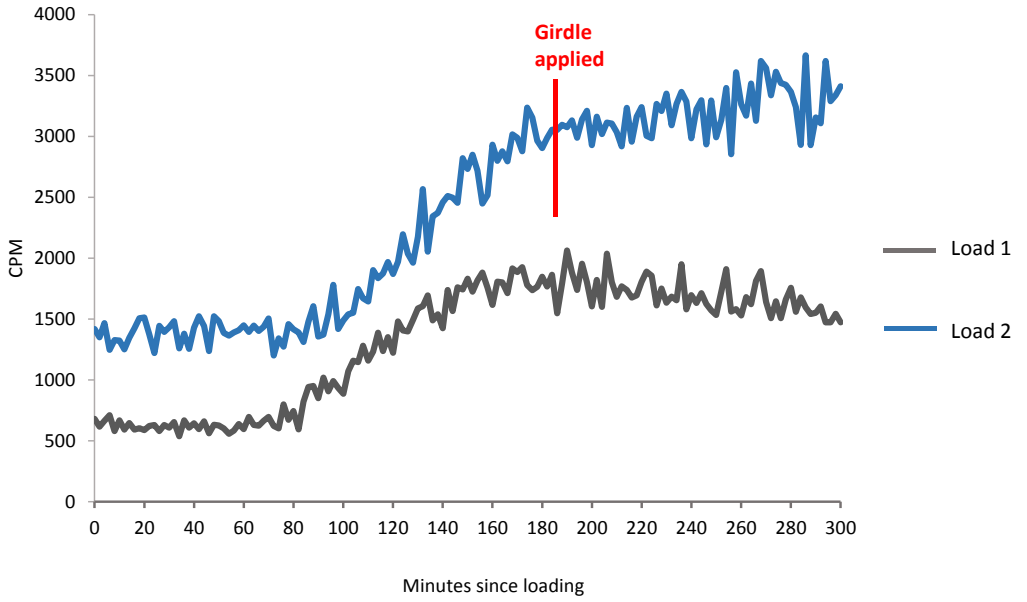


Figure 3.6: Counts per minute (CPM) of the ^{14}C isotope in the stem on T vine 4. Load 1, (dark grey) and load 2 (bright blue) shows CPM from loading to 300 minutes post loading. The second load went on 24 hours after the first load, the red line shows the timing of the girdle. The minutes since loading on the x-axis refers to the time since the source leaf was labelled with ^{14}C.

