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Sink-source and sink-sink relations during reproductive development in *Lolium perenne* L.

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Abstract

Spaced plants of *Lolium perenne* L. were labelled with ^{13}C at regular intervals from main ear emergence onwards in order to identify and measure the activity of source and sink organs during seed formation. The source activity of the various tiller groups within the plant roughly reflected the relative contributions of these groups to total plant dry mass. After anthesis there was little net exchange of ^{13}C-label between the older and younger tiller groups. From main ear emergence onwards the source activity of the leaves of the reproductive tiller declined sharply, from 95% of total tiller photosynthesis to 16% at final harvest. The ear became the main assimilating organ on the flowering tiller as the leaves aged. During anthesis the stem was a stronger sink than the seeds. At final harvest 70% of the label was located in the stem, when fixed during anthesis. Water-soluble carbohydrates accumulated in the stem, up to 25% of dry matter. After anthesis the sink strength of the developing seeds increased and that of the stem decreased and the stem remained a net sink organ up to about mid-seed filling. Pre-anthesis assimilates contributed 14% to final seed and spikelet carbon, when correcting for the palea and lemma that are present before anthesis. It is shown that the stem is a temporary storage organ that can support seed filling. Yet only a small amount of the stem reserves was used by the seeds. In contrast to carbon, nitrogen was largely redistributed from the stem and leaves to the seeds. At final harvest 59% of the nitrogen in the flowering tiller was located in the seeds.

Keywords: $^{13}CO_2$ labelling, *Lolium perenne* L., reproductive development, sink-source relations.

Introduction

Seed yields of crops of perennial ryegrass (*Lolium perenne* L.) are low and variable (Hebblethwaite *et al.*, 1980; Elgersma, 1990), with only 10–20% of the above-ground dry matter being harvested as seed. Competition between the seeds and

growth of new tillers is thought to reduce seed yield (Hampton *et al.*, 1983; Clemence & Hebblethwaite, 1984; Griffith, 1992). Also the elongating stem might be a factor reducing seed yield, as it might compete with the developing ear (Ryle, 1970). These questions can be investigated by monitoring the activity of leaves, stem, seeds and younger tillers as carbon-source or carbon-sink.

Between the start of stem elongation and ear emergence the export of carbon assimilates from the youngest leaf on the main tiller to the stem increases strongly. The export to the younger tillers decreases from 39% to 20% of the total export and that to the roots decreases from 35% to almost zero (Ryle, 1970; Parsons & Robson, 1981). The fraction received by the developing ear increases, reaching 66% at ear emergence (Ryle, 1970).

After ear emergence the ear becomes the main source organ on the flowering tiller. In *L. perenne* the relative importance of the ear as a source organ increases during seed filling when the leaves age (Ong *et al.*, 1978a; Clemence & Hebblethwaite, 1984; Colvill & Marshall, 1984). Ong *et al.* (1978a) found that the ear contributed 50% to the total amount of ^{14}C-label assimilated by the whole tiller at mid-grain filling.

^{14}C-labelling experiments in the field have shown that the amount of label exported to either the ear or the younger tillers varies. Clemence & Hebblethwaite (1984) found that the fraction of assimilated ^{14}C exported to the younger tillers increased from 10% to 24% during seed development. In their experiment part of the stem was labelled and harvest took place after 24 h. The export to the ear increased from 7% to 34%. Colvill & Marshall (1984) however, found much less label leaving the flowering tiller. When label was fixed by the main tiller at anthesis, at ripeness 16% of the label present was located in younger tillers and over 50% in the ear. According to Ong *et al.* (1978b) the flowering tiller exported only traces of ^{14}C-label at anthesis. These reports agree on the increasing export of current assimilates to the ear, but disagree on the fraction exported to the younger tillers. Furthermore, the role of stem reserves is not clear. This could be clarified by studying one genotype under greenhouse conditions, in order to reduce variation between plants.

Labelling whole plants with $^{13}CO_2$ enables one to follow the distribution of carbon through the plant (Yamagata *et al.*, 1987; Svejcar *et al.*, 1990) and thus to quantify the distribution between and within tillers of ^{13}C-label assimilated at regular intervals from ear emergence onwards. Monitoring the distribution of ^{13}C-label will give a qualitative pattern of the source-sink and sink-sink relations within the plant. ^{13}C-labelling was also used to measure the source activity, by viewing the fraction of ^{13}C-label fixed by a plant part as a measure of the actual carbon flux through that source (Farrar, 1992).

The objectives of the study were: to measure the relative activity of the source organs from ear emergence onwards, to determine changes in the pattern of sink-sink relations in the reproductive plant from ear emergence onwards, and to estimate the contribution of carbon fixed before anthesis to final seed carbon.

