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Reference

# Seasonal changes in starch and sugar content of poplar (*Populus deltoides* × *nigra* cv. Dorskamp) and the impact of stem girdling on carbohydrate allocation to roots

NICOLE REGIER,[1] SEBASTIAN STREB,[2] SAMUEL C. ZEEMAN[2] and BEAT FREY[1,3]

[1]  *Swiss Federal Research Institute WSL, Zürcherstrasse 111, 8903 Birmensdorf, Switzerland*

[2]  *Department of Biology, ETH Zurich, Universitätsstrasse 2, 8092 Zurich, Switzerland*

[3]  *Corresponding author (beat.frey@wsl.ch)*

**Summary**   Trees need to store reserves to allow their survival during winter and for bud flush and leaf growth in the following spring. In many tree species, these reserve functions are mainly covered by starch, which is degraded to soluble carbohydrates during the dormant season for maintenance respiration and in spring during bud flush. We conducted girdling experiments on poplar (*Populus deltoides* × *nigra* cv. Dorskamp) in order to elucidate how interrupted transport of carbohydrates to the roots during autumn affects plant survival during winter and bud flush in spring. We measured the content of starch, sucrose, glucose, fructose, raffinose and stachyose in stems (above and below the girdle), coarse roots and fine roots over 1 year. We found that, in response to girdling, carbohydrates accumulated in stems above the girdle. As a result of interrupted reserve allocation, girdled plants depleted their root starch reserves nearly to zero, whereas in stems below the girdle, reserves were maintained close to control values, presumably in order to facilitate dormancy release and re-sprouting from buds below the girdle. Furthermore, we showed that stachyose accumulated during winter also in the roots, even in girdled plants, consistent with its importance as freezing protectant. The lower stachyose content of roots compared with shoots was likely due to protection of the roots from cold by the soil.

*Keywords: carbohydrate allocation and storage, depletion, disturbance, root reserves, starch.*

## Introduction

Trees need to store reserves to allow their survival during winter and to fuel bud flush and shoot growth in the following spring until the leaves have developed sufficient photosynthetic capacity to support net carbon assimilation. Non-structural carbohydrate reserves (starch and soluble sugars) are essential for the survival of woody plants after stress or while overwintering (Kobe 1997, Canham et al. 1999, Kaelke and Dawson 2005) and commonly constitute more than 90% of the available C in plants, except for genera with significant lipid storage (e.g., *Pinus* and *Tilia*; Hoch and Körner 2003). Besides the reserve functions of carbohydrates, soluble sugars can also serve as freezing protectants. For example, sucrose, raffinose and stachyose have been shown to accumulate in birch (*Betula platyphylla* Sukat. var. *japonica* Hara) xylem in response to freezing, leading to enhanced freezing tolerance during winter (Kasuga et al. 2007). It has only been shown for *Picea abies* (L.) Karst. that raffinose accumulates in roots in response to freezing (Wiemken and Ineichen 1993). It remains to be investigated whether sucrose, raffinose and stachyose accumulate also in roots of deciduous trees during cold periods.

Roots are known to be essential for carbohydrate storage in several tree species. The amount of carbohydrate stored in the roots changes seasonally, with the lowest reserves in spring after bud flush and the highest reserves late in the season or during dormancy (Johansson 1993, Bollmark et al. 1999, Landhäusser and Lieffers 2003). For hybrid poplar (*Populus nigra* × *laurifolia*), it has been shown that a considerable amount of carbohydrate is allocated to the roots even late in the growing season (Nelson and Isebrands 1983). In pioneer species, such as poplar and birch, root reserves are more important than in other species, as they need a high re-sprouting ability due to a high probability of recurrent disturbances (Bollmark et al. 1999). Due to the seasonal differences in carbohydrate reserves, the capacity of poplar to re-sprout in the following season depends on the time of above-ground disturbance. The capacity for re-growth is much higher when the plants are disturbed in autumn, when the reserves in the roots are higher than in spring after bud flush and when the reserves in roots are depleted (Landhäusser and Lieffers 2002). Such disturbances can be fire, decapitation, browsing by animals or feeding of insects (Kosola et al. 2001, Carter et al. 2004, Luostarinen and Kauppi 2005), leading to a large source–sink imbalance.
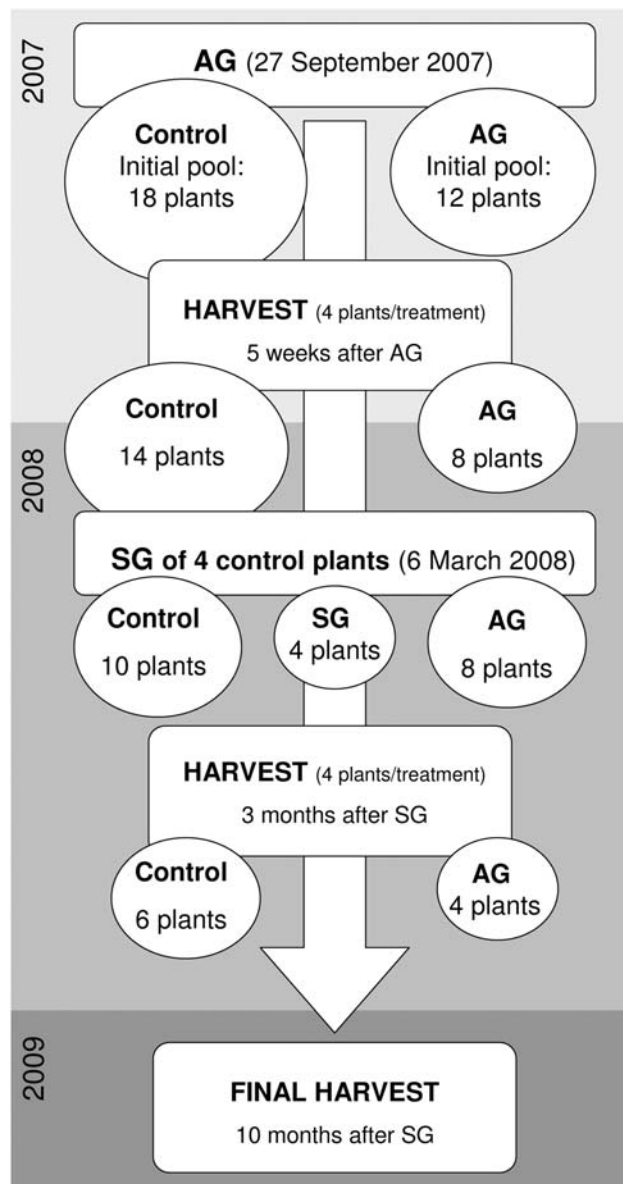
Figure 1. Flowchart showing experimental design. Ellipses represent pools of plants left in the experiment after each harvest. In spring, a subset of the control plants was taken for the second girdling treatment. Dates when only small tissue samples were taken, which was done monthly during the growing season, are not shown. AG, autumn girdling; SG, spring girdling.

