Tree girdling: a tool to improve our understanding of coupled sugar and water transport

Veerle De Schepper[1], Kathy Steppe[1]

[1]Laboratory of Plant Ecology, Ghent University, Coupure links 653, 9000 Ghent, Belgium
email: Veerle.DeSchepper@UGent.be

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Abstract (max 300 words)

Girdling can be used as a valuable research tool to improve our understanding of the integrated water (xylem) and sugar (phloem) transport. Therefore, girdling was applied in two ways on young oak trees (*Quercus robur* L.): manipulation of the sugar flow by mechanically removing a half band of bark (half-girdling) and a complete band of bark at two different heights (double girdling). The double girdling effects on both the water and sugar transport were investigated by analysing stem diameter variations, photosynthesis, xylem sap flow and concentrations of carbohydrates. The double-girdled oak trees could be divided in three stem zones: (1) the upper stem zone (U) still receiving new assimilates from the leaves, (2) the lowest stem zone (L) receiving only stored sugars from the roots, and (3) the middle stem zone (M) completely isolated from crown and roots. As downward carbon transport was interrupted by girdling, the stem expansion and carbohydrate content increased in U, indicating that U became the major sink instead of the roots. In contrast to U, stem expansion and carbohydrate content decreased in the two lower stem zones (M and L). Furthermore, a decrease in photosynthesis and sap flow rate was observed, which could be attributed to an indirect girdling effect. In the half-girdled trees, a labelling with radio-active ^{11}C was applied to visualise the sugar flow in small stem segments. The images of the labelled sugar flows showed that the translocation in the transport phloem was sectorial in both treated and untreated trees. However, half-girdling demonstrated that this sectorial behaviour was plastic and that lateral sugar translocation in the transport phloem was enhanced after wounding. In conclusion, different ways of girdling were successfully applied to test and confirm several in literature proposed hypotheses.

INTRODUCTION

The carbon status of a tree not only depends on the photosynthesis and the respiration rate, but also on the carbon translocation (carbon flow towards the growing organs). The translocation mechanism is often described by the well known Münch theory (Münch, 1930). According to this theory, the carbon flow in the phloem is induced by a gradient in turgor pressure which is caused by a difference in carbohydrate concentration between the regions of phloem loading and unloading (Münch, 1930). The preservation of this gradient relies on the balance between the loading and unloading processes (Ortega et al., 1988; Gamalei, 2002). The loading processes occur at the sources where carbohydrates are assimilated or produced from breakdown of stored products; whereas the unloading processes occur at the sinks where carbohydrates are diluted, stored or used for growth and respiration (Pritchard, 2007).

The main objective of the present study was to investigate the effects of a modified tree carbon status on the water and carbon transport in young oak trees. Therefore, we altered the carbon status of young oak trees (*Quercus robur* L.) by double and half-girdling of the stem. During the double girdling experiment carbon-related processes, such as stem diameter variations, photosynthesis and carbohydrate concentrations, were continuously measured together with sap flow. During the half-girdling experiment, the sugar flow was radio-actively labelled with ^{11}C to investigate some properties of the labelled flow. Hence, this paper presents the eco-physiological responses induced by double and half-girdling related to both the water and the carbon transport in the tree.

MATERIAL AND METHODS

Plant material and experimental setup

Two-year-old oak trees (*Quercus robur* L.) previously grown in an outdoor tree nursery were replanted in 50-l containers before spring. The trees were watered every two to three days to assure the potting mixture remained well humidified. As a first treatment, a double girdling experiment was performed during the growing season (DOY 244-284). On 10 September (DOY 253), two bands of bark were carefully detached from the xylem at a height of approximately 25 cm and 15 cm and it was checked that no residual phloem tissue was left after removal. Whereas double girdling is assumed not to hamper the upward water flow in the xylem tissue, it produces three distinguished horizontal zones with respect to downward phloem flow (Daudet et al., 2005). The upper stem zone (U) is positioned above the upper-girdled-zone and can still receive carbohydrates from the sources (i.e. the leaves). The middle stem zone (M) is on the other hand completely isolated from upward sources and downward sinks. As such, it can only use pre-existing local sugar reserves which are located in the bark and the xylem ray cells. The lower stem zone (L) is positioned below the lower-girdled-zone and may benefit from an upward sugar supply from the roots. Two trees were placed in a growth room: one tree was not double girdled and served as control tree. The growth room allowed control of the radiation level and the air temperature. Photosynthetic active radiation (PAR) was measured with a quantum sensor (Li-190S, Li-COR, Lincoln, NE, USA), mean PAR during the daytime period (from 06:00 h till 22:00 h) was around 134 µmol m^{-2} s^{-1}. Relative humidity of the air (RH) was measured with a capacitive RH sensor (Hygroclip S, Rotronic, AG Schweiz, Bassersdorf, Switzerland). RH fluctuated freely depending on radiation, air temperature and transpiration rate of the trees. Air temperature (T_{air}) was measured with a copper-constantan thermocouple (Omega, Amstelveen, the Netherlands). T_{air} was set at 21°C during the light period and at 19°C during the dark period. In a second experiment, the strip of bark was removed along only half of the stem outer circumference, henceforth referred as the half-girdling experiment. Before, the tree was half girdled on 12 October, it was placed in a lab set-up which allows visualisation of radio-active isotopes.