Materials and methods

Plant growing conditions

Vegetative tillers of *L. perenne* were clonally propagated on nutrient solution (Steiner, 1984). To obtain flowering tillers, one tiller per pot was vernalised for 14 weeks at 7 ± 1°C, at a photoperiod of 8 h and approximately 8 W/m^2 (PAR) using both fluorescent and incandescent light. This tiller will be referred to as the main tiller. After vernalisation the plants were transferred to a greenhouse at 15°C with the shading screen down and allowed to acclimatise for one week. The plants were then transferred to 1.75 l pots filled with a peaty soil. Per plant 240 mg N, 110 mg P_2O_5 and 180 mg K_2O was supplied. In the greenhouse natural daylength was extended to 17 h with incandescent bulbs (approximately 1.7 W/m^2). For 12 h per day supplemental lighting was provided by high pressure sodium lamps (Philips, AGRO SON-T, 400 W). The average daily incoming radiation at plant height was 2.7 MJ/m^2 (400 - 700 nm). The average day and night temperatures were 23°C and 17°C respectively. Iron wire was used to prevent the flowering tillers from lodging.

Experimental design

One clone (B1, selected from the Dutch cultivar Barlet) was used and the plants were grown in a randomised block design. Four other clones were used in the border rows and as pollinators. The time schedule of labelling and the number of plants used are shown in Table 1. After each $^{13}CO_2$ feeding four labelled plants were harvested immediately and at each subsequent $^{13}CO_2$ feeding until 42 days after main ear emer-

Table 1. Growth stage, time schedule of labelling and number of plants used.

Growth stage main ear	Pulse-chase labelling			Number of control plants	Number of plants labelled for ten minutes[1]
	Days after main ear emergence (DAEE)	Number of groups labelled (4 plants/group)	Harvest dates groups (DAEE)		
Main ear emergence	0	6	0, 14, 21, 28, 35, 42	15	5
Onset of anthesis	14	5	14, 21, 28, 35, 42	15	5
Mid-anthesis	21	4	21, 28, 35, 42	15	5
End of anthesis	28	3	28, 35, 42	15	5
3 Weeks after onset of anthesis	35	2	35, 42	15	5
4 Weeks after onset of anthesis	42	–	–	15	5

[1] Plants were labelled one day later than the pulse-chase labelling.

gence (DAEE), the final harvest. This means that after each $^{13}CO_2$ feeding the label could be chased for various periods until 42 DAEE. At each $^{13}CO_2$ feeding 15 unlabelled control plants (three plants times five replicates) were harvested to determine dry matter accumulation and distribution, water-soluble carbohydrate concentration, nitrogen concentration and control values of the ^{13}C stable isotope ratios ($\delta^{13}C$) in the plant organs studied.

$^{13}CO_2$ pulse-chase experiments

The first $^{13}CO_2$ feeding was applied when in 50% of the plants the top of main ear had emerged from the leaf sheath. Whole plants were labelled in a closed system consisting of a perspex chamber (80 × 80 × 80 cm) connected to a cooling device with a fan that kept the temperature at 20 ± 1 °C and also homogenised the air. The plants for the various harvest dates were randomised inside the labelling chamber. The light intensity during labelling was approximately 110 W/m^2 (PAR) at plant height provided by metal halide lamps (Philips HPI/T, 400 W). $^{13}CO_2$ was produced by adding a surplus of 0.5 M sulphuric acid to $Na_2{}^{13}CO_3$ (99 atom%, Campro Scientific, Elst, the Netherlands). The amount of $Na_2{}^{13}CO_3$ used at each $^{13}CO_2$ feeding was aimed at achieving sufficiently enriched plant organs ($\delta^{13}C > 0$) until the end of the desired chase period, hereby taking into account an estimated lowering of the $^{13}C/^{12}C$ isotope ratio through plant growth and respiration. Each $^{13}CO_2$ feeding period was ended when the $^{12}CO_2$ concentration in the chamber, measured by an infrared gas analyser had fallen to 100–200 µmol/mol.

Fixation of label by the source organs

In this experiment the fraction of label fixed by a plant part after labelling for ten minutes was considered to be a measure of the source activity of that part. Results from a pilot experiment, in which plants were labelled at mid-anthesis for ten minutes, had shown that the leaf blades exported 47% of the label within one hour. This means that labelling for a longer period would definitely underestimate the fixation by the leaves. Immediately after $^{13}CO_2$ feeding the plants were harvested, and the dissected plant parts placed on ice to minimise respiratory losses before drying. At the first harvest only the part of the ear that had appeared was separated from the stem. At the other harvests the spikelets and rachis were separated.

Harvest procedure

At each harvest the plants were divided into four groups of tillers (Figure 1): 1) the main tiller, 2) a homogeneous group of younger reproductive tillers, 3) tillers which had at least one fully emerged leaf, and 4) small tillers with a partly emerged first leaf and tillers present inside the leaf sheaths of the tillers in the first two groups. The second group of tillers consisted of all tillers other than the main tiller that were present one week after the end of vernalisation. These tillers were marked at that moment. The group 3 and group 4 tillers were not marked.