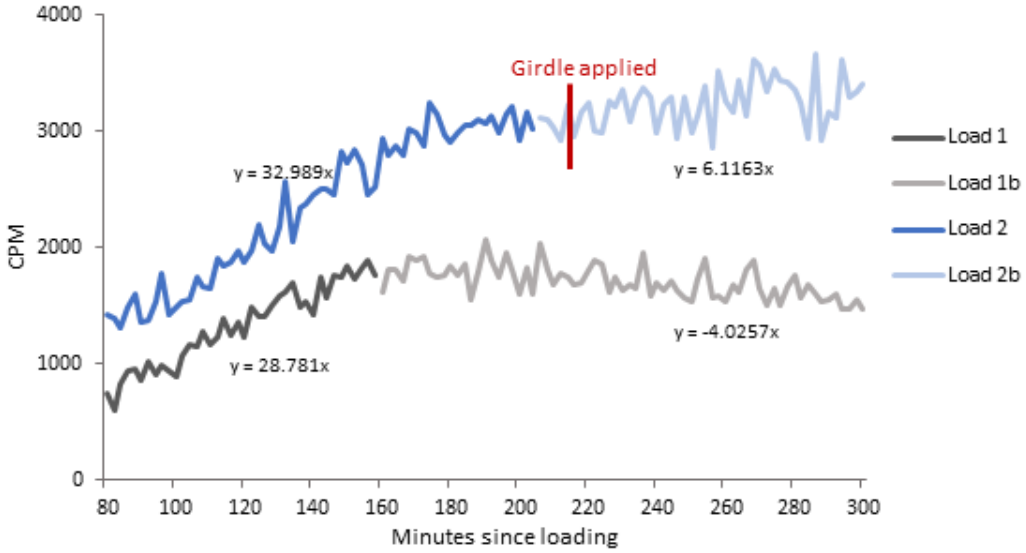


Figure 3.7: Counts per minute (CPM) of the ^{14}C isotope in the stem on T vine 4. Load 1, (dark grey), slope is y = 29x; load 1b (light grey) shows import rate after 160 minutes of the same load, slope is y = -4x. Load 2, (bright blue), slope is 33x; load 2b (light blue) shows the import slope after the girdle, y = 6x. The minutes since loading on the x-axis refers to the time since the source leaf was labelled with ^{14}C.

The roots showed little evidence of ^{14}C import. In five out of the total seven vines (C and T), there was no increase in counts from the roots under the detector. In two vines, one girdled and one non-girdled there was a low detection rate of ^{14}C import in the roots. As an example, (Figure 3.8) shows the girdled vine with a pulse of the radioactive isotope coming into the stem after both ^{14}C loading events, slopes (not shown), were not significantly different (ANOVA; $p>0.05$).

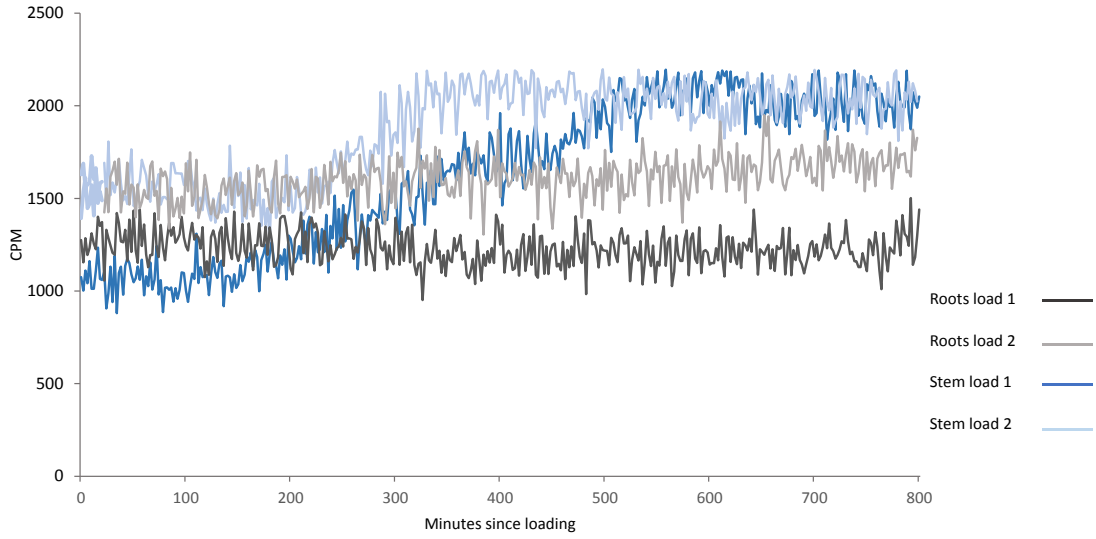


Figure 3.8: Counts per minute (CPM) of the ^{14}C isotope recorded in a control vine. The isotope was exported from the source leaf and it was transported through the stem (blue) of the vine towards the root system (grey). The minutes since loading on the x-axis refers to the time since the source leaf was labelled with ^{14}C.