Ecophysiological measurements during the double girdling experiment:

Stem diameter variations (ΔD) were continuously measured with Linear Variable Displacement Transducers (DF5.0, Solartron Metrology, Leicester, England). Three LVDTs were mounted with custom-made stainless steel frames on the girdled tree.

Net photosynthesis rate (P_n) was measured with an infrared gas analyser (IRGA-225-MK3 CO_2, ADC, Hertfordshire, England). A branch bag was installed on a second

order branch on both the girdled and control tree. Air from the growth room was blown into the branch bag at a rate of 4 l min^{-1}. During each sample period, the incoming and outgoing air of the selected branch bag were continuously measured, but only the stable readings during the last three minutes were averaged and used for calculations. Zero measurements were carried out to detect possible drifts in the zero point reading of the IRGA. For each day, day-time net photosynthesis (P_n) and night-time dark respiration (R_d) were calculated as averages over fixed time periods (P_n from 07:30 h till 21:00 h; R_d from 22:30 h till 06:00 h).

Sap flow rates (F) in the stem of the girdled and control tree were measured with sap flow sensors based on the heat balance principle (SGA10, Dynamax Inc., Houston, USA). F was calculated according to the approach of Steppe et al. (2005) to eliminate errors induced by heat storage effects.

Stem samples were collected for sugar and starch analysis. At the day of girdling, the removed bands of bark were collected and used for sugar analysis. At the end of the experiment, four samples were collected: two samples in U, one in M and one in L. These samples, taken at the beginning and the end of the experiment, represented the conditions before and after girdling, respectively. All samples were immediately frozen in liquid nitrogen and were stored at -80 °C. Soluble sugars were extracted from the ground bark samples with 80 % ethanol at 45 °C, followed by centrifugation at 5000 g for 10 min. Glucose, fructose and sucrose were analyzed using high pH anion-exchange chromatography with pulsed amperometric detection (Dionex; CarboPac MA1 column with companion guard column; eluent: 50 mM NaOH, 22 °C). The remaining ethanol insoluble material was washed twice with ethanol 80 % and the residual pellet was treated with HCl 1M for 2 hours at 95 °C to achieve starch hydrolysis. Starch was determined spectrophotometrically at 340 nm by the enzymatic reduction of $NADP^+$ (UV-VIS, Biotek Uvikon XL). From each sample, three repetitions were performed. The total amount of soluble sugar was calculated as the sum of fructose, glucose and sucrose.

All signals from online sensors were logged at 10 s intervals and 5 min means were stored using a data logger (DL2, Solartron Metrology, Leicester, England), except for the IRGA measurements for which 1 min means were stored.

Data collection and analysis during the half-girdling experiment

PlanTIS (Forschungszentrum Jülich, Germany) is a high-resolution PET scanner which allows the *in vivo* visualization of the positron emitter ^{11}C in plants by creating two (2D) or three (3D) dimensional images (Jahnke et al., 2009). Its detectors, frontend electronics and data acquisition architecture are based on the ClearPET™ system (Streun et al., 2006). The detectors are arranged in a horizontal plane to allow the plants to be measured in their natural upright position. Two groups of four detector modules stand face-to-face and rotate around the field-of-view (FOV). More details about this PET system can be found in Beer et al. (2010). The oak tree was placed in the PET system one day before the measurements to achieve acclimatisation of the tree. The FOV focused on an 11-cm-long section of the main stem. Pulse labelling of ^{11}C was achieved by adding ^{11}C labelled CO_2-gas to assimilating leaves of a first order branch. The radionuclide ^{11}C has a half life time of 20.4 min and the signal of the tracer was measured every 5 min for about three hours after labelling. Due to the fast decay of ^{11}C, two branches could be labelled per day.

