Partitioning of reserve and newly assimilated carbon in roots and leaf tissues of *Lolium perenne* during regrowth after defoliation: assessment by $^{13}$C steady-state labelling and carbohydrate analysis

A. MORVAN-BERTRAND,* N. PAVIS,* J. BOUCAUD & M.-P. PRUD’HOMME

U.A. INRA Physiologie et Biochimie Végétales, IRBA, Université, Esplanade de la Paix, 14032 Caen, Cedex, France

ABSTRACT

The relative significance of the use of stored or currently absorbed C for the growth of leaves or roots of *Lolium perenne* L. after defoliation was assessed by steady-state labelling of atmospheric CO$_2$. Leaf growth for the first two days after defoliation was to a large extent dependent on the use of C reserves. The basal part of the elongating leaves was mainly new tissue and 91% of the C in this part of the leaf was derived from reserves assimilated prior to defoliation. However, half of the sucrose in the growth zone was produced from photosynthesis by the emerged leaves. Fructans that were initially present in elongating leaf bases were hydrolysed (loss of 93 to 100%) and the resulting fructose was found in the new leaf bases, suggesting that this pool may be used to support cell division and elongation. Despite a negative C balance at the whole-plant level, fructans were synthesized from sucrose that was translocated to the new leaf bases. After a regrowth period of 28 d, 45% of the C fixed before defoliation was still present in the root and leaf tissue and only 1% was incorporated in entirely new tissue.

Key-words: *Lolium perenne* L.; C-13; defoliation; elongating leaves; fructans; photosynthates; regrowth; reserves; sheaths; water-soluble carbohydrates.

Abbreviations: RSA, relative specific allocation.

INTRODUCTION

The persistence of grassland species such as *Lolium perenne* is greatly dependent on their ability to grow after cutting or grazing. The stubble left behind after defoliation is mainly composed of fully expanded leaf sheaths and the enclosed elongating leaves. Growth of grass leaves is confined to their basal part: elongating leaves have successive zones of cell division, cell elongation and cell maturation and are a strong sink for photosynthate (Allard & Nelson 1991). The first leaf tissue exposed above the cutting level is the result of expansion of the cells located at the base of the growing leaf and consists therefore of cells which already existed at the time of defoliation. Anatomical characteristics of the growing leaf distal to the cutting level are similar to those of mature leaf blades and this region is photosynthetically active as it emerges from the whorl of the older sheaths (Wilhelm & Nelson 1978). Defoliation reduces the amount of leaf surface and, thereby, the supply of photosynthate to the leaf meristems. It is generally held that leaf growth after defoliation depends first on pre-defoliation reserves and then on post-defoliation assimilates (Alberda 1957; Davidson & Milthorpe 1966a; Danckwerts & Gordon 1987; Gonzalez et al. 1989; Johansson 1993; De Visser, Vianden & Schnyder 1997).

In *Lolium perenne*, the dry matter and C concentration of leaf growth zones transiently decreased during the two first days following defoliation (De Visser et al. 1997). This depletion indicates a decreased net influx of carbohydrates in the growth zone and was associated with use of pre-defoliation C. Pre-defoliation C might essentially refer to the carbohydrate reserves since depletion of carbohydrates in the stubble of grasses has been observed in many species during the initial phase of regrowth (Ward & Blaser 1961; Davidson & Milthorpe 1966a; Danckwerts & Gordon 1987; Gonzalez et al. 1989; Ward & Blaser 1961; Yamamoto & Mino 1987; Gonzalez et al. 1989; Fischer et al. 1997). This depletion was interpreted in terms of remobilization and use of reserves for leaf growth. However, in most of these studies no distinction was made between sheaths which do not grow but may serve as a source of C reserves to sustain the activity of leaf meristems, and leaf growth zones which are strong sinks for remobilized reserves and post-defoliation assimilates (Allard & Nelson 1991). Fructans accumulate preferentially in the elongating leaf bases (Schnyder & Nelson 1987; Schnyder, Nelson & Spollen 1988). Their concentrations decrease after cell elongation ceases and during active deposition of the cell wall (Allard & Nelson 1991). Only a few studies on refoliation
recognize the functional heterogeneity of stubble (Volenec 1986; Morvan et al. 1997; De Visser et al. 1997). Examining changes in concentration of carbohydrates in individual leaf sheaths and bases of elongating leaves from *Festuca* during regrowth after defoliation, Volene (1986) showed that fructan concentrations decline not only in leaf sheaths but also in elongating leaf bases. The same trend occurred in *Lolium perenne* where fructan depletion in the basal parts of regrowing leaves accounted for more than 50% of the decrease in carbohydrate level (Morvan et al. 1997).

