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# Phloem Transport and the Regulation of Growth of *Sorghum bicolor* (Moench) at Low Temperature

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## ABSTRACT

**Leaf expansion in *Sorghum bicolor* (Moench) was severely retarded by low night temperatures (5 C). However, this was not reflected in the early measurements of relative growth rate, indicating that the response was not associated with a deterioration of the photosynthetic system. For plants grown at 30/25 C (day/night) and subsequently held at an ambient temperature of 30 C, phloem transport, as measured either by the movement of $^{14}$C-photosynthate through a zone of controlled temperature or by accumulation of dry matter distal to this zone, was inhibited by temperatures below 10 C. The speed of movement of $^{32}$P through the temperature controlled zone was more sensitive to temperature with reductions apparent below 20 C. Although there was some recovery in the movement of $^{32}$P following 3 days equilibration at low temperature (1 to 10 C), the new values (approximately 100 centimeters per hour) were still only about one-third of those obtained in the high temperature controls. For plants held at an ambient temperature of 21 C, which is well below the optimum for growth, translocation was only inhibited by temperatures below 5 C. Although low temperature may reduce the carrying capacity of the phloem of *S. bicolor*, this is unlikely to be an important factor in regulating the growth of the plants at low temperatures.**

A number of interacting physiological processes may mediate the response of plants to low temperature. In recent years, considerable emphasis has been placed on the function of the photosynthetic system, and, in many species, including *Sorghum*, this would appear to play a critical role in plant survival at low temperature (2, 14, 16). However, there are still many situations, such as cloudy conditions or darkness, where the photosynthetic apparatus is not affected by low temperature; reduced growth may result from more direct effects of temperature on growing tissue or, possibly, less directly from the reduction of flow of photosynthate through the phloem. The latter possibility has been examined more extensively in the work that follows.

Although the transport system of many plants has been shown to respond to local changes in temperature (3, 5, 8, 20), a limitation in the carrying capacity of the conducting tissue has not generally been accepted as a factor in the control of growth (12). More recently, Geiger and Sovonick (6) have drawn a parallel between temperature effects on translocation and chilling sensitivity in plants. Unfortunately, little of the work on translocation in relation to temperature can be directly compared with studies on photosynthesis or growth, and it is difficult to establish the significance of these responses to the whole plant.

In the experiments described here on the subtropical C4 grass *Sorghum bicolor* (Moench), an attempt has been made to relate the repsonse of the transport system to localized cooling to other effects of temperature in the plant.

## MATERIALS AND METHODS

**Cultural Conditions.** Seedlings of *S. bicolor* (Moench), cv. Texas 610, were grown in a 1:1 mixture of Perlite to vermiculite in 12-cm pots. Unless otherwise stated, plants were established in a naturally lit glass house with the natural photoperiod extended to 16 h with incandescent lamps. They were supplied with a standard nutrient solution each morning and tap water at midday and each afternoon.

**Relative Growth Rate Studies.** Following germination and initial growth in a naturally lit glass house at 21/16 C (day/night) for a period of 10 days, groups of 25 plants were transferred to a range of experimental conditions in order to assess the effect of night temperatures on growth.

Between 08:00 h and 16:00 h all plants were held at 21 C in a glass house. Control plants were retained in the glass house under the standard 21/16 C regime. At 16:00 h groups of experimental plants were transferred to LB type cabinets (13) and subjected to the conditions illustrated in Table I.

Harvests were made at the time of the initial transfer and subsequently after 7 and 14 days treatment. Measurements were made of leaf area and shoot and root dry weight. From these values, the relative growth rate was calculated, based on combined shoot and root weights and relative leaf growth rate. These values and their SES were derived from the formulae described by McIntyre and Williams (11).

**Chl Determination.** Chl was determined after the method of Arnon (1), following extraction of leaves in 80% acetone at 5 C. OD was read on a Gilford 240 spectrophotometer at 645 and 663 nm, and total Chl was calculated from the formula 20.2 $OD_{645}$ + 3.02 $OD_{663}$.