Girdling is a well-established technique to disturb the carbohydrate allocation within a plant. It is widely used for research purposes but also for practical applications (Goren et al. 2003). Partial branch girdling has been applied to fruit trees in order to enhance fruit quality and yield. When the transport of assimilates downwards is decreased, they accumulate in the canopy and are available for fruit development, leading to lower fruit abscission rates and higher sugar content (e.g., Iglesias et al. 2006, Rivas et al. 2006). Trunk girdling is applied to disrupt the carbohydrate transport to the roots. This can be used to minimize the root respiration and

estimate the percentage contribution of root respiration to soil respiration (e.g., Högberg et al. 2001, Binkley et al. 2006, Frey et al. 2006). It is known that trees store large amounts of starch which, under normal conditions, are mobilized only partially. Jordan and Habib (1996) have applied trunk girdling to young peach (*Prunus persica* L. Batsch) trees to determine whether roots can mobilize older starch reserves and showed that starch in the roots was depleted in order to maintain the amount of soluble sugars at the same level as control trees.

The aim of our study was to determine how the carbohydrate status of the heterotrophic organs of poplar (*Populus deltoides* × *nigra* cv. Dorskamp) changes over the year. We hypothesized that girdling, which disrupts the allocation of reserves to the roots, would change the carbohydrate status in the heterotrophic organs of poplar plants and would lead to altered phenology. We wanted to elucidate whether poplar roots are able to mobilize older starch reserves and at which time of the year girdling has the strongest effects on the plant carbohydrate status. Therefore, we girdled one set of plants in early autumn and another set of plants in spring before bud break. In early summer, when the leaves were fully expanded, we removed all above-ground parts of half of the girdled and control plants to determine the extent to which the previous reserve depletion in girdled plants reduced the ability to re-sprout compared with control plants.

## Materials and methods

### Plant material and experimental design

Nineteen-month-old cutting-derived plants of *P. deltoides* × *nigra* clone 'Dorskamp' were used for this study. One hundred cuttings were grown in rows, with $0.5 \times 1.5$ m spacing in the experimental field of the Swiss Federal Research Institute WSL in Birmensdorf, Switzerland. Thirty plants were chosen for uniform height and stem diameter and were randomly assigned to the girdling treatment. A flow diagram of the experimental design is shown in Figure 1. Autumn girdling was conducted on 27 September 2007 by removing a 3-cm-wide ring of bark 30 cm above the ground using a sharp knife without injuring the xylem. In total, 12 plants were girdled and 18 plants were chosen as controls. Fine roots (<1 mm in diameter) and mature leaves were sampled from six plants on the day of girdling (zero time control) and then after 1, 2 and 4 weeks from each of six girdled and six control plants. For harvesting fine roots, we carefully uncovered part of the root system from soil and cut off only about 1–2 mg of fine roots in order to minimize the disturbance. On 1 November 2007 (5 weeks after girdling), four control and four girdled plants were harvested completely and divided into stem, coarse roots (>5 mm) and fine and medium roots (<5 mm). On 6 March 2008, four additional plants were spring girdled. Starting on 18 March 2008 (before bud break), samples of fine roots, coarse roots and stem bark (from above and below the girdle) were taken monthly each from four control plants,