The carbohydrate content of roots also decreased after defoliation (Davidson & Milthorpe 1966b; Gonzalez et al. 1989). Both C balance and 14C tracer studies suggest that roots remain a net sink for carbohydrates even immediately after defoliation (Davidson & Milthorpe 1966b; Danckwerts & Gordon 1987). Moreover, isolation of meristematic root zones in *Lolium perenne* showed that root growth after defoliation drew first on pre-defoliation reserves. Post-defoliation photosynthesis became the main source of carbon slightly later for root tips than for the leaf meristem (De Visser et al. 1997).

Our specific aims were (1) to establish the detailed partitioning of reserve and newly assimilated carbon in roots and leaf tissues during regrowth after defoliation and (2) to evaluate the relative involvement of fructans from leaf sheaths and from elongating leaf bases to the regrowth. To these ends, we took the functional heterogeneity of the shoot into consideration and we sampled the plants so that the parts left behind by defoliation could be distinguished from entirely new shoots and from parts regrowing from pre-existing tissues. Before being defoliated, the plants were supplied with 13CO2 in steady-state conditions in order to discriminate between the two sources of carbon for regrowth. We followed the fate of 13C in hexoses, sucrose and fructans to estimate the C availability in the reserves and the source of translocated C to sustain the shoot and root regrowth.

**MATERIALS AND METHODS**

**Plant material**

Seeds of *Lolium perenne* L. var. Bravo were germinated and grown for 8 weeks in a controlled environment on a nutrient solution (in 9 dm3 polyvinyl chloride pots) as described previously by Gonzalez et al. (1989). After 2 months of growth, the plants were defoliated at 4 cm above the ground level. Tillers of one plant were separated in order to obtain clonal plants. Clones were grown for 8 months, being defoliated and divided again every 2 months. After 10 months of growth, the plants were defoliated and transferred to a growth cabinet (E15; Conviron, Winnipeg, Canada) modified for 13CO₂ labelling, with a 16 h photoperiod (300 µmol photons m⁻² s⁻¹ at the top of the foliage) and a day : night temperature regime of 23 : 18 °C. In the growth cabinet, clonal plants were grown in 3-5 dm³ pots (three plants per pot) and the nutrient solution was replaced every fifth day.

**Labelling procedure**

A carbon labelling procedure was used to obtain plant matter labelled with 13C near their natural abundance. The 13CO₂ assimilation and labelling experiments were adapted from a procedure previously described by Avice et al. (1996). The 13C labelling began 4 weeks after the last defoliation and was continued for 3 weeks. Plants were transferred from natural atmosphere to air that was enriched to 1·278 atom percentage 13C (0·190% over natural abundance) by using 13CO₂. The CO₂ concentration was maintained constant at 450 mm³ dm⁻³ in the chamber. The 13C level of CO₂ was controlled by analysing triplicate samples of the atmosphere taken four times per 24 h (at the beginning and at the end of the light and the night periods) through an exit valve using previously evacuated 30 cm³ flasks. During labelling and chase periods, at the beginning and end of each photoperiod, CO₂-free air (obtained from ambient air compressed to approximately 8 MPa by a screw compressor, and passed through a NaOH column) was injected for 20 min into the cabinet to evacuate CO₂ and, thus to reduce plant reassimilation of 13CO₂. Moreover, in order to eliminate the plant isotope discrimination, the 13CO₂ exposure was started at the beginning of each light period and stopped 2 h before the light was switched off. Thus, the CO₂ had disappeared almost totally from the chamber atmosphere by the end of the light period. As a consequence, all CO₂ introduced into the chamber during the light period was assimilated and the plant isotope fractionation, which occurs only when the source of CO₂ is infinite, was avoided (Berry & Throughton 1974; Deléens, Cliquet & Prioul 1994). At the end of the labelling period, the assimilation cabinet was opened and quickly purged with ambient air. The plants were then defoliated at 4 cm above the ground level and allowed to regrow for up to 28 d in an atmosphere that was not 13C enriched (natural atmosphere with 350 mm³ dm⁻³ of CO₂ and a natural abundance of 1·108%).

**Leaf growth measurements**

Distances between the top of an elongating leaf and the level of defoliation visible on the surrounding leaf sheaths were measured with a ruler for 3d following defoliation in 10 tillers.

**Harvest procedures**

Plants were sampled at 0, 2, 4, 6 14 and 28 d after defoliation. Each day, triplicate pots containing three plants each, were harvested. Each plant contained between 15 and 40 tillers. Roots were separated from shoots and frozen in liquid nitrogen and the shoots were placed on ice to be dissected. The surrounding old sheaths were harvested on each sampling day, and, during regrowth, new leaf tissues developed and constituted new plant parts. Successively, eight new plant parts were distinguished according to their position in relation to the defoliation level: bases (from 0 to...
4 cm above the ground level) and tops (above 4 cm) of cut elongating leaves, sheaths and blades of cut mature leaves, bases and tops of new leaves, new mature leaves and new tillers. All plant tissues were frozen in liquid nitrogen, freeze-dried, ground to a fine powder and stored at +5 °C in a dessicator for subsequent analysis.