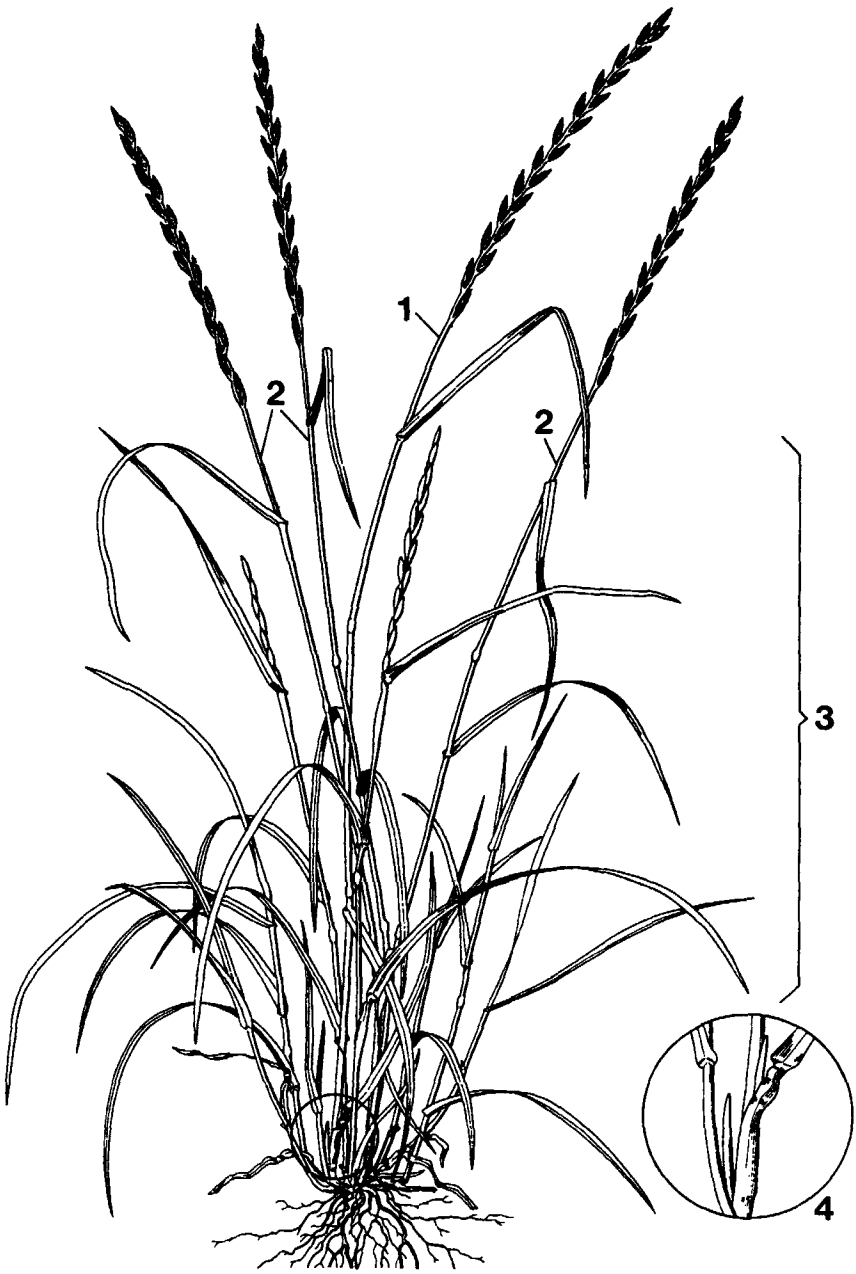

Figure 1. Representation of a flowering *Lolium perenne* plant, divided into four groups of tillers: 1) main tiller, 2) a homogeneous group of younger flowering tillers, 3) tillers with at least one fully emerged first leaf and 4) small tillers present inside the leaf sheaths of the older flowering tillers and tillers with a partly emerged first leaf. (From: Warringa & Kreuzer, 1996.)

The main tiller and group 2 tillers were divided into stem + rachis, green and yellow leaf and spikelets. At the first harvest partly emerged ears were taken out of the leaf sheath. At the last two harvests the seeds and empty florets of the main ear were separated, but not of the group 2 ears. The ears were stored at –20 °C until the number of seeds and empty florets were counted under a binocular microscope. Small top florets in a spikelet were regarded as non-fertile and omitted if they did not protrude beyond the subtending floret. The dry mass of the various plant parts was determined after drying at 70 °C for 48 h. Roots were not harvested.

Chemical analyses

For the determination of water-soluble carbohydrates (WSC) the plant parts were ground and extracted in water at 100 °C for 15 minutes. Total reducing sugars were measured colorimetrically after hydrolysis in sulphuric acid (0.23 M) with an automatic analysis device (Bran & Lübbe Analyzing Technologies, Inc., Elmsford, N.Y., USA). Starch was determined titrimetrically after enzymatic hydrolysis (Williams, 1984).

The ^{13}C isotope ratio ($\delta^{13}C$), carbon concentration and nitrogen concentration were determined with a continuous-flow isotope-ratio mass spectrometer (Roboprep-CN) and Tracermass (Stable Isotope Analyser) (Europe Scientific, Crewe, UK.). All replicates were pooled before chemical analysis.