Table 1. Stem height and circumference of control, autumn-girdled and spring-girdled plants. Stem, coarse root (>0.5 cm diameter) and fine/medium root (<0.5 cm diameter) biomass of control, autumn-girdled and spring-girdled plants. Values were considered significantly different at $P < 0.05$. $P$-values are given for all parameters. AG, girdled in autumn 2007; SG, girdled in spring 2008; n.s., not significant. $*P < 0.05$; $**P < 0.01$. Values are presented as the mean ± standard error; $n = 4$.

|  | Control | AG | SG | ANOVA | $P$-value |
|---|---|---|---|---|---|
| Stem height (m) |  |  |  |  |  |
| September 2007 | 4.3 ± 0.04 | 4.3 ± 0.06 | – | n.s. | 0.534 |
| November 2007 | 4.2 ± 0.06 | 4.1 ± 0.06 | – | n.s. | 0.167 |
| June 2008 | 4.8 ± 0.09 | 4.3 ± 0.06 | 4.6 ± 0.05 | n.s. | 0.086 |
| February 2009 | 5.0 ± 0.07 | 4.3 ± 0.09 | – | ** | 0.001 |
| Stem circumference (cm) |  |  |  |  |  |
| September 2007 | 8.8 ± 0.15 | 8.5 ± 0.23 | – | n.s. | 0.344 |
| November 2007 | 9.0 ± 0.21 | 8.5 ± 0.21 | – | n.s. | 0.134 |
| June 2008 | 11.4 ± 0.24 | 9.0 ± 0.21 | 9.5 ± 0.50 | ** | 0.001 |
| February 2009 | 11.6 ± 0.43 | 9.0 ± 0.54 | – | ** | 0.009 |
| Stem biomass (g) |  |  |  |  |  |
| September 2007 | – | – | – | – | – |
| November 2007 | 558 ± 16 | 520 ± 68 | – | n.s. | 0.597 |
| June 2008 | 995 ± 53 | 607 ± 56 | 762 ± 106 | * | 0.017 |
| February 2009 | 1342 ± 95 | 963 ± 136 | – | n.s. | 0.063 |
| Root (>0.5 cm) biomass (g) |  |  |  |  |  |
| September 2007 | – | – | – | – | – |
| November 2007 | 170 ± 11 | 163 ± 24 | – | n.s. | 0.816 |
| June 2008 | – | – | – | – | – |
| February 2009 | 321 ± 20 | 194 ± 36 | – | * | 0.021 |
| Root (<0.5 cm) biomass (g) |  |  |  |  |  |
| September 2007 | – | – | – | – | – |
| November 2007 | 40 ± 5 | 26 ± 2 | – | n.s. | 0.051 |
| June 2008 | – | – | – | – | – |
| February 2009 | 104 ± 17 | 45 ± 5 | – | * | 0.015 |

four autumn-girdled plants and four spring-girdled plants until September 2008. On 4 June 2008, the above-ground parts of four control, four autumn-girdled plants and four spring-girdled plants were harvested for determination of differences in growth and the ability of the stumps to survive. In early February 2009, all remaining trees were harvested completely and divided into stems, coarse roots and fine/medium roots. All samples were immediately frozen in liquid nitrogen and stored at −80 °C until analysis, except completely harvested trees, which were freeze-dried for 48 h or more in order to determine total biomass.

*Carbon, nitrogen and carbohydrate analysis*

The plant material used for the analysis of C and N concentrations was dried to constant weight at 60 °C and ground with a swing mill (Retsch, Haan, Germany). Six to eight milligrammes of plant material was weighed into tin cups and measured by a CN analyser (Carlo Erba Instruments, Milan, Italy).

Cross sections of the stems and roots were boiled in 80% ethanol, washed with water and stained for the distribution of starch with Lugol solution. For quantification of starch, ~0.2 g of plant material was homogenized in liquid nitrogen and extracted three times in 80% ethanol at 80 °C. Starch amount was determined by measuring the glucose released by treatment with α-amylase (EC 3.2.1.1) and amylogluco-

sidase (EC 3.2.1.3) as described by Smith and Zeeman (2006).

Soluble sugars in the pooled 80% ethanol supernatants obtained during the starch extraction were determined as previously described (Regier et al. 2009), with minor modifications. Sugars were analysed by high-performance anion-exchange chromatography coupled to a pulsed amperometric detector using a Dionex BioLC system (Dionex, Olten, Switzerland), equipped with a CarboPac PA-20 column according to the following conditions: eluent A, 100 mM NaOH; eluent B, 150 mM NaOH, 500 mM sodium acetate. The gradient was 0–15 min, 100% A (monosaccharide elution); 15–26.5 min, a linear gradient to 20% A and 80% B (maltose and oligosaccharide elution); 26.5–32.5 min, 20% A and 80% B (column wash step); 32.5–40 min step to 100% A (column re-equilibration). The flow rate was 0.5 ml min$^{-1}$. Peaks were identified by co-elution with known glucose, fructose, sucrose, raffinose, stachyose and maltose standards. Peak areas were determined using the Chromeleon™ software.