**Extraction and measurement of water soluble carbohydrates**

Soluble carbohydrates were extracted from freeze-dried tissues by first boiling under reflux (1 h) with 80% ethanol followed by boiling water under reflux (1 h). Extracts were dried under vacuum, combined and redissolved in water. After filtration through a 0.45 μm nylon membrane (Gelman Sciences, Ann Arbor, MI, USA), aliquots of carbohydrate extracts were passed through a column containing cation exchange resin (Dowex 50 W, H⁺-form, Sigma, St. Louis, MO, USA) and anion exchange resin (Amberlite IRA-416; Fluka, Buchs, Switzerland) to remove charged compounds (Smouter & Simpson 1991). The columns were eluted with water and samples were concentrated under vacuum and dissolved in water. Glucose, fructose, sucrose and fructans were quantified by high-performance liquid chromatography (HPLC) on a cation exchange column (Sugar-PAK, 300 mm × 6.5 mm, Millipore Waters, Milford, MA, USA) as described by Guerrand, Prud’homme & Boucaud (1996) using mannitol as an internal standard. The relative specific allocation percentage (RSA) is the proportion of newly incorporated atoms relative to total 13C input in the whole tiller:

\[
\text{RSA}_{\text{A}} = \left(\frac{Q_{13C_{\text{organ}}}}{Q_{13C_{\text{tiller}}}}\right) \times 100
\]

The relative specific allocation percentage (RSA) is the proportion of newly incorporated atoms relative to total atoms in the sample. Thus, by using the A% expression:

\[
\text{RSA} = \left(\frac{\text{A\%}_{\text{13C sample}} - \text{A\%}_{\text{13C control plant}}}{\text{A\%}_{\text{13C air supplied}} - \text{A\%}_{\text{13C control plant}}}\right) \times 100
\]

**Accuracy of the determination of the RSA parameter**

The accuracy of the determination of the RSA parameter is closely related to the maximum isotopic dilution range between control and labelled CO₂ and the precision of isotopic measurement (Cliquet et al. 1990). The long-term automatic CO₂ supply was successfully carried out at a constant δ¹³C value of 152.2‰ from the seventh day to the end of the exposure period. The first week was probably the time necessary for the complete loading of all the surface of the chamber and all the exchange surfaces of plant tissues. Thus, the maximum theoretical range for δ¹³C values in labelled plant organs was from –31.8‰ (control...
plants) to +152·2‰ (i.e. 184·0‰). The standard error on $\delta^{13}C$ determination was 0·2‰. As a consequence, the minimum significant value for the RSA value was 0·1% ($2/184 \times 100$). This experimental error was very small in comparison with individual plant variability (Deléens et al. 1994).

**RESULTS**

**Growth rate of the leaves of Lolium perenne after defoliation**

Plant tillers were defoliated 4 cm above the shoot/root junction. Defoliation was therefore relatively severe, since all blades were removed. The base of the expanding leaves enclosed within the whorl of mature sheaths was composed of the zone of cell division, the zone of elongation (3 cm long portion) and half of the maturation zone (3 to 5 cm above the point of leaf attachment) (unpublished results). The leaf bases continued to grow after defoliation. Leaf elongation proceeded at about 3 cm the first day and 2 cm d$^{-1}$ afterwards (Fig. 1). Thus, the base of the expanding leaves enclosed in the 4 cm high stubble left behind after defoliation reproduced its own length in less than 2 d.

**Defoliation effects on dry-matter production and relative distribution of $^{13}C$ within the plant**

Results are expressed on a tiller basis rather than on a plant basis to remove variations due to the differing number of tillers per plant.

Dry matter of the roots decreased during the first four days of regrowth and increased, thereafter, to values higher than those at the day of defoliation (Fig. 2a). The DW of the remaining sheaths (old sheaths), declined to approximately half their initial values following four days of regrowth and did not vary significantly afterwards so that

![Figure 1. Length of the elongating leaf that grew above the defoliation level during the first 3 d following defoliation in *Lolium perenne*. Plants were cut at 4 cm above the ground level. Vertical bars indicate $\pm$ SE ($n = 10$) when larger than the symbol.](image)