Calculations

Dry weight was analysed in an analysis of variance using the GENSTAT statistical package (Payne *et al.*, 1987). To calculate the amount of ^{13}C-label in the different plant organs the dry weight and the ^{13}C atom excess of the labelled plants were used (Svejcar *et al.*, 1990; Boutton, 1991). These results were expressed as a percentage of the total amount of ^{13}C-label present at that harvest, in the whole plant shoot or in a tiller.

The relative contribution of carbon fixed before anthesis to final carbon in the seeds of the main tiller and the spikelets in the group 2 tillers was estimated using the method described by Bidinger *et al.* (1977). The relative contribution to final seed and spikelet carbon of a growth interval between two $^{13}CO_2$ feedings was estimated using the fraction of the ^{13}C-label present in the seeds and spikelets at final harvest. The average fraction of the two enclosing $^{13}CO_2$ feedings was used. This fraction (FS) was then weighted using the changes in plant dry weight during this growth interval (equation 1; Hall *et al.*, 1989).

$$\Delta W_1 \cdot \overline{FS}_1 / [(\Delta W_1 \cdot \overline{FS}_1) + (\Delta W_2 \cdot \overline{FS}_2)] \quad (1)$$

In equation 1 ΔW denotes the change in plant dry weight, $\overline{FS}$ is the mean fraction of label in the seeds for a given period, and the subscripts 1 and 2 refer to the pre- and post-anthesis periods, respectively. The increase in plant dry weight was determined from the plant dry weight curve fitted with a negative exponential equation.

Results

Tiller growth and development

Because spaced plants were used, a hierarchy of tillers of different ages developed. Each plant had on average 7.6 ± 2.2 group 2 tillers. The number of group 3 tillers increased from 16 ± 4 at the first harvest to 43 ± 14 at the final harvest. As group 4 tillers developed they were reclassified as group 3 tillers by the next harvest, and therefore the number of group 4 tillers decreased from 8 ± 4 to 0.6 ± 0.8 per plant. The main ear started to flower at 41 ± 1 days after the end of vernalisation, the ears in group 2 started to flower several days later. At final harvest 44% of the group 3 tillers had an ear.

Figure 2 shows that total plant dry weight reached a plateau at 35 DAEE. The contribution of group 3 tillers to total plant dry weight increased from 25% at main ear emergence to 64% at final harvest. The dry weight of the group 4 tillers was negligible and decreased from 50 mg at main ear emergence to 4 mg at final harvest.

The main ear had 22 ± 1 spikelets and 212 ± 20 florets per ear. Seed set was 75% and the seed yield per ear 223 ± 54 mg with an average seed dry weight of 1.4 ± 0.2 mg. The pattern of dry matter distribution within both the main tiller and the average group 2 tiller was similar, but the main tiller was heavier and some days advanced in development (Figure 3). At final harvest the relative contribution of the spikelets to total tiller dry weight was 31% for the main tiller and 28% for the average group 2 tiller. The corresponding figures for the contribution of the stem were 45% and 46%.

Water-soluble carbohydrates (WSC), starch and nitrogen

The distribution pattern of the WSC and nitrogen amount were similar for both the main tiller and the group 2 tillers. Only the results of the main tiller are shown. From main ear emergence onwards the amount of WSC in the stem increased sharply (Figure 4). At final harvest the WSC concentration had risen to about 26% of the dry

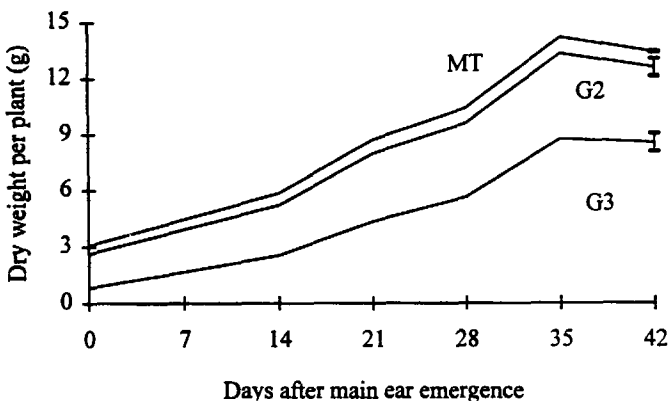

Figure 2. The cumulative dry weight of plants of *Lolium perenne*, divided into main tiller (MT), group 2 and group 3 tillers (see Figure 1). Vertical bars indicate LSD (0.05) values for a tiller group over time.