The PET data were reconstructed and converted by specific image reconstruction-tools to 3D-images that represent half-life corrected data (Beer et al., 2010). Based on the

position of three reference tubes, PET images of successive ^{11}C runs could be compared in time. The reconstructed PET data was additionally converted by Mevislab (version 2.1) to non half-life corrected ^{11}C tracer profiles, which mimic the output of ten virtual detectors along the measured stem segment with a thickness of approximately 1.1cm. The conversion is performed in a similar way as performed by Bühler et al. (2011): the spatial 3D-data were integrated over two dimensions and over a certain width in the third dimension. This third dimension was roughly the gravitational axis along the transport pathway.

Afterwards, the obtained ^{11}C tracer profiles were analysed with the mechanistic model of Bühler et al. (2011). This tracer model (Bühler et al., 2011) was fitted to the ^{11}C tracer profiles of the first five virtual detectors in order to obtain information about changes in the translocation pathway above the zone with bark manipulation. The estimated meaningful parameters of this model were the front velocity of the fasted ^{11}C particles in the transport pathway [mm min^{-1}] and the fractional loss in steadily fixed tracers along the transport conduit [% cm^{-1}] (Bühler et al., 2011).

In addition, the oak tree was placed in an MRI system (Forschungszentrum Jülich, Germany) to obtain water content images (De Schepper et al., 2012). A 1.5T MRI system was used consisting of a split-coil magnet (Magnex/Agilent, Oxford, UK) and a NMR imaging spectrometer (Varian/Agilent, Alto Palo, USA). Parallel magnetic field gradient-inserts at a separation of 120 mm were used (plate diameter 40 cm, gradient strength up to 800 mT/m). In between these two inserts a part of the oak stem was placed. A small solenoid radio-frequency (RF) coil was wound around the stem prior to the measurements. This results in a small RF coil which yields a much higher signal to noise ratio with respect to a standard whole body RF coil. Images were acquired using (multiple) spin echoes. Echo times were minimised to 5.4 ms which reduces T_2 effects, so that the images were a good approximation of water content. A slice thickness of 2.5 mm was used with a fixed in-plane resolution (pixel size) of 100 µm. Due to this fixed resolution, FOV varied between 18 and 20 mm.

RESULTS

Double girdling responses on ecophysiological processes

As shown in Fig. 1, double girdling had a lasting effect on the stem growth (ΔD) of the three stem zones delimited by double girdling (U, M, L). Before girdling, the daily growth rates of U, M and L were identical and almost constant. These three zones reacted almost immediately to the girdling event, but not in the same way. The girdled stem of zone U showed an excessive radial increase. In contrast to zone U, the stem diameters in zones M and L almost immediately stopped increasing after girdling. After a few weeks, even negative growth rates were noted at several times.

P_n of the girdled tree decreased significantly a few days after girdling (Fig. 2A). R_d of the girdled tree was significantly higher after girdling compared to R_d before girdling (Fig. 2B). R_d reached a maximum at the same time P_n started to decrease, afterwards R_d again gradually decreased. Furthermore, F was affected in a similar way as P_n: F significantly decreased after girdling (Fig. 2C). Noteworthy is that P_n of the control tree also decreased significantly after approximately one week (Fig. 2A). This decrease in control P_n (-8%) was however smaller than the decrease in P_n of the girdled tree (-19%). Additionally, P_n of the control tree remained higher than that of the girdled tree after girdling, which was not the case in the period before girdling. This suggests that the

additional decrease in P_n of the girdled tree most probably was caused by girdling. R_d and F of the control tree (Fig. 2B,C) did not change.

Double girdling clearly affected soluble sugars and starch content in the bark (Fig. 3). Just before girdling, soluble sugars and starch were equally distributed around the place of girdling, because no significant difference existed between both samples (sample a and b). The content of total soluble sugar after girdling (samples U, M and L) was significantly different from the content before girdling (Fig. 3A). The sucrose content was different for all stem zones after girdling: it increased in zone U and it decreased in zones L and M. Fructose and glucose content only changed significantly in U after girdling. The starch content significantly increased in zone U and decreased in zone M (Fig. 3B). In zone L, the starch content did not significantly change, but showed a tendency to increase. This slight increase in L could be attributed to the sprouting of new assimilating shoots that appeared at the stem base. Two samples were taken above the girdled band in zone U: the soluble carbohydrate content of U_{wound} increased in a similar way as the content in U (Fig. 3), while its starch content did not accumulate and reached a level similar to that of M.

