Sink to source transition in developing leaf blades of tall fescue

A. BRÉGARD AND G. ALLARD*
Département de Phytologie, Université Laval, Québec, G1K 7P4, Canada
Received 2 April 1998; accepted 7 September 1998

Summary
This study aims to characterize the translocation of photosynthates within and from developing tall fescue (Festuca arundinacea) leaves at the time of transition from sink to source. The developing leaf contains a source, the exposed tip, and a sink, the growing basal portion. When the exposed tip of the developing blade is labelled with $^{14}$CO$_2$, it exports photosynthates exclusively to sinks within the developing blade until the blade reaches 80% of its final length, when photosynthates begin to be exported from the blade and pass through the collar to reach the growing sheath and the next expanding leaf. Concurrently, the previous mature leaves reduce their level of photosynthetic export to the developing blade; export stops as soon as the sheath of the developing leaf elongates beyond 10 mm. Export from the mature leaves to the growing sheath and to the next expanding leaf blade increases rapidly. Thus, in a developing tall fescue leaf blade photosynthetic importation and exportation are exclusive events: the expanding blade imports photosynthesize from mature leaves until it reaches 80% of its final length, then exportation begins and importation ceases.

Key words: sink to source transition, leaf blades, Festuca arundinacea (tall fescue), $^{14}$C-labelling.

Introduction
A grass leaf is composed of a leaf blade and a sheath that grow successively. The growing part of the leaf, enclosed in the whorl formed by the older sheaths, consists of three different zones. Closest to the leaf base is the cell division zone (0–10 mm distal to the ligule), the cell elongation zone (10–30 mm) is more distal and the cell maturation zone extends up to 100 mm from the ligule (Schnyder & Nelson, 1988; Allard & Nelson, 1991; Skinner & Nelson, 1995). However, the cell division zone of the blade is 2 mm from the point of attachment of the leaf as meristematic cells of the sheath and the ligule constitute a 2-mm-wide ring of cells at the base of the leaf. Sheath meristematic cells stay quiescent until the sheath starts to grow.

Skinner & Nelson (1995) observed that, in tall fescue, sheath growth initiation occurs simultaneously with the development of the next leaf blade (Skinner & Nelson, 1995). Correlations between final lengths of the sheath and the next leaf blade have also been reported for Lolium perenne (Wilson & Laidlaw, 1985).

The gradient of development which is characteristic of developing grass leaves provides a model where sink and source are simultaneously located in the same organ with the emerged leaf tip already mature and photosynthetically active while the growing base is acting as a sink. The elongation zone is considered a strong sink since a large proportion of photoassimilates imported or synthesized in the leaf are used in this zone. Schnyder & Nelson (1988) measured that 50% of the d. wt in the elongation zone is water soluble carbohydrates, 70% of which are fructans (Spollen & Nelson, 1988).

During the early stages of leaf development, only the blade develops actively and leaf growth up to the emergence of the leaf tip is almost exclusively associated with blade elongation (Schnyder et al., 1990). However, as soon as the blade reaches a specific stage of development, the sink to source transition occurs in the leaf blade (Fellows & Geiger, 1974; Turgeon, 1989). This phenomenon is characterized by the end of photoassimilate import into the developing leaf blade and the beginning of export towards new sinks. The timing of these events in developing grass leaves has received markedly less attention than in dicot leaves. Giaquinta (1978) reported that leaf development in Beta vulgaris is accompanied by a rapid loss in the ability to import assimilates from a $^{14}$CO$_2$-fed source leaf. In this species, photoassimilate import declines sharply from the time a blade is 20–25% of its final length and stops by the time it
reaches 50% of its final length. In grass leaves, the balance between the amount of imported sugars and the amount of photoassimilates produced by the leaf blade evolves concurrently with the photosynthetic capacity of the leaf tip (Anderson & Dale, 1983). After the leaf transition from sink to source has been accomplished, other tissues will benefit from the increased availability of photosynthate exportation. Ryle & Powell (1972) working with Lolium temulentum demonstrated that specific meristematic sink regions are supplied when export from the developing leaf begins.