![Figure 2. Dry weights (a, b, c) and $^{13}C$ excess content (d, e, f) of the different plant parts of *Lolium perenne* in the 28 d after defoliation.](image)
their dry weight at the end of the regrowth period (28 d) was similar to their dry weight four days after defoliation (Fig. 2a). Bases of cut elongating leaves reproduced their own length in less than 2 d. Thus, at successive harvest days (2, 4 and 6 d after defoliation), they were at the same developmental stage but belonged to a different age group since their constituent cells were newly elongating. In plants that recovered from defoliation for 2 d, the bases of cut elongating leaves contained less dry matter than at the time of defoliation (Fig. 2b). Hence, immediately after defoliation, the rate of dry matter incorporation in leaf bases decreased. A similar decrease in dry matter happened between the second and the fourth day of regrowth and occurred thereafter until the 14th day after defoliation. Part of this decrease could be explained by the fact that some cut elongating leaves had become mature and so had been considered as ‘bases of new leaves’ (Fig. 2b). Bases of cut elongating leaves showed a transient 6% decrease in C concentration over the first six days following defoliation (371–347 mg g\(^{-1}\) DW). Carbon concentration slightly increased (<2%) between the sixth and the 14th day of regrowth. Cut leaves which were elongating at the time of defoliation became completely mature between the 14th and the 28th day of regrowth. Therefore, shoot fractions including the bases and tops of cut elongating leaves, no longer existed at the 28th day (Fig. 2b). On the sixth day of regrowth, the new elongating leaves emerged from the stubble (Fig. 2b) and became mature after the 14th day following defoliation (Fig. 2c).

\( ^{13}C \) excess content

During the first week of regrowth, \( ^{13}C \) excess content dropped by 29% in roots and 61% in old sheaths (Fig. 2d). In the bases of cut elongating leaves, \( ^{13}C \) excess content also declined from the very beginning of regrowth (Fig. 2e). The contribution of the pre-defoliation C to the production of new leaves declined rapidly after defoliation, as shown by \( ^{13}C \) excess content which dropped by 46% during the initial two days of regrowth and by 60% during the two following days. In the tops of cut elongating leaves emerged from stubble after two days of regrowth, the \( ^{13}C \) excess content was 2-5-fold less than in the leaf bases on the day of defoliation (Fig. 2e). Considering the growth rate of new leaves (5 cm in 2 d) and the height of cutting (4 cm), it follows that about 80% of cut elongating leaf tops that emerged from the stubble on the second day of regrowth was already present before defoliation. Hence, during the two first days, cells of leaf bases lost about 50% of their \( ^{13}C \) excess content when they were displaced from the basal to the distal end of the elongating leaf. Part of the \( ^{13}C \) might have been lost through respiration and part might have been the source of C for new tissue in the leaf base. On the fourth day of regrowth, an excess of \( ^{13}C \) was found in plant tissues that did not exist at the time of defoliation, such as the bases of new leaves (Fig. 2e & f). This shoot fraction, together with the tops of new leaves, lost their excess content in \( ^{13}C \) progressively throughout the regrowth period. At the end, the \( ^{13}C \) excess content still present in plants accounted for less than 45% of the total \( ^{13}C \) incorporated before defoliation.

Relative specific allocation

In order to appreciate the relative contribution of pre-defoliation reserves and currently absorbed carbon for shoot and root growth after defoliation, we used the RSA parameter, defined as the proportion of newly incorporated C relative to total C in a given sample (Cliquet et al. 1990). During the 3 weeks labelling period before defoliation, RSA varied from 0 (no incorporation) to 100% in shoots (new material built with labelled element only) and from 0 to 72% in roots (Fig. 3). After defoliation, the RSA of old sheaths did not change significantly until the sixth day of regrowth. However, between the sixth and 14th day of regrowth, the RSA dropped by 20%.

In roots, the RSA did not vary significantly during the first four days and declined thereafter until the end of regrowth.

Two days after defoliation, the cells in the bases of cut elongating leaves had an RSA of 91% which means that a high proportion of their carbon had been assimilated before the defoliation. The remaining 9% might have been provided by shoot tissues that had emerged from the stubble, which with an RSA of 89% were probably already photosynthetically active.

On the fourth day of regrowth, some leaves which were elongating on the day of defoliation became mature; their RSA was high (89%) compared with the RSA of the bases and tops from the remaining cut elongating leaves (40 and 61%, respectively). The new C was acropetally distributed along the axis of the leaves. On the fourth and sixth days of regrowth, the quantitative contribution of reserves to the carbon required for the growth of new leaves was similar to that of current photosynthate (RSA = 45–50%).

The sheaths and blades of cut mature leaves exhibited RSA values that declined progressively throughout the regrowth period. The RSA of sheaths was always smaller than that of the corresponding blades and this difference increased with time. Current photosynthate became the quantitatively dominant source of carbon for new leaves between the sixth and 14th day. At the 28th day, the dependence of growth on stored C reserves was almost negligible, as shown by RSA values of new tillers and of new elongating leaves of less than 1%.

Partitioning

While RSA quantifies carbon turnover in a given tissue, partitioning represents the proportion of the labelled C in a given part, relative to the total labelled element in that plant.