*Statistical analyses*

We tested our data for homogeneity of variances using the Levene test. Depending on the results of this test, we used ANOVA or the Brown–Forsythe test to determine the effects of girdling on growth parameters and carbohydrate content.
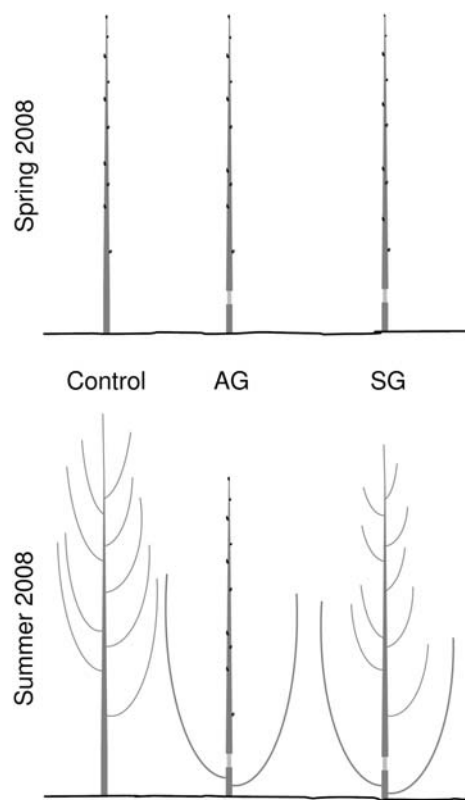
Figure 2. Schematic illustration of poplar plants in early spring before bud break and in summer 2008. Leaves are omitted to increase clarity of the illustration. AG, girdled in autumn 2007; SG, girdled in spring 2008.

Where we had data for control, autumn-girdled and spring-girdled plants, we applied post hoc tests; least significant difference was applied when variance homogeneity was given and the Tamhane test for unequal variances. Time, treatment and time × treatment interaction effects were tested using the general linear model repeated-measures procedure. Statistical tests were considered significant at $P < 0.05$. SPSS 16.0 for Windows statistical software package (SPSS Schweiz AG, Zurich, Switzerland) was used for all analyses.

## Results

### Plant development and growth

After girdling of 12 plants in September 2007, there was no observable difference between autumn-girdled and control plants in the time of onset of leaf senescence until leaf abscission. There were also no significant differences in stem height and circumference below the girdle and stem and root biomass of the plants harvested in November 2007, when most of the leaves had already been shed (Table 1).

In 2008, all plants started bud break late in March, and until the third week of April, there was no difference between the treatments. Then, in autumn-girdled plants, the development of new leaves stalled and the buds started drying out. Between spring-girdled and control plants, no difference

could be observed at this stage. Below the girdle of autumn-girdled and spring-girdled plants, bud break started in the middle of April, while in control plants, the comparable buds did not break (Figure 2).

On 4 June 2008, the stems of four autumn-girdled, four spring-girdled and four control plants were harvested in order to compare the growth. The spring-girdled plant biomass was intermediate between the control and autumn-girdled values, but was not statistically significantly different from either (Table 1). Control plants were significantly ($P < 0.05$) higher than autumn-girdled plants, but there were no significant differences between the height of control and spring-girdled as well as autumn-girdled and spring-girdled plants. Stem circumference of control plants was larger than that of girdled plants, but between autumn-girdled and spring-girdled plants there was no significant difference. Widths of the tree rings formed in 2008 were significantly different between the treatments. The ring width of spring-girdled plants was $0.21 \pm 0.04$ mm and of control plants $0.34 \pm 0.01$ mm ($P < 0.05$). Autumn-girdled plants did not form a new ring above the girdle. All stumps cut in June formed new buds that produced only small and slim shoots with small leaves compared with the shoots that had emerged in spring. The leaves of the cut plants showed premature senescence in the middle of September, while the leaves of uncut autumn-girdled and control plants remained on the plants until the end of October.

In February 2009, the remaining plants were harvested. Control plants were significantly higher than autumn-girdled plants and had a significantly greater stem circumference and stem and root biomass than autumn-girdled plants (Table 1). While control and autumn-girdled plants had vital and branched root systems, root systems of all plants that had been cut in June were dead; they were dark brown and brittle (Comas et al. 2000, Richter et al. 2007).

### C and N content

We did not find effects of girdling on C content in stems above the girdle and roots, except for the March 2008 sampling where the C content in stems below the girdle was significantly lower in autumn-girdled and spring-girdled plants than in controls (Table 2). In roots, C content was slightly lower than in stems. In March 2008, N content was lower in autumn-girdled stems below the girdle than in controls. In August 2008, N content in stems above the girdle was higher in autumn-girdled than in control plants, whereas in coarse roots, it was higher in control than in autumn-girdled plants. Both C and N content significantly differed between different plant parts ($P < 0.001$), and N content additionally varied over time ($P < 0.001$).