3.3.2 Import by the shoot apical meristem

An effect of the girdle on carbon import by the shoot meristem was seen in two treatment vines. The import slope of ^{14}C was steeper after the stem girdle was applied in comparison to the pre-girdle slope ($p<0.0001$; Figure 3.9 A), treatment vine 2. In the remaining two girdled vines the ^{14}C import rates into the meristem

after the loading episodes were not significantly different (ANOVA; p>0.05; Figure 3.9 B), treatment vine 1. In the non-girdled vines the import rate into the meristems was variable between vines and loading episodes. In two vines the import rates into the shoot apical meristem were different after the two loading events (ANOVA; p=<0.001; Figure 3.10), in the other vines there was no difference in the import slopes (p=>0.05) between the two loads.

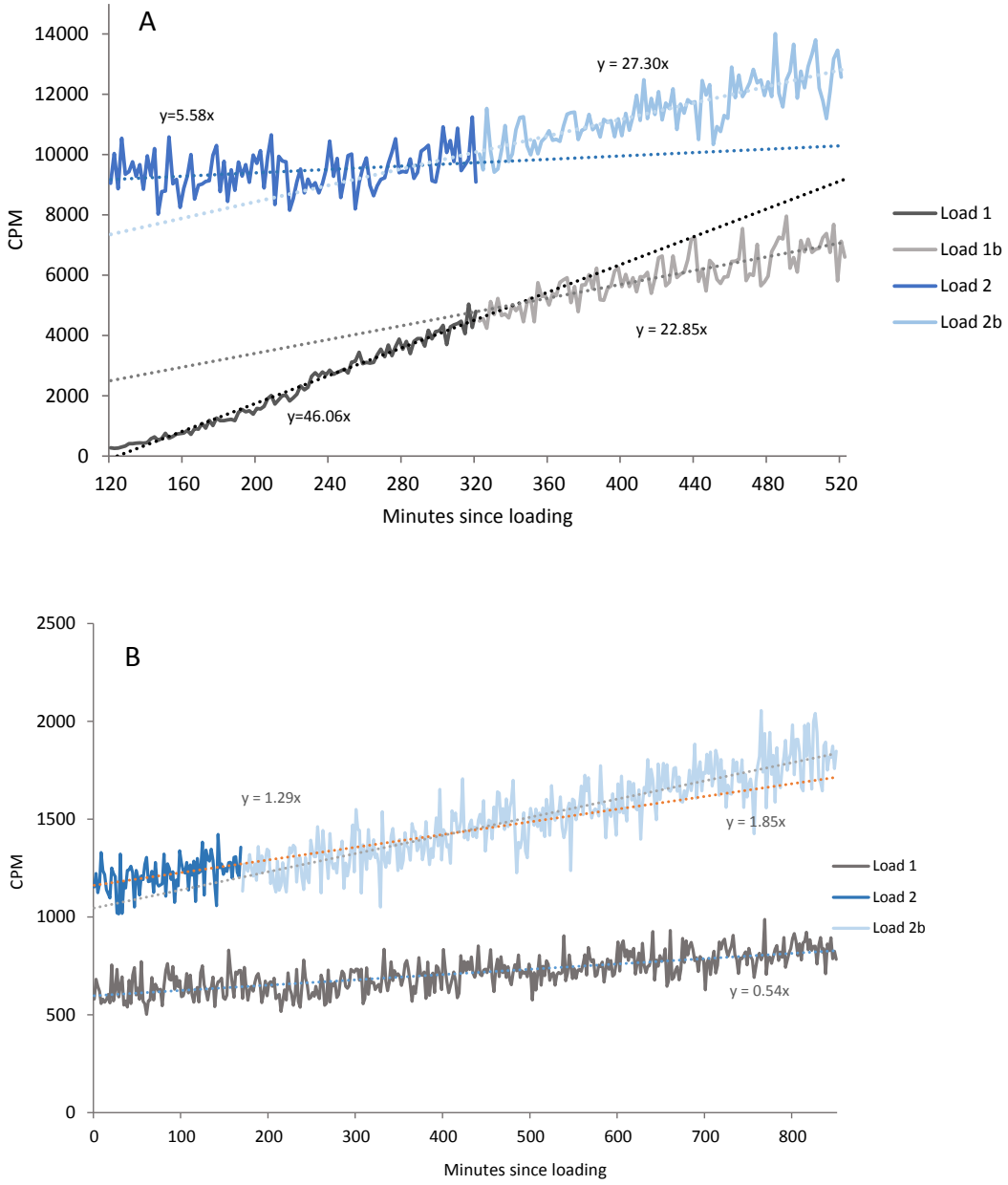


Figure 3.9: Counts per minute (CPM) of the ^{14}C isotope recorded in the shoot apical meristems following two loads of ^{14}C. The first load is denoted by the grey lines and the second load by the blue lines. Monitoring of ^{14}C accumulation was carried out following load 1 with no girdle applied to the plant. 24h afterwards,