Values for labelled C partitioning at the end of the regrowth period are given in Table 1. The data show that the younger the shoot tissues were, the less pre-defoliation C they contained. Approximately 87% of \( ^{13}C \) was found in tissues left behind by defoliation, namely the old sheaths.
and most of the roots, whereas less than 1% was incorporated in entirely new shoot. The remaining 12% was present in blades of cut mature leaves part of which was already in the bases of elongating leaves at the time of defoliation. Leaves which elongated on the 28th day of regrowth relied no more on pre-defoliation C but drew only on current assimilate.

**Effect of defoliation on carbohydrate levels and fructan composition within the plant**

**Sucrose**

In old sheaths, the sucrose concentration decreased to approximately 46% of its initial concentration following six days of regrowth (Fig. 4a). In roots, sucrose was depleted during the first two days of regrowth and replenished from the second to the 28th day (Fig. 4a). The new shoot tissue (bases of cut elongating leaves) produced after defoliation contained 2·3 times less sucrose at the second day than the bases of elongating leaves present on the day of defoliation (Fig. 4b). At the fourth and sixth days the sucrose concentration was at the lowest level but it started to increase by the sixth. This trend also occurred in the bases of new leaves (Fig. 4b). The tops of cut elongating leaves (Fig. 4b) that emerged from stubble at the second day contained 85% less sucrose in their dry matter than the bases of elongating leaves from which they were mainly issued (Fig. 4b). Sucrose concentration did not vary significantly until the sixth and 14th days and reached a similar value to the sucrose concentration in blades of corresponding leaves (Fig. 4c). However, the sucrose concentrations in these tissues were 20 times lower than in new mature leaves at the end of the regrowth period (Fig. 4c).

**Hexoses: glucose and fructose**

In old sheaths, glucose and fructose concentrations dropped by 93 and 86%, respectively, during the first six days of regrowth (Fig. 4d & g). In roots, glucose and fructose concentrations were very low (less than 1 mg g⁻¹DW) when

---

**Table 1.** Partitioning of C incorporated during the labelling period before defoliation and remaining at the end of the regrowth period (28 d after defoliation). Values are the mean ± SE of three replicate determinations

| Partitioning (%) | Roots 53·2 ± 4·3 | Old sheaths 33·7 ± 4·5 | Sheaths of cut mature leaves 0·6 ± 0·2 | Blades of cut mature leaves 12·2 ± 0·6 | Bases of elongating leaves 0 | Tops of elongating leaves 0·1 ± 0·1 | Sheaths of new leaves 0·2 ± 0·2 | Blades of new leaves 0 | New tillers 0 |

compared with old sheaths (Fig. 4d & g). They also decreased after defoliation. Glucose level was the lowest at the sixth day and fructose at the fourth. In bases of cut elongating leaves where new cells were produced, glucose and fructose concentrations decreased during the four first days of regrowth. At the fourth day, both hexose concentrations were the lowest, then they increased rapidly and reached their initial concentrations at the sixth day. In bases of new leaves (Fig. 4e & h), glucose and fructose levels were low at days 4 and 6. The main increase occurred between the sixth and 14th day. The tops of cut elongating leaves that emerged from the stubble at day 2 existed already at the time of defoliation in bases, but contained 7-8 times less glucose and 10 times less fructose in their dry matter than on the day of defoliation (Fig. 4e & h). In sheaths and blades of cut mature leaves (Fig. 4f & i), glucose and fructose concentrations were always lower than in bases and tops of growing leaves (Fig. 4e & h). In both leaves, the hexose concentrations increased by the sixth day of regrowth.

© 1999 Blackwell Science Ltd, Plant, Cell and Environment, 22, 1097–1108

Figure 4. Concentrations of sucrose (a, b, c), glucose (d, e, f), fructose (g, h, i) and fructan (j, k, l) in the different plant parts of Lolium perenne in the 28 d after defoliation. (□—□), roots; (■—■), old sheaths; (●—●), bases of cut elongating leaves; (○—○), tops of cut elongating leaves; (▲—▲), bases of new leaves; (△—△), tops of new leaves; (●—●), sheaths of cut mature leaves; (○—○), blades of cut mature leaves; (◇—◇), new mature leaves; (△—△), new tillers. Vertical bars indicate ± SE (n = 3) when larger than the symbol.
Fructans