### Carbohydrate content

The content of carbohydrates in the plants varied markedly between different seasons and treatments. Lugol staining re-

Table 2. Carbon (C) and nitrogen (N) content of control, autumn-girdled and spring-girdled plants. Mean values ± standard errors followed by the same lower case letter for C or N within a line are not significantly different. Different capital letters indicate significant differences over time. Significance of time, girdling, plant part and interaction effects on C and N content was according to repeated-measures multivariate analysis of variance. T, time; G, girdling (treatment); P, plant part; AG, girdled in autumn 2007; SG, girdled in spring 2008; n.s., not significant. *$P < 0.05$; ***$P < 0.001$. Values are presented as the mean ± standard error; $n = 4$.

| | C (mg $g_{DW}^{-1}$) | | | N (mg $g_{DW}^{-1}$) | | |
|---|---|---|---|---|---|---|
| | Control | AG | SG | Control | AG | SG |
| Stem above the girdle | | | | | | |
| November 2007 | 46.41 ± 0.25 a A | 46.16 ± 0.24 a A | – | 1.02 ± 0.06 a A | 1.05 ± 0.06 a B | – |
| March 2008 | 47.88 ± 0.57 a A | 47.37 ± 0.52 a A | 46.02 ± 0.29 ab | 1.05 ± 0.08 a A | 1.03 ± 0.02 a B | 0.99 ± 0.08 a |
| August 2008 | 47.81 ± 0.31 a A | 47.19 ± 0.38 a A | – | 0.56 ± 0.06 b B | 1.32 ± 0.02 a A | – |
| Stem below the girdle | | | | | | |
| November 2007 | 46.29 ± 0.08 a A | 46.58 ± 0.20 a B | – | 0.92 ± 0.05 a A | 0.89 ± 0.06 a A | – |
| March 2008 | 48.13 ± 0.57 a A | 45.64 ± 0.45 b B | 45.59 ± 0.19 b | 0.98 ± 0.04 a A | 0.80 ± 0.03 ab A | 0.92 ± 0.06 a |
| August 2008 | 46.71 ± 0.06 a A | 46.86 ± 0.34 a AB | – | 0.51 ± 0.02 a B | 0.48 ± 0.03 a B | – |
| Coarse root | | | | | | |
| November 2007 | 43.61 ± 0.16 a A | 44.37 ± 0.42 a A | – | 0.74 ± 0.07 a A | 0.60 ± 0.01 a A | – |
| March 2008 | 42.06 ± 0.76 a A | 40.07 ± 1.29 a B | 40.21 ± 1.01 a | 0.69 ± 0.08 a A | 0.63 ± 0.01 a A | 0.80 ± 0.08 a |
| August 2008 | 43.11 ± 1.46 a A | 42.02 ± 0.52 a AB | – | 0.49 ± 0.02 a B | 0.36 ± 0.01 b B | – |
| Fine root | | | | | | |
| November 2007 | 43.89 ± 0.53 a A | 43.23 ± 1.39 a A | – | 1.15 ± 0.02 a A | 1.06 ± 0.04 a A | – |
| August 2008 | 41.33 ± 1.09 a A | 41.97 ± 0.35 a A | – | 0.79 ± 0.07 a B | 0.88 ± 0.04 a B | – |
| T | n.s. | | | *** | | |
| G | n.s. | | | n.s. | | |
| P | *** | | | *** | | |
| T × G | * | | | *** | | |
| T × P | *** | | | *** | | |
| G × P | n.s. | | | *** | | |
| T × G × P | n.s. | | | *** | | |

Table 3. Significance of time, girdling and interaction effects on content of metabolites (starch, sucrose, glucose, fructose, raffinose and stachyose) in stems above and below the girdle, coarse roots and fine roots according to repeated-measures multivariate analysis of variance. T, time; G, girdling; n.s., not significant. *$P < 0.1$; **$P < 0.05$; ***$P < 0.01$; ****$P < 0.001$; $n = 4$.

| | Above the girdle | | | Below the girdle | | | Coarse root | | | Fine root | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Parameter | T | G | T × G | T | G | T × G | T | G | T × G | T | G | T × G |
| Metabolite (mg $g_{DW}^{-1}$) | | | | | | | | | | | | |
| Starch | **** | n.s. | **** | **** | n.s. | * | **** | *** | n.s. | **** | **** | ** |
| Sucrose | **** | n.s. | *** | **** | n.s. | n.s. | **** | n.s. | * | **** | *** | ** |
| Glucose | **** | **** | **** | **** | n.s. | n.s. | **** | *** | *** | **** | ** | **** |
| Fructose | *** | n.s. | n.s. | ** | n.s. | n.s. | *** | *** | ** | **** | ** | **** |
| Raffinose | **** | **** | *** | *** | n.s. | * | **** | n.s. | ** | **** | n.s. | n.s. |
| Stachyose | **** | *** | **** | **** | ** | *** | **** | n.s. | **** | **** | n.s. | n.s. |

vealed that the major starch amount was located in the bark of stems and time effects on stem starch content were highly significant ($P < 0.001$; Table 3). In stems above the girdle, starch content was significantly higher in autumn-girdled than in control plants. It reached a maximum of 130 mg $g_{DW}^{-1}$ compared with 80 mg $g_{DW}^{-1}$ in April 2008, thereby following the same pattern in both treatments (Figure 3). Then, starch content significantly decreased in both treatments and values of autumn-girdled plants fell below those of control plants. Spring girdling led to a significant starch accumulation in stems above the girdle, leading to a pattern differing from the other treatments. In stems below

the girdle, there were no differences in starch content between the treatments, but the same variation over time ($P < 0.001$) as in stems above the girdle. In coarse roots as well as in fine roots, starch content decreased in response to girdling. Overall, starch content was lowest in fine roots. In leaves, we did not find any effect of girdling on the starch content (data not shown).