load 2 was applied to the plant and once a linear import rate was established a girdle was applied to the trunk. A) shows an effect of the girdle which was applied approximately 300 minutes after the second ^{14}C load. Following the girdle the import rate into the meristem increases from 5.58 CPM/min to 27.30 CPM/min. B) is an example of a plant which had no effect of the girdle in import rate to the meristem. A girdle was applied approximately 180 minutes after the second ^{14}C load. The accumulation rate before the girdle was 1.29 CPM min^{-1} and 1.85 CPM min^{-1} after the girdle.

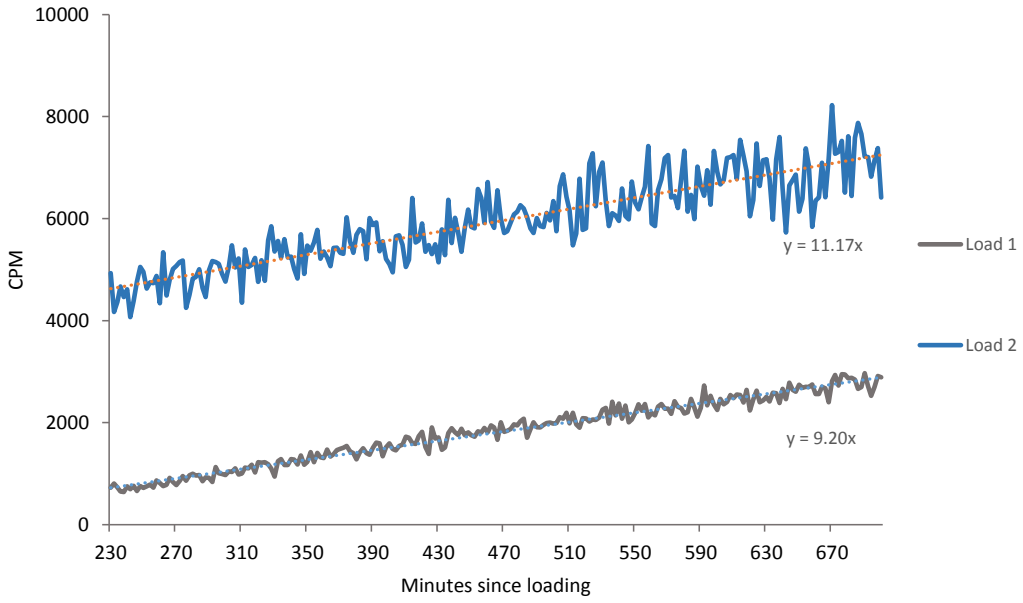


Figure 3.10: Counts per minute (CPM) of the ^{14}C isotope recorded in the shoot apical meristem on control vine 3. Load 1, (grey) and load 2 (blue) show the CPM from 230 minutes after each labelling event. The calculated import slope shows import slope after each load of ^{14}C was applied to the source leaf. The minutes since loading on the x-axis refers to the time since the source leaf was labelled with ^{14}C.