Fructan concentration was highest in the sheaths at the onset of the study and declined rapidly throughout the first days of regrowth. It dropped by 50% within two days and by 95% within six days (Fig. 4j). Fructan depletion in sheaths (~0·66 mg tiller\(^{-1}\)) accounted for 13% of the decrease in dry matter that occurred in sheaths after defoliation (Fig. 2a). In roots, the fructan concentration was 10 times less than in sheaths, and decreased from 1·8 to 0·3 mg g\(^{-1}\)DW within four days at the beginning of regrowth (Fig. 4j). Fructan depletion in roots (~0·06 mg tiller\(^{-1}\)) represented only 1% of the decline in root dry matter. In the bases of cut elongating leaves, fructan also declined as a response to leaf blade removal (Fig. 4k). Nevertheless, given that every two days most of the constituent cells of the bases were newly elongating, the decrease in fructan concentration should not be interpreted in terms of hydrolysis. Rather, it indicates that at the second, fourth and sixth days after defoliation, new tissues were still able to store part of the incoming carbon, regardless of the fact that all the active photosynthetic apparatus has been removed. It is only after the sixth day of regrowth that fructan concentration increased and at the 14th day reached similar values to those observed at the time of defoliation. Comparison of fructan concentrations in tops of cut elongating leaves at day 2 and in bases of cut elongating leaves at the onset of the study (Fig. 4k) showed that fructans that were present on the day of defoliation in bases (19 mg g\(^{-1}\) DW) were not recovered in tops at day 2 (2·7 mg g\(^{-1}\) DW) suggesting that a high proportion were hydrolysed. Concentration of fructans increased in tops of cut elongating leaves by day 4 (Fig. 4k). At the same time it also increased in the blades of cut mature leaves (Fig. 4l). Between the sixth and 14th days, fructan concentration increased four times in the sheaths of cut mature leaves (Fig. 4l) and 10 times in bases of new leaves although it remained at a low level in the tops of the new leaves (Fig. 4l).

At the end of the regrowth period, half of the fructans occurred in the sheaths of new leaves and in bases of elongating leaves (Table 2). In blades and tops of new leaves, fructan concentrations were relatively low, between 4·71 and 7·14 mg g\(^{-1}\) DW. However, these shoot parts contained 37·3% of total fructans because leaf blades represented half of the plant dry matter. Remaining fructans were located in roots (6·9%) and in sheaths (1·2%) that were cut at the onset of the study but were not totally depleted in carbohydrates during the regrowth period.

### Table 2. Relative distribution and concentration of fructan in the different plant parts of *Lolium perenne* at the end of the 28 d regrowth period. Values are the mean ± SE of three replicate determinations

<table>
<thead>
<tr>
<th>Plant Part</th>
<th>Relative distribution of fructan (%)</th>
<th>Fructan concentration (mg g(^{-1}) dry weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roots</td>
<td>6·9 ± 0·7</td>
<td>2·60 ± 0·32</td>
</tr>
<tr>
<td>Old sheaths</td>
<td>1·2 ± 0·2</td>
<td>2·19 ± 0·18</td>
</tr>
<tr>
<td>Sheaths of cut mature leaves</td>
<td>4·0 ± 0·5</td>
<td>6·35 ± 0·55</td>
</tr>
<tr>
<td>Blades of cut mature leaves</td>
<td>7·2 ± 0·8</td>
<td>4·71 ± 0·52</td>
</tr>
<tr>
<td>Bases of elongating leaves</td>
<td>20·1 ± 2·5</td>
<td>42·23 ± 5·22</td>
</tr>
<tr>
<td>Tops of elongating leaves</td>
<td>16·6 ± 2·6</td>
<td>7·14 ± 0·81</td>
</tr>
<tr>
<td>Sheaths of new leaves</td>
<td>30·5 ± 3·2</td>
<td>36·41 ± 4·25</td>
</tr>
<tr>
<td>Blades of new leaves</td>
<td>13·5 ± 1·6</td>
<td>5·64 ± 0·54</td>
</tr>
</tbody>
</table>

Defoliation effects on 13C distribution within different carbohydrate pools

In old sheaths, the RSA value of fructan remained constant until the fourth day following defoliation (Fig. 5j) and declined thereafter. The RSA values of sucrose, glucose and fructose had decreased by the second day of regrowth (Fig. 5a, d & g).

In roots, the RSA value of sucrose was near to 100% whereas that of fructan, glucose and fructose was only about 50% (Fig. 5a, d, g & j) at the end of the labelling period. During the first two days of regrowth the RSA values dropped by more than 50%.

In bases of cut elongating leaves, the RSA values of carbohydrates declined rapidly throughout the first days of regrowth. Two days after defoliation, the fructans contained in the bases of cut elongating leaves were synthesized from both pre-defoliation and post-defoliation carbon as indicated by their RSA value of 53%. The RSA value of sucrose was similar (54%) to the RSA value of fructan. By contrast, RSA values of glucose and fructose were higher (68 and 78%, respectively), suggesting that glucose and fructose did not come solely from the immediate hydrolysis of sucrose or if so, were not used directly.