Half-girdling responses on labelled phloem flow positions

When two different branches were labelled before half-girdling, a different flow position depending on the position of the labelled branch was observed in the stem cross-section (Fig. 4). Fig. 4A illustrates schematic the labelled assimilate flow in the tree, while Fig. 4B shows images of the tracer flow. The angels between the nodes of the two labelled branches were around 90°. In addition, a two dimensional cross-section is overlaid on a MRI-water content image of a similar oak tree (Fig. 4C) to illustrate that the labelled tracer is located in the phloem region of the stem which is located in the white band of the MRI-image (De Schepper et al., 2012). Because PlantTIS detects γ-rays that are produced after collision of a positron with an electron, the detected region is only near the position of the positron emitting ^{11}C. Therefore, the visualized ^{11}C flow should be interpreted as a cloud around the actual radio-active ^{11}C flow. Furthermore, the labelled carbon flow did not change its position in time before half-girdling (Fig. 5A). Fig. 5A compares the ^{11}C flow of a non-girdled tree after five days and shows that its position remained the same.

When the bark of the oak trees was manipulated, the labelled carbon flow in the region of this manipulation changed position compared to the position before manipulation. Fig. 5B shows the radio-active carbon flow of branche 1 in Fig. 4 before and after half-girdling. The ^{11}C flow of branch 1 was positioned before manipulation in the stem zone were the bark afterwards was cut off during half-girdling. The grey flow in Fig. 6B represents ^{11}C transport that is labelled before manipulation. During this measurement, the bark was manipulated resulting in the interruption of the imaged ^{11}C flow (Fig. 5B) because the bark and the labelled carbons within it were removed during manipulation. The image of this ^{11}C flow (Fig. 5B) illustrates that the ^{11}C transport changed its position already one day after half-girdling.

The ^{11}C tracer profiles derived from these images were analysed to detect changes during three days after half-girdling. The mechanistic modelling approach extracted information of the ^{11}C transport located in upper half of the 11-cm long stem segment, positioned above the half-girdle. These analyses revealed changes in time of the calibrated parameters values (Fig. 6). A change in transport velocity in both branch 1 and 2 after manipulation was observed (Fig. 6A), whereas the loss only changed in branch 2

(Fig. 6B). The transport velocity before half-girdling was between 8 and 12 mm min^{-1}. The labelled carbon flow of branch 1 (Fig. 6) was positioned within the zone of removed bark and it corresponds with the carbon flow of branch 1 depicted in Fig. 4B. The ^{11}C flow of branch 2 (Fig. 6) was not located in the manipulated bark zone and corresponds to the flow of branch 2 shown in Fig. 4B. The time profiles of velocity indicate that carbon flow velocity of branch 1 decreased continuously after manipulation while the flow velocity of branch 2 increased temporarily (Fig. 6A). The carbon loss of labelled flow of branch 1 substantially increased shortly after girdling and then restored to normal values within two days (Fig. 6B). The loss of branch 2 seemed to be unaffected by the half-girdling event.

DISCUSSION

Double girdling affects both the carbon and water status

Double girdling completely blocks the downward phloem transport from the leaves to the roots, while the upward water flow from the roots to the leaves is maintained (Daudet et al., 2005; Johnsen et al., 2007). Accordingly, the carbon status of the trees was changed, since the roots could no longer function as a major sink for new assimilates after double girdling. The results indicate that the stem adapted accordingly in order to cope with the excess amount of carbohydrates. Therefore, stem growth and carbohydrate content strongly changed in the stem depending on their position above or below the girdled zone (Fig. 1 and Fig. 3).

After double girdling, soluble carbohydrates accumulated in zone U due to the phloem blockage (Fig. 3). Note that the accumulation of carbohydrates was more pronounced closer to the upper-girdled-zone. Hence, it is likely that double girdling expedited the carbon unloading of the stem tissue close to the phloem blockage. Some girdling studies observed a similar increase in soluble carbohydrates in zone U (Li et al., 2003), while other studies did not (Daudet et al., 2005; Cheng et al., 2008). Cheng et al. (2008) and Li et al. (2003) performed girdling (DOY 262 and 268, respectively) close to the girdling date of our experiment, while Daudet et al. (2005) girdled earlier in the growing season (DOY 193). It should be mentioned that all these cited studies used different types of trees. The observed increased amount of soluble carbohydrates probably induced a higher conversion to storage carbon, because an increase in starch content was also observed in the bark of U (Fig. 3). This increased starch content is consistent with previous girdling studies (Myers et al., 1999; Li et al., 2003; Daudet et al., 2005). In comparison with the sample taken at 0.5 cm above the upper-girdled-zone (Uwound), the wound tissue displayed a different storage behaviour (Fig. 3B). Since this wound tissue contains less starch, it seems to have a higher respiration rate and/or growth rate.