The aim of this study was to characterize the displacement of photoassimilates in developing leaves of tall fescue during the brief period of the sink to source transition. Different leaves were fed with 14CO2 in order to determine which ones were the sources of carbon for the developing leaf blade. Thus, by visualizing carbon partitioning during leaf expansion we would be able to determine the time when the sink to source transition is triggered. Consequently, we evaluated the percentage of leaf blade expansion when the sink to source transition occurred in the leaf blade. Finally, we studied the displacement of 14C-labelled assimilates in the growing sheath and inner newly expanding leaf, both considered major areas of meristematic activity in addition to the developing blade. Anatomical characteristics of leaves in transition will be useful to select tall fescue leaves at the precise time of sink to source transition in order to study further physiological and biochemical aspects.

**Material and Methods**

**Plant culture**

Three vegetative tillers of tall fescue (Festuca arundinacea Schreb.) were grown per 125-mm diameter pot containing vermiculite, perlite, dark soil and peat moss (1:1:2:2). After a 3 wk exposure to a 16-h photoperiod in a glasshouse, plants were transferred to a growth chamber set at 21°C and 70% r.h. with continuous light (24 h) under a photosynthetic photon flux density (PPFD) of 380 μmol m−2 s−1. Pots were watered daily and fertilized every 5 d with a nutrient solution equivalent to 60 kg ha−1 N-P2O5-K2O. Stems were cut as necessary to keep plants in a vegetative state. After 1 month in these constant growing conditions, plants were used for experiments. Independent series of plants were used for each experiment.

**Leaf elongation rate (LER)**

Tillers with their fourth leaves newly emerged were selected for LER determination. The fourth leaf blades were measured daily from the top of the third leaf sheath to the tip of the fourth leaf blade. Elongation data were plotted for each leaf and LER for the population of tillers was determined using the linear portion of the elongation curves.

**Leaf measurements**

Sheaths and blades of the third and fourth leaves, and the fifth blades when present, were measured at different stages of development on tillers with mature or developing fourth leaves. For each measurement, tillers were separated from the subtending plant and the sheaths of the older leaves surrounding the third and fourth leaf removed. Sheath lengths were measured from the point of attachment to the stem to the collar, i.e. the ligule zone, and blade lengths were measured from the collar to the tip of the leaf blade. Sheaths (S) and leaf blades (B) of the third and fourth leaves were respectively named S3, B3, S4, B4 (Fig. 1). The fifth newly expanding leaf blade was named leaf B5.

Based on a correlation calculated between sheath length of the third leaf (S3) and length of the fourth leaf blade (B4), a population of tillers with similar S3 sizes was selected. Consequently it could be assumed that the fully expanded B4 from these tillers would have similar lengths. Developing B4 blades could therefore be compared with mature B4 blades to evaluate their level of development simultaneously with S4 growth.

**Leaf blade selection for 14C-labelling**

The second or third mature leaf or the fourth developing leaf of different tillers were labelled using 14CO2 during the various experiments. As soon as the fourth leaf blade was visible, the third leaf blade was considered mature since tall fescue leaf blades grow one at a time (Skinner & Nelson, 1995). The fourth leaves ranged from approx. half expanded to fully developed leaves.

**Leaf labelling procedure**

Four comparable blades from different plants were labelled simultaneously with 14CO2 using a closed system containing an air reservoir. The 150- to 250-mm distal part of the leaves were enclosed in vertical leaf chambers. The four chambers were connected in parallel and a pump was used to maintain an air circulation rate of 11 min−1 per leaf chamber. A small flask included in the system was used to introduce 14CO2 (40 μCi) generated by addition of HCl to NaH14CO3. An InfraRed Gas Analyser (Li-Cor 6200, Lincoln, NE, USA) continuously measured the CO2 concentration. After 10 min of labelling, which corresponded to a decrease of c. 200 ppm, the system was opened and the gas bubbled through a 1 N NaOH solution. Labelled leaves were carefully removed from the chambers. Plants were kept in the
growth chamber for the duration of the experiment, i.e. during the labelling procedure and for an additional 8 h to allow translocation of photoassimilates within the tiller.