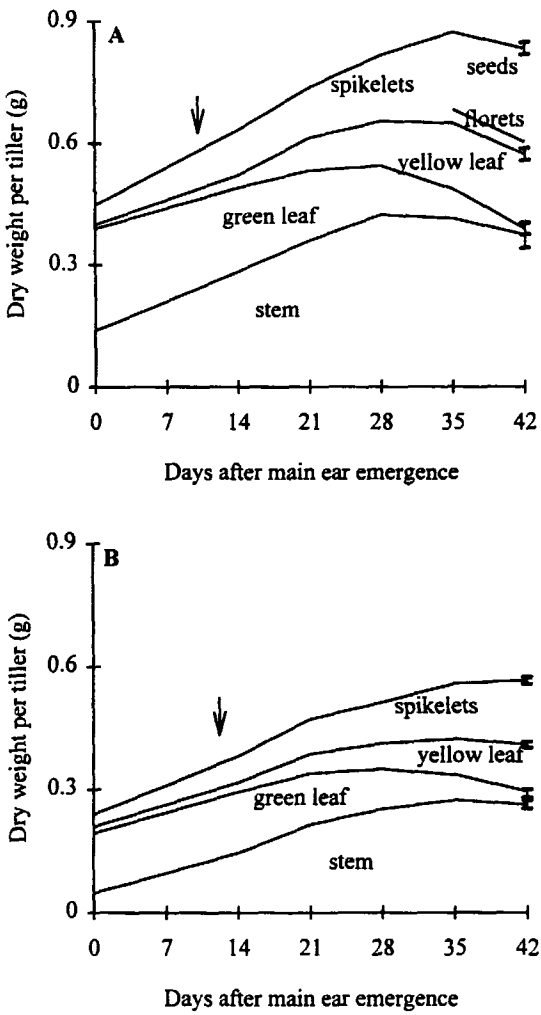

Figure 3. The cumulative dry weight of the main tiller (A) and of the average group 2 tiller (B) in plants of *Lolium perenne*. Onset of anthesis is indicated by the arrows. Vertical bars indicate LSD (0.05) values of the various plant organs over time.

weight in the main stem and 24% in the stems of the group 2 tillers. Between main ear emergence and the beginning of anthesis both concentration and amount of WSC in the leaves and the spikelets decreased. After onset of anthesis the amount of WSC in the leaves and the spikelets increased again. From the moment the seeds started to accumulate starch the amount of WSC in the spikelets declined. The seeds in the main ear had a starch concentration of 30% at 35 DAEE and 36% at final harvest. The WSC concentration of the whole plant increased from 11% at main ear emergence to 17% at final harvest.

The amount of nitrogen decreased in all the main tiller organs except the spikelets. At final harvest 59% of the nitrogen in the main tiller was located in the seeds, which had a nitrogen concentration of 2.1%. Total plant nitrogen concentration fell

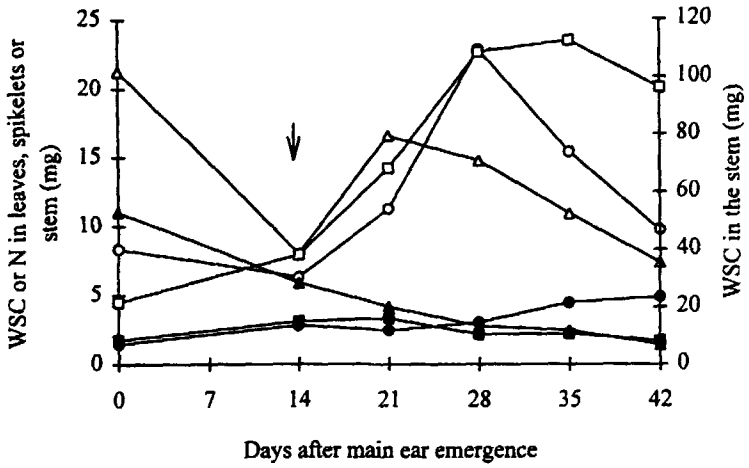

Figure 4. Changes in the amount of water-soluble carbohydrates, WSC (open symbols) and nitrogen (closed symbols) in the spikelets, stem and leaves of the main tiller over time after main ear emergence in plants of *Lolium perenne*. (○,●) spikelets; (□,■) stem; (△,▲) leaves. Arrow indicates onset of anthesis.

from 2.7% at main ear emergence to 1.0% at final harvest.

Nitrogen was also redistributed between tiller groups. From main ear emergence until the end of flowering of the main ear, at 28 DAA, the fraction of nitrogen in the tillers of group 3 increased from 27% to 67% on a whole plant basis. The fraction of nitrogen in the main tiller fell from 17% to 5% and that in the tillers in group 2 fell from 54% to 28%.

Fixation and distribution of ^{13}C-label between tiller groups

The absolute amount of ^{13}C-label fixed in ten minutes by the whole plant rose from 0.52 mg at main ear emergence to 0.98 mg at 29 DAEE and then declined to 0.6 mg at 43 DAEE. These values, in the same order, represent 0.9%, 0.8% and 0.5% of the amount of label supplied.

Figure 5 shows the relative ^{13}C-fixation by the different tiller groups immediately after labelling for ten minutes and the relative distribution at final harvest after pulse-chase labelling for 1.5–2.5 h. The difference between the two lines gives information about the redistribution of label between the tiller groups. The fraction of total assimilated ^{13}C-label that was fixed by the main tiller and group 2 tillers decreased during plant development, whereas the fraction fixed by the group 3 tillers increased sharply (Figure 5). The small group 4 tillers fixed only traces of ^{13}C-label (<1%). These results roughly reflected the pattern of relative contributions to total plant dry weight of those tiller groups (Figure 2).