In M, radial stem growth was no longer observed after girdling (Fig. 1), indicating that carbon reserves in the stem were not sufficient to sustain growth. Since no radial growth was observed, the reserves of stored carbohydrates (Fig. 3) might be mainly used in order to maintain respiration of the living stem cells (Bryce and Thornton, 1996). In contrast to the findings of Daudet et al. (2005) no recovery of radial growth in M was observed. This might be caused by the continuous removal of newly formed axial phloem strings in our study. The formation of wound tissue indicates the presence of starch reserves in the xylem ray parenchyma (Zapata et al., 2004; Carlquist, 2007), which can be mobilised whenever a shortage of carbohydrate supply occurs (Carlquist, 2007). Wound tissue was rapidly growing by using the available sugars for structural growth and not for storage (Fig. 3). The small decrease in stem diameter, which was observed in M (Fig. 1B)

might be attributed to a decrease in water content: the decrease in soluble carbohydrate content attracted less water in the bark cells.

Due to the undisrupted phloem connection between L and the roots, previously stored carbohydrates in the roots remained available for zone L after girdling (Von Fircks and Sennerby-Forsse, 1998; Zapata et al., 2004). This availability might explain the higher amount of soluble carbohydrates observed in L compared to M (Fig. 3) and can explain why the radial growth rate in L decreased more slowly compared to M (Fig. 1). After approximately one month new shoots and leaves were observed at L, indicating an important remobilization of carbon stored in the roots as a response to the above-ground disturbances (Rodgers et al., 1995; Landhausser and Lieffers, 2002). Similar basal shoots have been observed in the girdling study of Wan et al. (2006) who related these shoots to a blocked auxin transport in the phloem. This newly formed assimilating tissue might also explain why the starch content tended to increase in L after girdling. Such an increase was never observed before in other girdling experiments (Li et al., 2003; Daudet et al., 2005; Wang et al., 2006).

Girdling is often seen as a manipulation which only affects the downward carbon flow and not the upward water flow (Murakami et al., 2008). However, this hypothesis is questionable, since in the present study the upward sap flow rate decreased a few days after girdling (Fig. 2). The decrease in upward sap flow was probably caused by a feedback inhibition of P_n (Williams et al., 2000; Cheng et al., 2008; Domec and Pruyn, 2008). The observed trends of a decrease in P_n and an increase in R_d after girdling (Fig. 2A,B) suggest such a feedback inhibition. Several girdling studies (Iglesias et al., 2002; Bondada and Syvertsen, 2005; Johnsen et al., 2007; Cheng et al., 2008; Rivas et al., 2008) have reported this P_n inhibition which they attributed to the decreased sink demand in girdled trees. In literature two processes are described which might trigger inhibition of P_n: stomatal closure (Williams et al., 2000; Cheng et al., 2008; Domec and Pruyn, 2008) and damage to the photosynthesis system (Myers et al., 1999; Bondada and Syvertsen, 2005; Rivas et al., 2008). Cheng et al. (2008) observed that these two processes occur simultaneously. The decreased sap flow in our study suggests that the inhibition of P_n was accompanied with stomatal closure.

Sectorial phloem flow in oak trees becomes plastic after half-girdling

The flow of radio-active carbon had a different position in the phloem ring depending on the position of the nodes of the labelled branches (Fig. 4), which demonstrates as such the sectorial behaviour of phloem flow in the stem of young oak trees. Similar sectoriality between shoot and roots has been reported earlier in herbaceous species (Stieber and Beringer, 1984; Marshall, 1996; Jahnke et al., 2009). In general, sectoriality arises when the vascular connections between sources and sinks restrict the movement of assimilates in such a way that carbon fixed by a leaf remains primarily within its orthostichy (Fetene et al., 1997; Preston, 1998). Leaf orthostichy means that source leaves preferentially support sink leaves directly above or below them on the stem, because they have direct vascular connections (Taiz and Zeiger, 2002). Therefore, the allocation pattern of assimilates from a source leaf to a particular sink becomes quite predictable from basic information on the geometry of the leaf arrangement on the stem (Marshall, 1996). Based on ^{14}C methods and indirect clipping experiments, sectoriality in phloem flow was most commonly found in trees and dicotyledonous herbs (Watson and Casper, 1984; Vuorisalo and Hutchings, 1996).