Two days after defoliation, the tops of cut elongating leaves exhibited a RSA value of 89% but carbohydrates had already low RSA values comprised between 12 and 33% (Fig. 5b, e, h, k). More especially, the very low level of labelled sucrose (RSA 16%) shows that new shoot tissues became rapidly able to fix atmospheric unlabelled CO₂ and to synthesize sucrose. Moreover, the RSA value of sucrose in bases was also strongly reduced between day 0 and day 2. Fructans present in emerged tissues exhibited an RSA value close to the sucrose RSA (12%) but much lower than the RSA value of fructan from leaf bases at the time of defoliation (100%) (Fig. 5k & j).

Tops of new leaves appeared on the sixth day of regrowth. At day 4, the new leaves were totally enclosed within stubble and consequently had to import their carbon from other plant organs. This might be the reason why the...
Partitioning of reserve and newly assimilated carbon in Lolium perenne after defoliation

DISCUSSION

Transition from reserve dependence to current assimilate dependence for shoot growth after defoliation

The physiology of plants after defoliation has two distinct phases. The first one is a transient period during which previously stored reserves are used to rapidly replace lost tissues. The second phase is a readjustment of physiological activity when reserve pools are progressively replenished. According to many studies, the phase of reserve mobilization...
tion lasts for about 1 week (Alberda 1957; Davidson & Milthorpe 1966a; Davies 1988; Gonzalez et al. 1989; Ourry, Bigot & Boucaud 1989; Thornton et al. 1993; Johansson 1993). This estimation is based on the time course of N and C reserve depletion in stubble. A similar result was obtained when foliar tissues of stubble were separated according to their developmental stage and therefore to their function. In Festuca arundinacea the fructan contents decreased significantly during the first week in sheaths that were left behind by defoliation whereas they had increased in new sheaths by day 6 (Volene 1986).

The present data showed that in Lolium perenne, the fructan concentration declined rapidly during the first six days of regrowth in sheaths and started to accumulate in new growing zones and in new sheaths by day 6, as well. Therefore, the re-establishment of a positive whole-plant carbon balance was achieved in 6 d. It does not mean, however, that reserves were the sole source of C for refoliation. As outlined recently by De Visser et al. (1997), the functional heterogeneity of stubble must be taken into consideration to appraise the relative significance of remobilized and current assimilates for regrowth. In Lolium perenne photosynthesis became the most important C source for leaf dry matter accumulation within three days after defoliation as indicated by the predominance of newly fixed carbon in the biomass of the elongation and maturation zones (De Visser et al. 1997). Predominance of the newly fixed carbon over reserve carbon might happen even earlier since growth zone biomass represents the composition of assimilates accumulated in the plant prior to sampling (De Visser et al. 1997). Our data on new to pre-defoliation carbon ratios in sucrose show that the carbon imported by the shoot growing zones was already half new on the second day of regrowth. Therefore, the quantitative contribution of current photosynthates to the carbon required for shoot regrowth, starts to outweigh that of the carbohydrate reserve by the second day following defoliation.

Partitioning of reserve and recently fixed carbon within the plant

In roots

According to previous studies, the supply of carbon to roots is reduced immediately after defoliation, not only because of reduced photosynthesis due to the removal of leaves but also because of a greater allocation of carbon to shoot meristems (Hartt, Korschark & Burr 1964; Ryle & Powell 1975). Measuring the time course incorporation of currently absorbed carbon within sucrose should provide new insights on carbon allocation priorities occurring as a response to defoliation. On day 4, the proportion of post-defoliation photosynthate in the sucrose of roots was much lower than in the sucrose of the growing shoot. This illustrates the fact that roots remain a sink for carbon after defoliation and that carbon was mainly allocated to leaf growth regions. However, during the first two days of regrowth, the RSA of sucrose dropped from 91 to 36% in roots but only from 100 to 54% in growing leaf tissues. In roots, the decline in fructan concentrations might be a source of carbon for sucrose. Thus, the lower value of sucrose RSA in roots is probably related to the fact that RSA of fructan was only 59% at the time of defoliation. The decline in fructan concentration is in accordance with other studies which indicate that the rapid decrease in soluble carbohydrate concentrations is not only the result of reduced allocation from the shoot system but is also a consequence of continued utilization of these reserves by root maintenance and respiration (Davidson & Milthorpe 1966b; Dankewerts & Gordon 1987). Furthermore, the RSA of fructans found on day 2 in roots was similar to the RSA of sucrose. This suggests that the fructans stored in roots at the time of defoliation were extensively hydrolysed during the first days of regrowth and that the fructans present on day 2 were the products of synthesis from sucrose rather than the products of initial fructan breakdown. Despite a negative whole-plant carbon balance, fructans were accumulated in roots.