Net exchange of ^{13}C-label between the tiller groups occurred mainly after labelling at 15 and 22 DAEE, i.e. during anthesis. In the last two $^{13}CO_2$ feedings there was little net exchange of ^{13}C-label between the tiller groups (Figure 5).

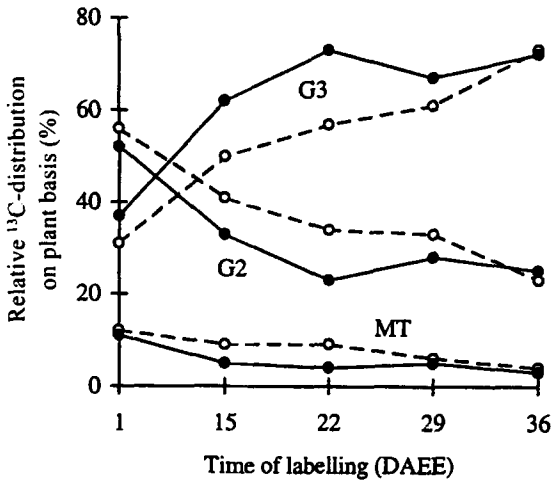

Figure 5. The relative fixation of ^{13}C-label after labelling for ten minutes (○) and the relative distribution at final harvest (42 DAEE) after labelling for 1.5–2.5 h (●) between groups of tillers in *Lolium perenne* plants. MT = main tiller, G2 = group 2 tillers, G3 = group 3 tillers. The time of labelling is indicated on the horizontal axis.

Fixation and distribution of ^{13}C-label within the flowering tiller

In both the main tiller and the average group 2 tiller the fraction of ^{13}C-label fixed by the leaves declined sharply from more than 95% at main ear emergence to 16% at final harvest. The fraction of ^{13}C-label fixed by the stem increased steadily, while that of the ear rose to a plateau of 30-40% after anthesis (Figure 6).

The relative distribution of ^{13}C-label within the main tiller and within the group 2 tillers was similar, therefore only the results of the former are shown (Figure 7). The ^{13}C-label in the leaves declined sharply between each $^{13}CO_2$ feeding and final harvest both in relative and absolute terms. When the ^{13}C-label was fixed later in development, more was exported to the spikelets and less to the stem. But the distribution of ^{13}C-label fixed during early anthesis (14 DAEE) was in contrast to this trend.

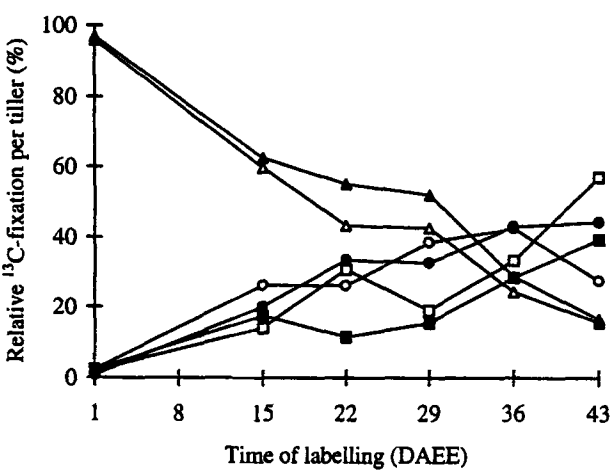

Figure 6. The relative fixation of ^{13}C-label by the ear, stem and leaves of the main tiller (open symbols) and the average group 2 tiller (closed symbols) in plants of *Lolium perenne* after labelling for 10 minutes. Time of labelling is indicated on the horizontal axis. (○,●) spikelets; (□,■) stem; (△,▲) leaves.

Then the leaves exported to the stem and not to the spikelets. From labelling at 28 DAEE onwards the leaves exported only to the spikelets.

Until about 28 DAEE the stem imported ^{13}C-label from the leaves. An increasing