The downward ^{11}C flow halted immediately after half-girdling. However, one day after half-girdling, a downward ^{11}C flow could already be detected (Fig. 5B). Most likely, the sugar flow found its way towards the roots by changing its position towards the undamaged phloem. Because the translocation pattern changed towards the undamaged tissue above the manipulated bark, it seems that the translocation pattern is not absolutely determined by the vascular architecture of the tree (Preston, 1998). The apparent fixed sugar pathway of a certain leaf to a certain sink is altered after half-girdling (Fig. 4), probably causing the leaf to nourish another sink. As such the original sectoriality, based on the sieve tube architecture, was altered by the applied bark manipulation. Other studies (Gent, 1982; Aloni and Peterson, 1990; Preston, 1998) have observed a similar breakdown of sectoriality after manipulation of the source-sink relations. Hence, it seems that the barriers to lateral flow in the sieve tubes are not absolute and that sectoriality is plastic (Preston, 1998; Orians et al., 2005).

The observed transport velocity (Fig. 6A) was in range with the velocities of phloem water flow (12-24 mm min^{-1}) observed in poplar trees with MRI (Windt et al., 2006). The decreased velocity in the flow of branch 1 is probably related to the increased resistance which is induced by the lateral translocation occurring after bark cutting. It seems plausible that the resistance of sieve plates in the sieve tubes, which were destroyed after half-girdling, was lower than the lateral transport resistance. The changes in pathway loss for the flow of branch 1 (Fig. 6B) suggest that the lateral transport appears by modified plasmodesmata (Orians et al., 2005). According to this hypothesis, an active change in translocation pathway might be made to enhance lateral translocation. Hence, the amount of energy delivered by assimilates has to increase temporarily to fuel the vascular changes, e.g. an increased lateral plamosdesmatal conductance. Once the changes are completed the required amount of energy will reduce again. Therefore, this hypothesis seems consistent with the pathway loss that first increases and afterwards decreases. Probably new labelled assimilates were consumed in the wounded stem when the changes were made that may have contributed to the temporal increase in pathway loss.

The temporary increased velocity of the flow of branch 2 after manipulation (Fig. 6A) was probably attributed to a temporary higher sink demand. After half-girdling, the roots most likely received less carbohydrates from branches with their sugar flow originally located in the manipulated bark zone (e.g. branch 1). The higher observed loss of the labelled flow produced by branch 1 suggests that a smaller fraction of assimilates, produced by this branch reached the lower stem and roots immediately after half-girdling. After two days, the flow velocity of branch 2 restored more or less to velocities observed before girdling which coincided with the decrease in loss of branch 1. This indicates that the recovery of the reduced sink translocation of assimilates, produced by branches such as branch 1, coincides with the reduction of the increased translocation velocity of assimilates produced by branches, such as branch 2. When the loss of disturbed flows of branches as branch 1 decreased, the amount of their assimilates reaching the sinks probably increased. Therefore, the reduced sink strength exercised by the roots on branches as branch 2, probably triggering the reduced translocation velocity of their assimilates.

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Figures

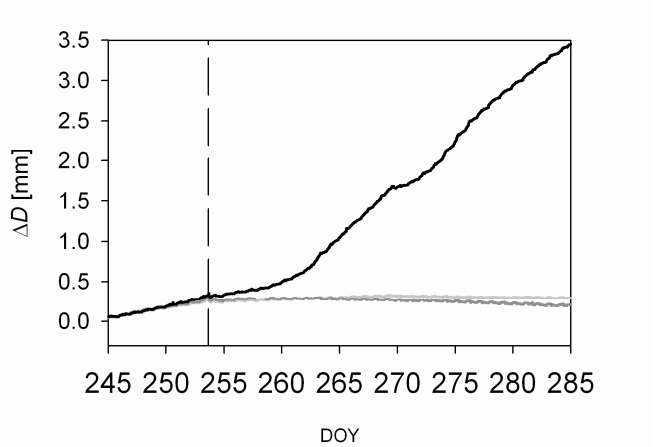

Fig. 1. The typical, detailed patterns of stem diameter variations (ΔD) for the upper (U), the middle (M) and the lower (L) stem zone of the double girdled tree. The vertical long dashed line indicates the moment of double girdling. Note that stem growth of the M and L zones immediately ceased after girdling. (DOY: Day Of the Year).