Sucrose concentrations were lower than starch content in all tissues but also varied over time ($P < 0.001$; Table 3). In general, sucrose concentration was highest in coarse roots, irrespective of the treatment, with the values up to 60 mg $g_{DW}^{-1}$ during winter. In stems above and below the girdle and in fine
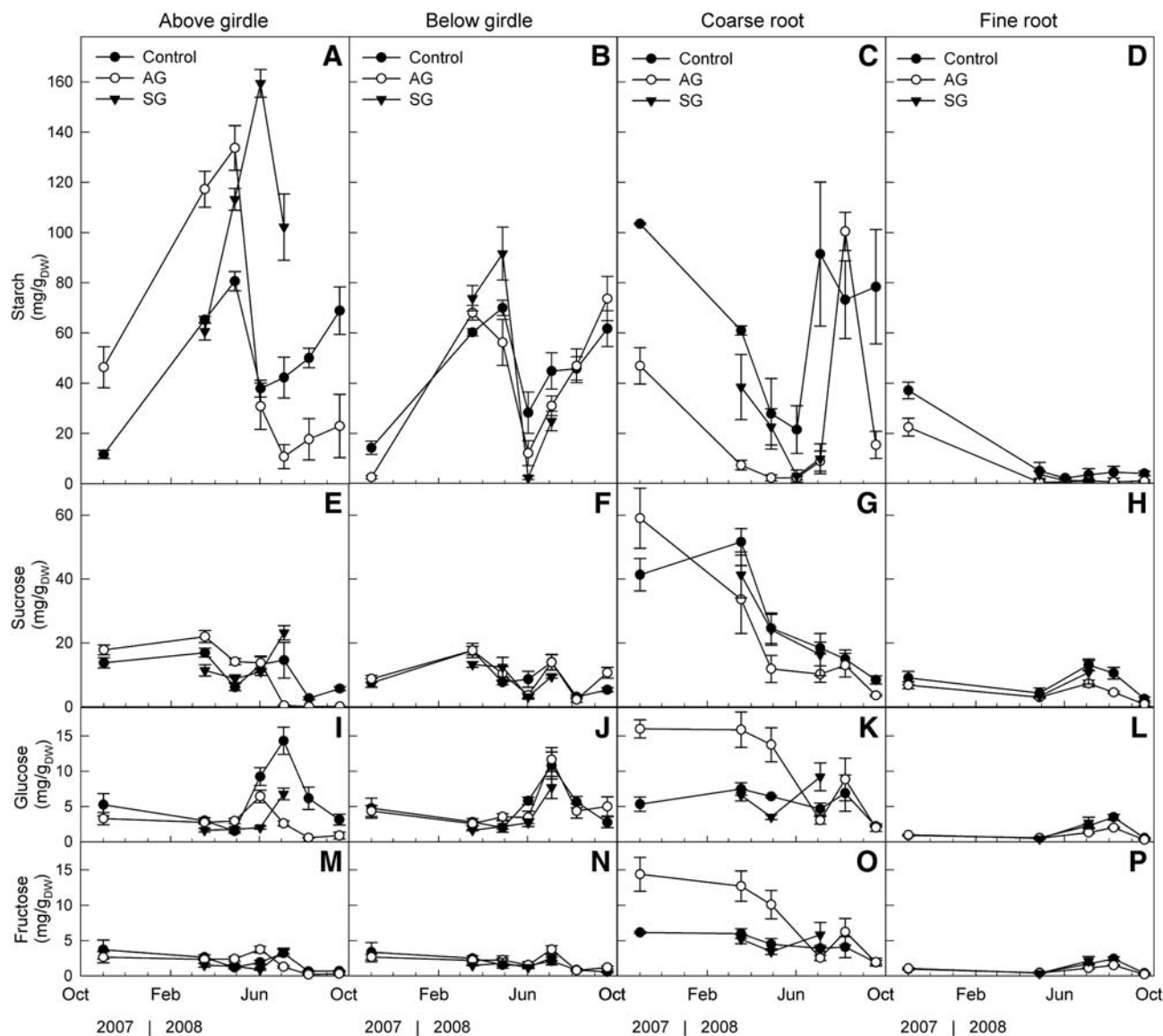
Figure 3. (A–D) starch, (E–H) sucrose, (I–L) glucose and (M–P) fructose content of stems above and below the girdle, coarse roots and fine roots of control, AG and SG cutting-derived poplar from November 2007 until September 2008. AG, girdled in autumn 2007; SG, girdled in spring 2008. Values are presented as the mean ± standard error; $n = 4$.

roots, sucrose concentrations never exceeded 25 mg $g_{DW}^{-1}$ (Figure 3).