3.4 Discussion

3.4.1 Girdle effects on export of carbon from the source leaf

There was no evidence that the girdle had an effect on export rates from the source leaf. The export rate of radiolabelled isotope (^{14}C) was variable and showed no obvious trend between the two ^{14}C loading episodes in C or T vines. Export of labelled ^{14}C began within minutes of loading the source leaf, the peak period of export began soon after loading and reduced gradually over the following 24 hours. Given the natural geometrical variability within the source leaves of each vine, the sink tissues, and the variability in sink strengths (depending on age and respiration requirements) it is not surprising that the rate of export varied between vines. As the export rate of carbon from the source leaf did not decline after the phloem girdle had isolated the roots from the source leaf, presumably there is more carbon available for respiration and growth in alternative sinks. Increase in carbon allocation to fruits is the main reason for applying spring and summer girdles to kiwifruit vines when vine canopies are mature (Currie *et al.*, 2011a; Patterson & Currie, 2011b), but it has not been known previously if the response would be the same when a pre-flower girdle is applied for the purpose of reducing the incidence of budrot and the canopy is immature and shoots are still developing (Currie *et al.*, 2011b).

3.4.2 Girdle effects on cycling of carbon from source to sink tissues

In this study there was no evidence of remobilisation of ^{14}C labelled carbon from the roots to sinks either before or after the girdle. This was undoubtedly affected by the fact that very little radioactivity was at the level required for detection by the X-ray detectors viewing the roots. The roots of the kiwifruit vines in this study had a large biomass and the proportion of the root system that was visible to the X-ray detector was small. This would have diluted the isotope throughout the root mass, reducing the percentage of isotope within the

detection range of the X-ray detector. However, in a previous study on *Actinidia arguta* a remobilisation of radioactive carbon was reported. The study found a pulse of radiolabelled isotope moving to the roots during daylight which then appeared to move back up the stem to the shoot overnight, possibly in the xylem (Boldingh *et al.*, 2015).

3.4.3 Girdle effects on carbon partitioning

The main effect resulting from the stem phloem girdle was a significant increase in the ^{14}C import rates to the shoot apical meristem and an accumulation of carbon above the girdle in two of the four girdled vines. This gives partial support to the hypothesis that the trunk girdle will result in an accumulation of carbon above the girdle and that more carbon is then available for alternative sink tissues.

Post girdle the X-ray detector positioned directly above the stem girdle detected an increase in the counts per minute (CPM) of radioactive isotope in two of the girdled vines. The continued increase of CPM in the stem after the girdle could be indicating an accumulation of carbon above the girdle caused by unloading of carbon (Li *et al.*, 2003). The carbon accumulation in these two vines suggests the phloem girdle has halted the import of carbon into sinks below the girdle and unless photosynthesis is prohibited (Cheng *et al.*, 2008; Setter *et al.*, 1980; Williams *et al.*, 2000), more photosynthates should be available to alternative sinks. The shoot apical meristem in these vines showed an increased import slope post girdle, this also implies that more carbon was made available to alternative sinks post girdle. Accumulation of CHO above the girdle has been reported in other studies involving trunk girdling. For example carbohydrates increased above the girdle in poplar species (Regier *et al.*, 2010), in pine (López *et al.*, 2015) and in kiwifruit (Currie *et al.*, 2005).

In the remaining two girdled vines there was no effect of the girdle. In these vines and the control vines the import slopes were variable and showed no

accumulation on carbon above the girdle, or increased export rate into the apical shoot meristem post girdle.

3.4.4 Limitations

This study was limited by low repetitions which means further work on the girdling effects on carbon partitioning is needed to confirm the results found in this study. In further work the methods would need refining so that a larger proportion of the root system available to the plant is able to be monitored for X-ray detection. This proved to be an issue in this project with a large root mass and only a small percentage of this visible to the X-ray detector. An alternative method of looking at carbon partitioning may be to isolate the shoot apical meristem with a girdle, in this project it was a strong sink. The accessibility of the shoot apical meristem would enable it to be monitored for changes in carbon import through the Bremsstrahlung technique. Given more time it would have been useful to use potted kiwifruit vines that had flower buds, this would add an extra alternative sink to the shoot.