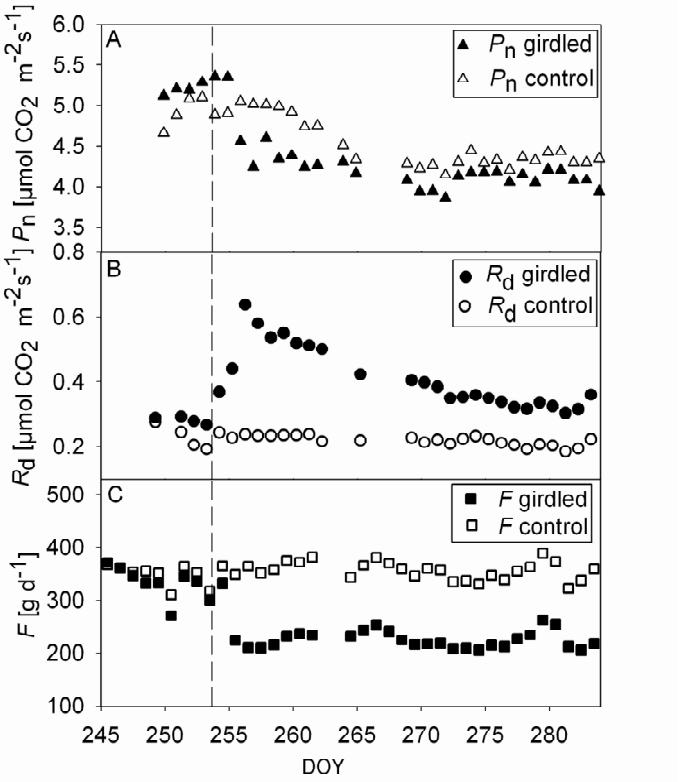

Fig. 2. A: Average leaf net photosynthesis rate during the daytime period (P_n) (from 07:30 h till 21:00 h); B: average leaf dark respiration rate (R_d) (from 22:30 h till 06:00 h); and C: daily xylem flow rate (F) (from 07:30 h till 21:00 h) of the double girdled and control tree. The moment of girdling is represented by the dashed line (DOY: Day Of the Year).

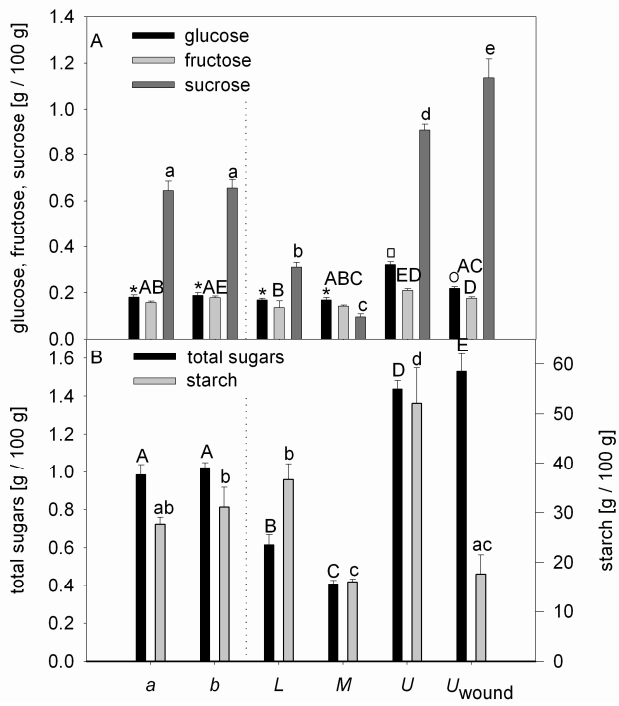

Fig. 3. A: Changes in the spectrum of soluble sugars due to double girdling. Error bars represent the standard deviation; significant differences ($p<0.05$) between different samples are indicate with symbols, capital and small letters for glucose, fructose and sucrose, respectively. B: The total soluble sugars and starch content. The samples a and b were collected before girdling (DOY 253). The other samples were collected after girdling (DOY 294): two in the upper stem zone (U and U_{wound}), one in the middle (M) and one in the lower stem zone (L). 'U_{wound}' represents a sample of wound tissue formed just above the upper-girdled-zone. Capital and small letters indicate significant differences ($p<0.05$).

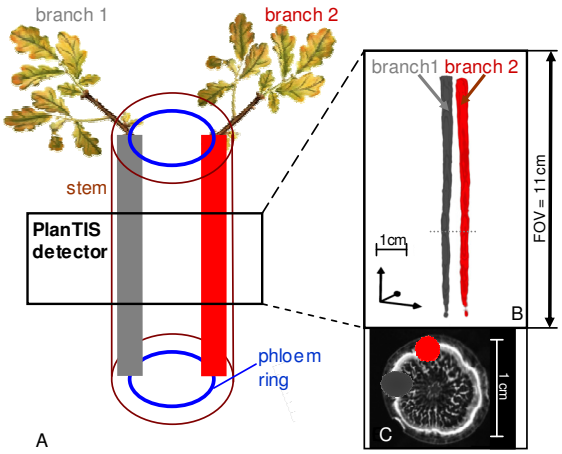

Fig. 4. A: Schematic presentation of how the tree's structure related to the images obtained by PET; B: 3D-PET-images of the labelled carbon; and C: a 2D-horizontal cross-section of this carbon flow overlaid on an MRI image of a similar oak tree. The dotted lines indicate the position in the 3D-image where the 2D-cross-section was made.