**Determination of $^{14}$C**

Samples were harvested 8 h after labelling. Leaf segments, 5-mm long, were sampled from different sections of each labelled tiller (Fig. 1): (1) portions of the cell division zone and the cell elongation zone of the fourth developing leaf, i.e. 0–5 mm and 10–15 mm distal from the ligule, respectively; (2) the newly grown sheath of the fourth leaf, when the sheath was >10 mm, two samples were harvested, one at the top and one at the base, activities found in the top and the base were linearly extrapolated to the full length of the sheath to give an approximation of the whole sheath activity; (3) the whole new leaf expanding within the fourth leaf; and (4) the basal portion of the fed leaf blade, i.e. the second, third or fourth leaf depending on the experiment. Radioactivities of sections 1, 2 and 3 were weighted on the basis of the respective labelling intensity of each tiller determined by the radioactivity level in section 4, which gives weighted $^{14}$C activity ratios (dpm sections 1, 2 or 3/dpm section 4).

Samples were dried for 36 h at 65°C then transferred in 0.5 ml of NCS solubilizer (Amersham, Baie d’Urfé, Québec, Canada) and 0.05 ml H$_2$O for a minimum of 12 h at 55°C using a dry bath (Type 16500-Dri-Bath, Thermolyne, Dubuque, Iowa, USA). After this solubilization step, 2.45 ml of water were added and the samples were homogenized with an ultrasonic homogenizer (36260 series, Cole Palmer, Chicago, IL, USA). Finally, 2 ml of water and 10 ml of liquid scintillation cocktail (Ready Gel, Beckman, Fullerton, CA, USA) were added and the whole sample was counted in a liquid scintillation counter (1217 Rackbeta, LKB Wallac Oy, Turku, Finland). Results available in counts per minute were converted in disintegrations per minute (dpm) using a $^{14}$C standard curve.

**RESULTS**

**Leaf growth**

When they emerge from the whorl of older sheaths, leaves reach their maximum growth rate which corresponds to the phase of linear growth of the leaf (Skinner & Nelson, 1995). The LER averaged 1.39 mm h$^{-1}$ (33.5 mm d$^{-1}$), a value slightly greater than 1.17 mm h$^{-1}$ reported by Allard & Nelson (1991) but similar to Schnyder & Nelson (1988) with 1.33 mm h$^{-1}$.

**$^{14}$C-labelling**

**Labelling of developing leaves.** When the emerged tip of the developing fourth leaf was labelled, radioactivity was detected in the growing zone of the leaf blade and beyond the ligule, in the inner expanding leaf B5 and the growing sheath S4 (Fig. 2a,b). The labelled assimilates were observed in these sinks only when the sheath was >8 mm long. This illustrates that the export of assimilates from the developing leaf blade towards the growing parts of the tiller is correlated with the growth of the leaf itself. Moreover the subtending sheath S4 received more labelled assimilates than the inner leaf B5.
Figure 2. Measurement of $^{14}$C activity of tall fescue (*Festuca arundinacea*) when the fourth leaf blade (B4) was labelled as the fourth sheath (S4) was growing. (a) inner expanding fifth leaf (B5), and (b) growing fourth sheath (S4).

Figure 3. Measure of $^{14}$C activity in the elongation (●) and division (○) zones of the developing fourth leaf blade (B4) of tall fescue (*Festuca arundinacea*) tillers while the fourth sheath (S4) is elongating; labelling either (a) the second leaf blade (B2) or (b) the third leaf blade (B3).