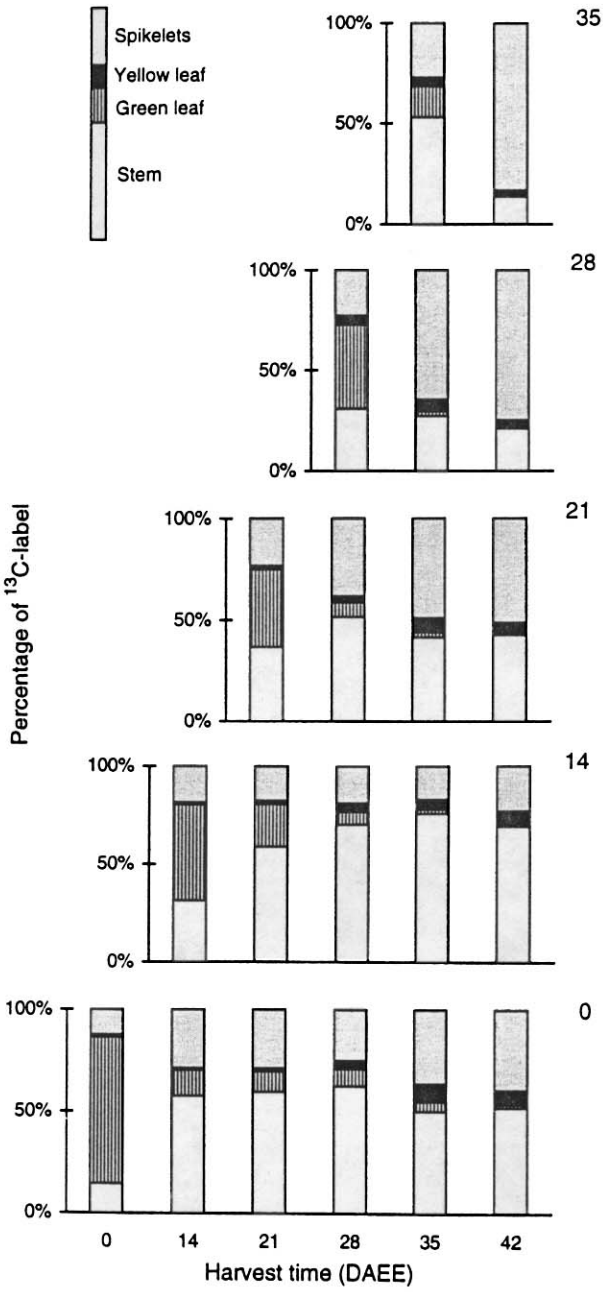

Figure 7. The relative distribution of ^{13}C-label within the main tiller of plants of *Lolium perenne*, between spikelets, green and yellow leaf and stem. Plants were pulse-labelled at 0, 14, 21, 28 and 35 days after main ear emergence (DAEE), as indicated next to the graphs. Plants were harvested regularly up to the final harvest (42 DAEE), as indicated on the horizontal axis.

fraction of the ^{13}C-label fixed by the stem after 28 DAEE was exported to the spikelets. In the first $^{13}CO_2$ feeding some of the ^{13}C-label imported by the stem was redistributed after 28 DAEE to the spikelets. The fraction of ^{13}C-label in the stem decreased by about 10%.

Towards later stages of development label fixed by the stem and leaves was rapidly distributed to the spikelets. After labelling at 35 DAEE the fraction of label in the spikelets of the main tiller increased from 27% to 83% at final harvest one week later (Figure 7) and in the group 2 tillers it increased from 35% to 84%.

The distribution of ^{13}C-label at final harvest within the spikelets of the main tiller between empty florets and the seeds shifted towards the seeds when fixed later during development. The fraction of ^{13}C-label in the seeds, as a percentage of the total amount in the spikelets, increased from 72% after labelling at main ear emergence to more than 96% in the last $^{13}CO_2$ feeding.

Estimation of the relative contribution to seed carbon of pre-anthesis assimilates

Using equation 1 the estimated pre-anthesis contribution to final carbon in the seeds of the main tiller and the spikelets in the group 2 tillers was 40%. This is, however, an over-estimation because the palea and lemma are present already at anthesis. They contribute about 26% to total seed dry weight in the genotype used (Warringa *et al.*, 1998). This means that only 14% of the final seed and spikelet carbon was redistributed after anthesis.

Discussion

In the present study growth analysis was combined with pulse-chase labelling of whole plants in order to estimate the contribution of pre-anthesis assimilates to final carbon in the seeds. A flaw of this method is that redistribution from the roots is ignored (Bidinger *et al.*, 1977; Schnyder, 1993). However, loss of leaves after anthesis which can be a problem in field experiments, did not occur in the greenhouse.

Furthermore, monitoring the distribution of ^{13}C-label resulted in a qualitative estimate of the changes in sink-sink relations within the plant. Under the assumption that ^{13}C will behave similar to ^{12}C in the plant an indication of the relative changes in sink strength (e.g. stem vs. ear) is obtained. Carbon input lost through respiration is not measured. Ideally, steady-state labelling is used which would enable a quantification of the partitioning of carbon within the plant (Yamagata *et al.*, 1987; Schnyder, 1993).

Fixation of ^{13}C-label and its distribution between tiller groups

The increase in plant dry weight from main ear emergence onwards was mainly due to the increase in number and dry weight of the group 3 tillers (Figure 2). This pattern was reflected by the increasing source activity of these tillers as development proceeded (Figure 5).

During anthesis of the main tiller the group 3 tillers were a net sink (Figure 5), because of their increasing number, as the relative amount of ^{13}C-label per individual tiller hardly changed (data not shown). During the seed filling phase, after 28 DAEE, there was only a minor net exchange of ^{13}C-label between the tiller groups and tillers seemed mutually independent.

These results agree with Colvill & Marshall (1984), who found 16% of the label to be located in the younger tillers at ripeness when fixed during anthesis. Clemence & Hebblethwaite (1984) however, found that the fraction of the assimilated label exported from the main tiller to the younger tillers within 24 h increased up to 24% during seed development. And according to Ong *et al.* (1978b) the flowering tiller exports only traces of label at anthesis. Clemence & Hebblethwaite (1984) grew plants under high nitrogen availability compared with the other studies mentioned and lodging was severe (Marshall, 1985). This demonstrates that sink strength of the younger tillers is affected by external conditions.