3.5 Conclusions

Despite reducing the carbohydrate demand by a trunk phloem girdle and removing the roots as a carbon sink, the carbon is still exported from the source leaf at the same rate. This is an important finding as this means the availability of photosyntates to alternative sink tissues is increased, the fall in demand for carbon did not affected the supply. For pre-flower girdled vines this is important as the timing of this girdle occurs when demand for carbon in the developing canopy is high.

The trunk girdle has the ability to alter carbon transportation and partitioning within the young 'Hayward' vines. However, in this study the results were variable. Some vines showed an increased export to the alternative sink once the stem girdle was applied and others showed no response. It is not clear what the reason could be for the high variability in the response to the girdle. It may be partly due to small differences in the vines physiological development in one or more organs effecting how it responds to a girdle.

This study did not show remobilisation of carbon from the roots to sinks either before or after the girdle. The dilution of the isotope in the total root mass meant the counts of ^{14}C were below the detection levels of the X-ray detectors.

4 Chapter four – *Conclusions*

The goal of this thesis was to examine how a trunk phloem girdle impacts carbon partitioning in kiwifruit vines, particularly during the pre-flowering period when the shoots are transitioning from being carbon sinks to carbon sources. This is also a time when root pressure reaches high levels and may have an important role in vine development, including the movement of resources from roots to shoots (Black *et al.*, 2012a; Clarkson, 1993; Loescher *et al.*, 1990). It was known that root pressure is affected by the pre-flower trunk girdle, but the mechanism for this is unknown (Clearwater *et al.*, 2007). The results of this thesis confirm the effect of the trunk girdle on root pressure, including a characteristic delay between girdle application and the reduction in root pressure in girdled compared to control vines. Interestingly, the findings also indicate that the mechanism for the reduction in root pressure is not a simple depletion of total carbohydrate reserves in the roots that follows a reduction in carbohydrate supply caused by the girdle. Instead there were more subtle changes in root tissue and xylem carbohydrate composition, suggesting that the girdle may be affecting shoot to root signalling rather than causing large changes in total carbohydrate partitioning (Li *et al.*, 2003; Setter *et al.*, 1980). Regardless of the effect of the girdle, the findings also reveal new information about the mechanism of spring root pressure generation in kiwifruit.

Fructose seems to be an important CHO in early spring. With twice the osmotic value of sucrose on a weight basis, fructose has the ability to create a lower water potential in the xylem, and generate a higher hydrostatic pressure (Enns *et al.*, 2000; Enns *et al.*, 1998). This is important as through winter dormancy embolisms have formed in the xylem. To support vine and fruit growth the embolised xylem vessels need to refill so that xylem sap flow connects the underground vine organs with the above ground canopy. Conversion of starch and oligosaccharides such as sucrose to a monosaccarhide such as fructose will maximize the root pressure for a given investment in carbon based solutes (Kameli & Losel, 1995).

The development of pressure in the xylem was not even with height up the trunk. Pressure in the xylem developed lower in the trunk first, sometimes taking several days to develop pressure as little as 40cm further up the trunk, as seen in control vine 5, figure 2. This is consistent with the hypothesis that xylem vessels are loaded with CHO originating from root reserves, and that this process has a significant role in driving out air embolisms from the xylem of the shoots. With the fine rather than the coarse roots declining in soluble CHO and starch, this study suggests that the source of solutes necessary to create the required increase in osmolality within the xylem originates from the fine roots. The fine roots have direct contact with the soil nutrients and water, whilst the coarse roots are not directly responsible for water uptake and are therefore less in a position to be responsible for the generation of spring xylem pressure.