Labelling of mature leaves. When the third or second leaf was labelled, similar levels of radioactivity could be detected in the division and the elongation zones of the fourth leaf blades (Fig. 3a,b). This illustrates the import of assimilates into the developing blade from the older leaves. Nevertheless, as soon as the sheath S4 started to grow, the level of radioactivity decreased progressively within the blade; it was only a fraction of the initial dpm by the time the sheath reached 10 mm in length. Conversely the level of radioactivity detected in the expanding inner leaf B5 and in the sheath S4 increased progressively (Fig. 4a, b), showing the growing sink activity of these tissues.

When B3 leaves were labelled, the levels of radioactivity detected in the inner B5 leaves were slightly higher than those measured in the S4 sheaths while levels were similar in both sink tissues when B2 leaves were labelled.

Leaf measurements

The lengths of the expanding fifth leaf and the growing fourth sheath were strongly correlated, for S4 < 80 mm in length, $r^2 = 0.87$. The B4 lengths could be separated into two groups: S4 is either $> 90$ mm in length. The value of 90 mm was determined as the mean sheath length when the blade stopped elongating. The two groups are illustrated by two regression lines (Fig. 5). Regression lines were used to estimate the mean length of B4 before and after the growth of S4 stopped. We determined that B4 averaged 311 mm (intercept of regression line (1)) when S4 was not elongated ($x = 0$), and that B4 averaged 395 mm (regression line (2)) when S4 was close to full elongation ($x = 100$). Hence by calculating the ratio between both values (311/395 = 0.79) we determined that the leaf blade was 80% developed when the sheath started to grow.
blade (B4) and the fourth sheath (S4) of tall fescue (Festuca arundinacea) tillers with the fourth sheath length varying from a meristem (regression line 1) to full expansion (regression line 2).

**Figure 5.** Correlation between lengths of the fourth leaf blade (B4) and the fourth sheath (S4) of tall fescue (Festuca arundinacea) tillers with the fourth sheath length varying from a meristem (regression line 1) to full expansion (regression line 2).

**DISCUSSION**

The sink to source transition of developing tall fescue leaf blades coincides with sheath elongation and occurs when leaf blades are 80% elongated. Hence, mature leaves supplied developing leaf blades until 80% of full development, which seems to indicate that tall fescue leaf blades depend on mature leaf carbon supply longer than dicots. Leaves, usually described to be 30–60% developed when importation ends (Turgeon, 1989). To our knowledge, no previous study has reported the percentage of development of a grass leaf blade at the time of the sink to source transition. Grass leaf blade autotrophy possibly occurs later than in dicots, because of lower photosynthetic capacity since a large proportion of the developing grass leaf is enclosed in the whorl of older leaves. However, the tip of the blade is structurally mature and photosynthetically competent by the time it emerges and it exports towards the growing basal part where assimilates are also imported from mature leaves. This is in agreement with Ryle & Powell (1972) who determined that, in Lolium temulentum leaves, 60–80% of the assimilates produced in the emerged leaf tip are used in the basal growing zone when the blade is one third to one half developed. Similarly, Anderson & Dale (1983) mentioned that 60% of the carbon required by developing barley leaves at the time of emergence is imported from the mature leaves, whereas the additional 40% is produced by the developing blade itself.

Interestingly, the amount of labelled photoassimilates imported in the inner expanding leaf (B5) differed from the amount imported by the growing sheath (S4) when the third leaf (B3) was the supplier (Fig. 4b). This concurs with Anderson & Dale (1983) who reported that each barley leaf (Bn) tends to be the predominant supplier of carbon for the second next developing leaf (Bn+2) before the developing leaf emerges. After emergence, however, partitioning changes and all mature leaves might supply the developing leaf. In meadow fescue mature leaves, Butcher et al. (1987) stipulated that sheath sink-strength tends to be lower than that of the leaf blade. However the fourth leaf blade L4 supplied more photoassimilates to the subtending sheath S4 than the inner leaf B5 which is probably because of its continuity with the labelled leaf blade B4. By contrast, amounts of 14C-carbohydrates were similar in the division and the elongation zones, which could indicate that nearly equal proportions of carbon issued from the mature leaves were exported to both cellular zones (Allard & Nelson, 1991). This agrees with the idea that the division and the elongation zones are both strong sinks and that vascular bundles are continuous between these two different cellular zones, unlike the fourth sheath and new inner leaf which are two distinct organs with separate vein networks.