Several authors (Hampton *et al.*, 1983; Clemence & Hebblethwaite, 1984; Griffith, 1992) have suggested that younger tillers compete with the seeds for assimilates. This seems unlikely, given the large amount of carbohydrates in the stem (Figure 4; Spiertz & Ellen, 1972). Indeed, stem carbohydrate reserves even increased concomitantly with the large increase in number of group 3 tillers (Figures 2, 4).

Not only ^{13}C-label but also some nitrogen was redistributed from the flowering tillers to the group 3 tillers. Whether or not an increased sink strength of younger tillers (carbohydrates and/or nitrogen) can depress seed yields remains to be seen. Tillering and growing conditions need to be manipulated in order to investigate this.

Fixation and distribution of ^{13}C-label within the flowering tiller

The source activity of the leaves of both the main tiller and average group 2 tiller declined sharply after main ear emergence, while that of the ear and stem increased (Figure 6). The reduction in source activity of the leaves was due to an 80–90% reduction in green leaf weight (Figure 3) that was accompanied by the nitrogen concentration of the remaining green leaf falling from 3.5% to 1.1%. Generally a positive correlation between leaf nitrogen and carbon-assimilation capacity is found (Evans, 1989; Sinclair & Horie, 1989).

In accordance with results of Ong *et al.* (1978a) and Clemence & Hebblethwaite (1984) we found an increasing source activity of the ear during seed development as the leaves aged. The former found that the source activity of the ear decreases quickly at ripeness (Ong *et al.*, 1978a); this agrees with results for the main tiller shown in Figure 6. Our results confirm that the ear is the main source organ on the flowering tiller (Ong *et al.*, 1978a; Clemence & Hebblethwaite, 1984; Colvill & Marshall, 1984).

Up to about 28 DAEE the stem remained a net sink organ (Figure 7). During anthesis the stem was a stronger sink than the spikelets, i.e., carbon from the leaves was imported by the stem and not by the spikelets. After anthesis the sink strength of the developing seeds increased and exceeded that of the stem. The stem then became a net source organ. Our results confirm the role of the stem as a temporary storage

organ (Colvill & Marshall, 1984; Griffith, 1992) that can support seed filling.

Assimilates exported from the leaves enter the stem, which then either stores them, uses them for growth or exports them. The increase in amount of WSC in the stem between ear emergence and anthesis, together with the decrease in the leaves and spikelets (Figure 4), indicates that the elongating stem used more assimilates than it produced. At that time the stem was still elongating and increasing in dry weight (Figure 3). During stem elongation the stem is the dominant sink on the flowering tiller (Ryle, 1970), whereas young seeds are a very weak sink.

It is not clear if this pattern of assimilate distribution reduces the number of seeds. Under reduced light intensity during stem elongation, and limited assimilate availability, the number of florets per spikelet is reduced (Ryle, 1967). However, abortion under non-stress conditions is thought to have genetic and cytological causes associated with outbreeding and not to be caused by competition for assimilates (Elgersma & Sniezko, 1988; Marshall & Ludlam, 1989).

In contrast to WSC, nitrogen did not accumulate in the stem or the leaves but was translocated to the seeds in such a way that at final harvest most of the nitrogen in the flowering tiller was located in the seeds (Figure 4). Accumulation of nitrogen in seeds is thought to be regulated independently of that of carbon (Jenner, 1980; Swank *et al.*, 1982).

Estimation of the relative contribution to seed carbon of pre-anthesis assimilates

Assimilates fixed before anthesis contributed about 14% to final seed and spikelet carbon, when correcting for the palea and lemma that develop before anthesis and constitute 26% of the final seed dry weight. Expressed on the basis of the caryopsis itself (seed without palea and lemma) this would mean a contribution of assimilates fixed before anthesis of about 19%. In wheat and barley about 5–15% is accounted for by carbohydrates stored prior to anthesis under non-stressed conditions. Wheat seeds are harvested without the palea and lemma but in barley the husk constitutes about 8% of the final seed dry weight (Bidinger *et al.*, 1977). The contribution of pre-anthesis assimilates to seed carbon seems somewhat higher in *L. perenne* than in wheat and barley.

Under conditions unfavourable for photosynthesis such as drought this contribution increases to 27–44% (Bidinger *et al.*, 1977; Austin *et al.*, 1980; Schnyder, 1993). Reducing the assimilate availability by shading the plant during seed filling led to a sharp fall in stem reserves in *L. perenne* but had a small effect on seed yield (Warringa & Marinissen, 1996). This indicates support from stem reserves to seed yield.

The relatively low utilisation of available stem reserves during seed filling indicates that seed yield potential is high and not limited by the availability of assimilates. The results presented here have shown that the stem of the flowering tiller does not compete with the filling seeds, but contains large amounts of soluble sugars and exports assimilates as seed filling proceeds. The seeds do not seem to be able to use these reserves fully.

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