The pre-flower girdle had a delayed, prolonged effect on the mean maximum positive xylem pressure. Xylem root pressure remained low, at around zero kPa for a perod of two weeks, and the recovery occurred 34 days after the girdle was applied. The lack of a decline in root soluble carbohydrates or starch indicate that this reduction in root pressure is not caused by depletion of root reserves. There are two possible explantions. The first is that the delayed response is associated with the distruption of transport of substances other than carbohydrates in the phloem. The phloem is known to transport more than CHO, including growth regulators, enzymes, proteins, nucleic acids and organic acids (Turgeon & Wolf, 2009; Wind *et al.*, 2010). One of these classes of compound could well be an import signalling molecule that impacts the functioning of the roots. The second explanation is the difference in positive pressure between the two xylem pressure probes indicates a major disruption in the xylems ability to refill vessels in response to girdling. It is likely the girdle interfered with the vines ability to repair embolisms that can occur in the xylem with daytime transpiration and low pressure (Enns *et al.*, 2000).

At a time of high CHO demand, the roots – primarily recognised for their role in plant nutrition, starch storage and hydraulic function, showed no short-term effects of the pre-flower girdle. The CHO reserves located closer to the high-demand sink tissues were utilised in preference to starch and soluble CHO in the roots (Loescher *et al.*, 1990). Roots have been considered priority storage organs, but in fact it may just be that it is more energy efficient to supply CHO 'locally', thereby saving on the energy costs of long distance transport when energy is in short supply.

At a time when leaves were beginning to export carbon, the leaves from girdled vines were either accumulating photo-assimilates - unable to resume photosynthate transport, or they were not yet autotrophic (Piller *et al.*, 1998; Richardson *et al.*, 2016). The results from the ^{14}C carbon transport study did not show a reduced export of carbon from the source leaf after the stem girdle. It is possible that the leaves from the girdled vines in the on orchard study had delayed development, they were not exporting fully but retaining their carbohydrates while the ungirdled vine leaves began exporting sugars by the 15/11/2017. This is important as a delayed transition to an autotrophic status could have an effect on the development of vine organs at a critical developmental stage.

4.1 Gaps and future research

1. Though this study identified mobile CHO, in particular fructose as an important sugar in developing spring root pressure, the origin of the increase in xylem solutes is not clearly identified. As soluble CHO is often found in xylem sap, especially in spring, the solutes may arise from parenchyma cells and be loaded into the apoplast of the xylem. This was an avenue of research that could not be explored in the current study but which may be a worthwhile area for further research.
2. This study shows the CHO are probably the most important factor when it comes to maintaining xylem root pressure. Further research is needed to

determine the reason for delayed, prolonged loss of root pressure resulting from a phloem trunk girdle. If the girdling is causing increased levels of embolism in the shoots, the disruption to transport of essential nutrients could impact development of vine organs. Research is needed to identify signal molecules or mechanisms that are related to maintaining root pressure, and determining the impact on their function that occurs with loss of phloem flow.

3. The time period of this study did not encompass the future effects of applying a pre-flower girdle. The phenological development of the vine and fruits examined within the range of this study was not impacted by the girdle. However, it is not known what the effects will be on final fruit quality, or on aspects like return bloom the following season. If this girdle was to be applied regularly, on top of the three girdles already applied during spring and summer, there could be impacts on the vine that compound with repetition. For example, effects on root reserves, the impacts on fruit and flowering quality – these are areas that need to be looked at in the future by following the long term development of the vine with and without application of a pre-flower trunk girdle.
4. Due to the limitations in the ^{14}C carbon transport study it was not possible to identify whether the xylem was acting as a transport pathway for assimilates being remobilised from the roots to the shoots. Because this study found the shoot apical meristem to be a strong sink tissue, attracting direct transport of carbon from the source, an alternative method would be to isolate the shoot apical meristem with a girdle rather than the roots. Import into the roots, or to an alternative sink like a bud could then be monitored.
5. If further study of carbon transport to roots is to be considered, it will be necessary to modify how the plants are produced and observed during labelling so that the isotope signal is not diluted in a large root mass. This may mean root pruning, or the selection of younger rootstocks with a smaller volume of root tissue.

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