It is possible to calculate the time required for the sink to source transition because the delay between the decline of import by the blade and the initiation of export is related to sheath elongation. The sink to source transition occurs when the sheath elongates from 2 to 10 mm and therefore we suggest that the time required for a cell to move from 2 to 10 mm from the base is equivalent to the sink to source transition period. In view of Schnyder & Nelson’s (1987, 1988) results, we calculated by extrapolation that 2.4 d are necessary for a cell to move from 2 to 10 mm from the base. Concurrently while the sheath grows, the blade continues to elongate since it has to grow a further 80 mm on average to reach its final length. This corresponds with the assessment by Schnyder et al. (1990) indicating that sheath cells are produced and elongate long before the leaf blade ceases elongation. Therefore the leaf blade is still elongating even after the sink to source transition has been completed and the leaf blade is more than self-sufficient in carbon to elongate a further 20%.

Further, the end of import from mature leaves and the beginning of export through the ligule zone seem to be exclusive events. This agrees with an observation reported by Allard (1989) that no assimilates issued from the lamina tip can move through the ligule when only the leaf blade is elongating. It has been previously reported that bidirectional assimilate transport occurs in the distal part of the developing leaf blade as the elongation zone is simultaneously supplied by the mature part of the developing leaf and mature leaves (Schnyder et al., 1988; Allard & Nelson, 1991). The phloem of F. arundinacea is composed of major and minor veins, disposed alternately (MacAdam, 1988) which made Allard & Nelson (1991) suggest that each type of bundle might drive carbon flux in an opposite direction. Moreover, MacAdam (1988) observed that minor veins are too thin to pass through the narrow collar of the ligule, whilst major veins seem to
maintain continuous phloem connections between the sheath and the leaf blade. Hence we suggest that minor veins may translocate assimulates within the leaf blade whereas major veins could translocate sucrose through the ligule, for export or import depending on the stage of development of the leaf.

Bidirectional transport in leaves has been reported by several authors including Lush (1976) who concluded that, in *Lolium temulentum* and *Panicum maximum*, export outside the leaf occurs through major veins, while loading and short distance transport were possible through minor veins. Turgon (1989) also, mentioned that minor veins are the distribution network of the dicot leaves.

The assumption that carbon transport to or from a leaf blade occurs through only major veins implies that the direction of carbon flux may change at the time of sink to source transition in this type of vein. The time needed for all veins to change the direction of transport corresponds to the sink to source transition period. Inversion of flux might occur progressively and basipetally in each vascular bundle as soon as cells are fully mature and stop importing. This is reported by Turgon (1989) who suggested that the developmental control may induce the restriction of the phloem unloading capacity. This will result in a diminution of import in leaves. Ho (1988) also mentioned the importance of vein maturation on sink to source transition events. Recently, Evert *et al.* (1996) studied the implication of the suberin lamella development in maize leaf sink to source transition. Giaquinta (1983) stated that particular biochemical differentiation of the phloem membranes could coincide with the acquisition of loading and export capacity. Hence our results suggest that sucrose accumulation in the mature part of major veins might induce a mass flow which drives the translocation flux in the opposite direction (Fellows & Geiger, 1974).

The direction of carbon flux may also change in minor veins but as minor veins probably do not go through the ligule zone (MacAdam, 1988; Evert et al., 1996), photoassimilate movements in these veins are not associated with the sink to source transition of the leaf in relation to the other leaves or to its sheath. However, even after the sink to source transition, sucrose is still transported within developing leaf blades from the mature part to the basal section which is completing its development.

### REFERENCES