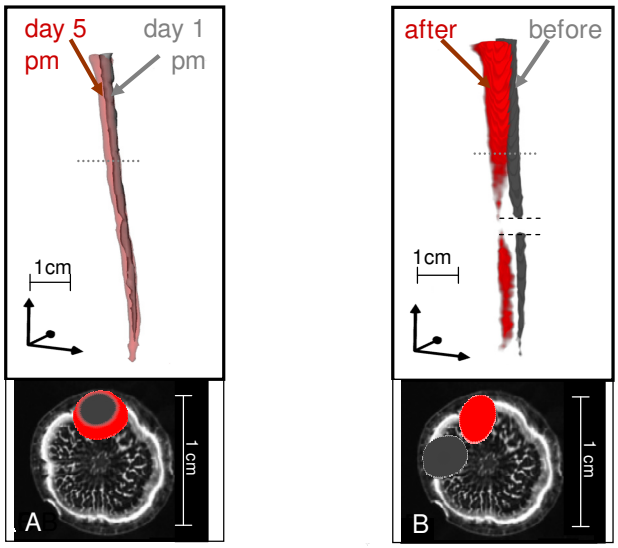

Fig. 5. A: Before bark manipulation carbon flow occurred at a fixed position in the cross-section of oak tree stems. 3D-PET-images of the labelled carbon flow and a 2D cross-section of this flow overlaid on an MRI-image of a similar oak tree to illustrate the same position in the half girdled oak tree before bark manipulation. B: Both flows were visualised by labelling branch 2 in Fig A at different times. Labelled carbon flow in the zone of bark manipulation changes its position in the phloem ring after manipulation. 3D-PlanTIS-images of the labelled carbon flow and a 2D cross-section of this flow overlaid on an MRI-image of a similar oak tree to illustrate that the position of the ^{11}C flow changed its position after bark manipulation. The flow in grey was measured before manipulation and the flow in red was measured after manipulation. The grey ^{11}C flow detected before manipulation shows an interruption representing the removed bark. The dotted lines (A and B) indicate the position in the 3D-image where the 2D-cross-sections are made, whereas the position in between the two dashed lines roughly corresponds with the location of bark manipulation.

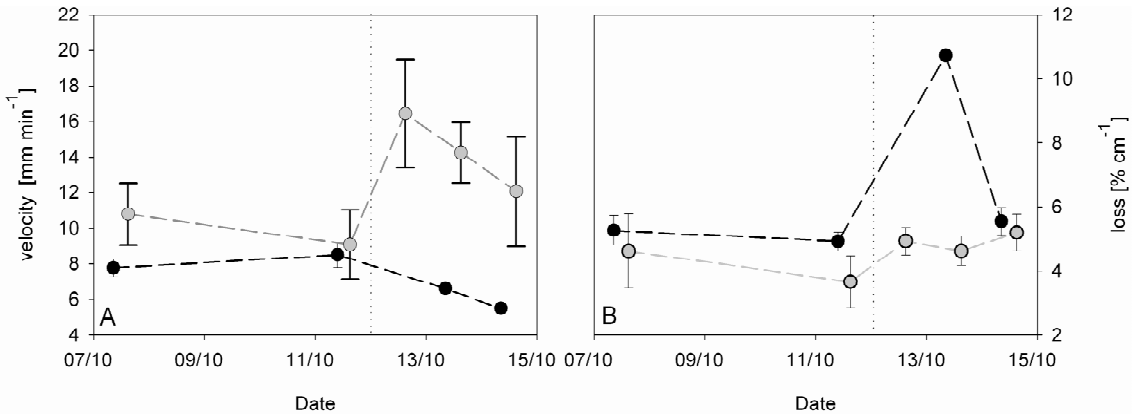

Fig. 6. A: the velocity of the transported assimilates; B: about the amount of steadily fixed tracers lost along the transport conduit (loss); the lines and symbols represent parameter values of the ^{11}C flow of branch 1 (black) which originally flowed through the girdled bark and of the ^{11}C flow of branch 2 (grey) which never flowed through the girdled bark zone. The dotted line represents the moment of half-girdling.