In sheaths

As a response to defoliation, sheaths that remained in the stubble were converted from sink to source status (Volene 1986). In Lolium perenne, they lost 37% of their carbon, 91% of their soluble carbohydrates and 96% of their fructans during the first six days of regrowth. Moreover, there was no loss of label from fructans over the first four days following defoliation. Together, these observations indicate that fructans from leaf sheaths acted as a source of carbon for sink parts of the plants at the beginning of regrowth. By contrast, post-defoliation C was detected in sucrose, glucose and fructose on the second day of regrowth. It accounted for 9 and 21% of the total carbon in sucrose and hexose, respectively. Four days later, it represented about 70% of the water-soluble carbohydrates. Surprisingly, there was also 70% of newly fixed carbon associated with the small pool of fructan present. Therefore, between the fourth and sixth day of regrowth, fructans were probably even more hydrolysed than indicated by the differences in concentrations.

The labelling period was long enough with respect to carbon turnover in shoots but not with that in roots. Therefore, dilution of labelled C in shoots did not necessary mean that post-defoliation assimilation was providing C. Unlabelled C could also be provided by roots. However, as the root system continued to import carbon from the shoots after defoliation, as outlined above, unlabelled C found in sheaths (and also in new leaves) within the first days of regrowth most probably resulted from current photosynthesis rather than translocation from roots to shoot. Sheaths themselves are capable of photosynthesis and it has been estimated that their C fixation rate was about 5% of the photosynthesis rate in blades (Borland & Farrar 1985). Unlabelled C could also be provided by the photosynthetic activity exhibited by the new leaves that have emerged.
from stubble. It is difficult, however, to take this hypothesis into account since shoot meristems represented high-priority sinks towards sheaths for carbon acquisition, according to our data and to other studies (cf. Richards 1993).

In leaf bases

After defoliation, the leaf bases reproduced their own length in less than 2 d. It does not mean, however, that all the cells located in the bases were newly synthesized because leaf bases include the cell division zone and contain more cells per unit of leaf length than the tops of elongating leaves. The time needed for a cell to cross the division zone restricted to the basal 0–0.5 mm was predicted to be approximately 2 d, whereas two further days were necessary for displacement of a cell from the proximal end of the elongation zone to the limit of the stubble (40 mm high) (from unpublished results). It follows that at the second day of regrowth, cells located above the division zone were already present in the division zone at the time of defoliation. Amongst the 91% labelled carbon found in elongating leaf bases, the whole carbon was not issued from reserve mobilization since a fraction of it was part of the pre-existing cells. However, this fraction was probably very small because the elongation zone represents the most active sink for carbohydrate and nutrient deposition (Schnyder & Nelson 1987).

At the beginning of regrowth, leaf elongation rate was to a large extent dependent on mobilization of the pre-defoliation C. Fructans were hydrolysed in sheaths and they were also hydrolysed in leaf bases as indicated by the difference in fructan concentrations between leaf bases at the time of defoliation and tops of elongating leaves on the second day of regrowth. As already pointed out for the sheaths, differences in fructan contents give an underestimation of fructan breakdown in bases since the proportion of currently fixed C was very high in the fructans of the tops. These data indicate that the fructan that was present in the emerged parts of elongating leaves were products of synthesis rather than products of hydrolysis. Fructans were also accumulated in leaf bases, when the whole-plant carbon balance was negative as also shown in roots and sheaths. Similarly, net fructan deposition occurred at the location of most active growth, at low irradiance (Schnyder & Nelson 1989). This indicates that the most actively growing tissue was supplied with C assimilates far in excess of growth needs, whatever the whole-plant carbon balance was. It would be interesting to address the question of whether there is a threshold in carbon content below which fructans are no longer accumulated; that is, under which imported sucrose is exhaustively used for growth.

Three possible roles have been ascribed to fructans in elongating leaf bases. By removing sucrose from the cytosol, they might function to maintain a sucrose gradient between the phloem and the shoot meristems, allowing phloem unloading to proceed (Schnyder & Nelson 1987). Because of their low mean degree of polymerization, fructans of elongating leaf bases might also contribute to the osmotic potential necessary to support cell expansion by moving water into them (Volenc 1986; Spollen & Nelson 1988). They might also serve as a short-term carbohydrate storage since they are recycled for a biosynthetic process in the maturation zone where they provide about half of the C needed for secondary cell wall and lignin formation (Allard & Nelson 1991). The present data shows that on the second day of regrowth, pre-defoliation C content in fructose was 1.5 times more important than in fructan or sucrose. The relatively slow turnover of fructose could be attributed to the fact that part of the fructose pool found in the new shoot was supplied with the breakdown of pre-defoliation fructan to support the genesis and growth of new cells. Defoliation might, thus, initiate a shift in allocation priority of fructan degradation products to the synthesis and elongation of meristematic cells. This change might partly explain why in the early regrowth period, the incorporation of currently assimilated C was slightly slower in the elongation zone than in the maturation zone (De Visser et al. 1997).

REFERENCES


Received 18 December 1998; received in revised form 5 March 1999; accepted for publication 5 March 1999