The glucose and fructose concentrations in stems and roots of control plants were low during the dormant season, but after bud flush, the glucose concentration increased in stems concomitantly with the decrease in starch (Figure 3). Girdling did not have an effect on glucose and fructose concentrations in stems below the girdle, but in coarse roots of autumn-girdled plants, glucose and fructose were significantly higher during the dormant season than in control plants (Table 3, Figure 3). There were no effects of girdling on the sugar contents of leaves (data not shown).

Raffinose and stachyose concentrations were high during the dormant season, dropped between March and April 2008 concomitantly with bud flush, remained low during summer

and began to increase again in early autumn (Figure 4). Overall, in the stems of control plants, the raffinose and stachyose concentrations during winter were higher than in roots. In autumn-girdled plants, the raffinose and stachyose concentrations in the stems were lowered to values comparable to those of roots.

## Discussion

In our study, we combined analysis of short-term and longer-term responses of trees to girdling and determined the changes in the carbohydrate status of all heterotrophic parts of poplar over 1 year with high resolution. The effects of girdling on plant morphology occurred only after several
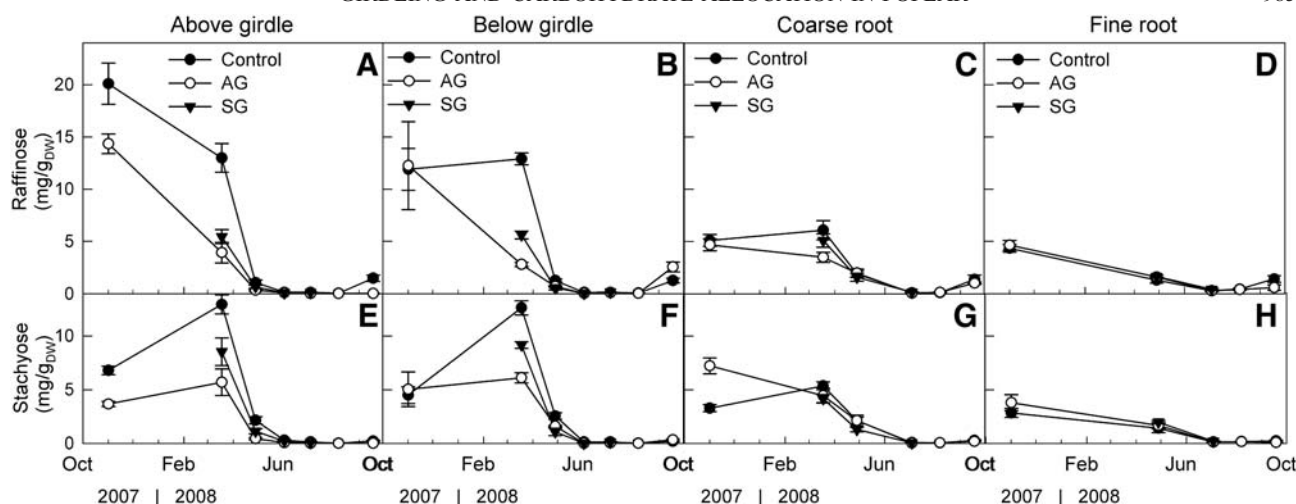
Figure 4. (A–D) raffinose and (E–H) stachyose content of stems above (A, E) and below (B, F) girdle, coarse roots (C, G) and fine roots (D, H) of control, AG and SG cutting-derived poplar from November 2007 until September 2008. AG, girdled in autumn 2007; SG, girdled in spring 2008. Values are presented as the mean ± standard error; $n = 4$.

months. After the onset of bud break, above-girdle shoots of autumn-girdled plants dried out. Injury to the cambium by girdling inhibited the formation of a new tree ring above the girdle in autumn-girdled plants, leading to insufficient water supply of the buds. Spring-girdled plants had started to produce early wood before girdling, which was sufficient to supply the shoot with water. The risk that plants or plant parts can be damaged or even die after girdling, as observed in our study, can be a limitation of this method for practical applications (Goren et al. 2003). Both autumn-girdled and spring-girdled plants produced sprouts below the girdle, while in control plants, vigour was highest at the apex and decreased downwards along the stem (Figure 2). This suggests that girdling broke apical dominance, as has also been shown for conifers by Wilson and Gartner (2002). Even in spring-girdled plants, which also sprouted above the girdle, dormancy of the buds at the base of the stem was removed. In an earlier study, it also has been shown that girdling of poplar promotes sprouting at the stem base (Wan et al. 2006). Cutting of the stems close to the soil in early summer led to dieback of 100% of the cut plants by next spring, irrespective of the previous treatment. This contrasts with previous studies, which have shown that poplar and other pioneer tree species have a high re-sprouting ability after aboveground disturbance (Landhäusser and Lieffers 2002, Wan et al. 2006). However, Auclair and Bouvarel (1992) found that poplar shows a high mortality after 4 years biannual coppicing.

The C content of roots was slightly lower than the C content of shoots (Table 2). Bert and Danjon (2006) found that the C content in root wood of maritime pine was lower than in stem sapwood and suggested that this might have been due to the starch concentration. In our plants, however, this could not be the case, as the starch content in roots was not lower than in the shoot. Possibly, the difference in C content between roots and shoots was because of differences in

structural compounds like cellulose or lignin. We found a significantly increased N content in stems above the girdle of autumn-girdled plants in August 2008 (Table 2). Siebrecht and Tischner (1999) have shown that nitrate is an important transport form of N in poplars. The majority of N assimilation occurs in the leaves of poplar by nitrate reductase, whereas in stems and roots, nitrate reductase activity has been shown to be generally low (Black et al. 2002). This suggests that, in autumn-girdled plants, nitrate has still been transported to the shoot, but could not be assimilated due to the absence of leaves and accumulated in stems above the girdle during spring and early summer 2008. The lower N content in coarse roots of autumn-girdled than control plants in August 2008 might be explained by strongly decreased N uptake of girdled plants, as has been shown for peach (Jordan et al. 1998).

In previous studies, it has been shown that starch amount in stems and roots is highest from autumn during the dormant season, decreases in spring during bud break and is then replenished during summer and early autumn (Johansson 1993, Bollmark et al. 1999, Kaelke and Dawson 2005), which is consistent with our findings in all analysed tissues (Figure 3). These results can be explained by changes in the source–sink balance within a tree throughout the year (e.g., Dickson 1991, Dickson et al. 2000). On one hand, current assimilates are partitioned into growth and storage, which explains that reserve filling of the heterotrophic organs occurs mainly late in the growing season, when growth has been completed. On the other hand, heterotrophic plant organs can change between being a sink for assimilates during the growing season and being a source during the dormant season and until bud flush has finished (Dickson 1991). Girdling prevented the transport of assimilates towards the roots, leading to an accumulation of starch in stem tissue above the girdle, with a concomitant depletion of starch in coarse roots, as has been observed previously (Dunn and Lorio 1992, Jordan and Habib 1996). In fine roots, starch amount also decreased in response

to girdling, but not as drastically as has been shown by Frey et al. (2006) in fine roots of sweet chestnut (*Castanea sativa* Mill.). In our plants, the starch amount in fine roots was much lower than in coarse roots. Barbaroux et al. (2003) found that, in *Fagus sylvatica* L. and *Quercus petraea* (Matt.) Liebl., starch reserves of coarse roots were higher than in fine roots, but not to such an extent as in our poplar plants. Possibly, poplar has a higher turnover rate for fine roots than other tree species and, therefore, does not store large amounts of reserves in fine roots. Until now, there have been no studies available comparing fine root turnover rates of different deciduous tree species, but it has been shown that fine root turnover of poplar is twice as high as that of pine (Coleman et al. 2000).

In control plants, the starch amount in coarse roots decreased to a minimum of about 20 mg $g_{DW}^{-1}$ in early June (Figure 3). This agrees with the findings of Lacointe et al. (1993) who showed that only the most recently formed starch was mobilized in oak roots during the dormant season and sprouting in spring and that older starch reserves were maintained. They concluded that these reserves could be of importance for the plants in the case of exceptional stress like total defoliation. As starch reserves in coarse roots of autumn-girdled plants were completely depleted during spring, we assume that poplar also has longer-term reserves, which can help the plants to survive in cases when they lose source tissue. This can happen, for example, by fire, insect feeding or browsing by animals (Kosola et al. 2001, Carter et al. 2004). Interestingly, in stem tissue below the girdle, we did not find any differences between the treatments. We propose that girdled plants preferentially deplete their root reserves while maintaining reserves in stem tissue below the girdle. As the apical dominance of the plants was broken due to girdling, the remaining starch reserves in stems below the girdle might facilitate sprouting at the stem base and, as a result, a faster replenishment of the reserves in roots.

The concentrations of raffinose and stachyose were high during winter in all tissues and treatments (Figure 4). Raffinose has been shown to increase in response to chilling in poplar leaves, leading to an enhanced freezing tolerance (Renaut et al. 2004). In roots of spruce, raffinose has also been shown to increase in response to cold (Wiemken and Ineichen 1993). In birch xylem and cortical tissue, it has been shown that raffinose and stachyose increased during winter, protecting the tissue from freezing (Kasuga et al. 2007). In our study, we could show that, also in poplar roots, raffinose and stachyose accumulate during winter, correlating with temperature decrease and the need for freezing protectants. However, concentrations of these sugars were lower in roots than in shoots, presumably because roots are protected by the soil and, therefore, exposed to milder frost than stems.

## Conclusion

In conclusion, we were able to show that poplar responds to girdling much faster on the metabolic than on the phenolog-

ical level. Mobilization of root starch to maintain soluble sugar concentrations for maintenance respiration occurred already a few weeks after autumn girdling, whereas the phenology was not altered until the next spring. Only by the end of April did we find visible differences between control and girdled plants. Carbohydrates accumulated above the girdle, and roots of autumn-girdled plants depleted their reserves, presumably in order to maintain reserves in stems for sprouting below the girdle in the following spring. Nevertheless, raffinose and stachyose accumulated during winter even in autumn-girdled plants, which is consistent with their proposed importance in protecting plants from cold, even under resource-limited conditions. The complete dieback of plants cut in summer suggests that roots rely heavily on support from the shoot during summer, whereas in winter, they can maintain themselves due to the stored reserves.

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