**Translocation of $^{14}$C-Photosynthate.** A 10.5-cm length from the distal part of the blade of the last fully mature leaf from each of 8 plants was enclosed in a Plexiglas photosynthesis chamber 2 × 10.5 cm in cross-section. In four of the plants, a 2-cm length of the leaf below the assimilation chamber was enclosed in a controlled temperature jacket (17). In the remaining four control leaves, aluminum foil was used to cover the leaves in the same position

Table I. *Experimental Conditions for Relative Growth Rate Studies*

| Condition | Temperature |
|---|---|
| | *C* |
| Light | |
| 08:00–16:00 | 21 |
| Dark | |
| 16:00–18:00 | 13 |
| 18:00–06:00 | 4, 7, 10, or 13 |
| 06:00–08:00 | 13 |
| Light | |
| 08:00– | 21 |

as the jacket. When the temperature of the jacket had equilibrated at the appropriate level, all eight leaves were allowed to assimilate $^{14}CO_2$, containing 50 $\mu$Ci $^{14}C$, for a period of 2 min. After labeling, excess $^{14}CO_2$ was scrubbed from the circulating system, and the photosynthesis chamber was removed from the leaves. Translocation was then allowed to proceed for a period of 4 h. At harvest, the youngest emerged growing leaf was dissected out from the surrounding mature leaf sheaths and cut into 1-cm lengths. These lengths were mounted on aluminum planchets, dried, and assayed directly for radioactivity with a Tracerlab-Omniguard end-window, gas-flow Geiger counter. The mean counts for the four plants, in which the jacket temperature was below ambient, were expressed as a percentage of the mean counts obtained in the four ambient controls; this was considered to be a measure of the effect of the jacket temperature on the transport of photosynthate.

**Translocation of $^{32}P$.** A solution of $^{32}P$-orthophosphate (2 $\mu$Ci $^{32}P$ in 25 mM $KH_2PO_4$) was applied in a small vial to a reverse flap cut between the midvein and the edge of the leaf. This method of application was used to give entry of $^{32}PO_4^{3-}$ along the xylem of longitudinal veins towards the tip of the leaf, with subsequent lateral movement of $^{32}P$ across the leaf to uninterrupted vascular strands and export back down the leaf in the phloem. Two sealed, end-window G-M tubes, 18 cm apart, were located on either side of a 10-cm-wide temperature control jacket which had been placed across the leaf proximal to the $^{32}P$ feeding area. The G-M tubes were covered with lead caps having a 5-mm-wide slit. These detectors were used to determine the time of arrival of the tracer front on either side of the jacket, and, from this, an estimate was made of the speed of $^{32}P$ movement down the leaf. The wider jacket was used to increase the precision of the measurements of speed.

**Leaf Dry Weight Changes.** Plants were transferred from either a 21/16 C or a 30/25 C glass house to an artificially lit cabinet under conditions of continuous light (650 $\mu$E $m^{-2}$ $s^{-1}$ in the range 400 to 700 nm) and a constant relative humidity of 85% for a period of 12 h. Cabinet air temperatures were held at either 21 C or 30 C.

A 10-cm length of leaf blade near the sheath was enclosed in the temperature control jacket that was also used for the $^{32}P$ experiments. To determine the change in dry weight with time, leaf tissue distal to the jacket was sampled by punching 1.4-$cm^2$ discs at 0, 6, and 12 h after adjustment of leaf temperature under the jacket. For each temperature, the responses were recorded in five replicate leaves, and the mean change in dry weight of these leaves was always compared with the change in dry weight of five control plants in which a 10-cm length of aluminum foil was used to enclose the base of the leaf blade. For reference, the effects of temperature on the accumulation of dry matter were compared with the effect of completely blocking phloem transport by steam killing the tissue across the base of the leaf blade.

## RESULTS

**Effect of Night Temperature on the Growth of *S. bicolor*.** The effect of varying night temperature on both the relative growth rate and relative leaf growth rate of *S. bicolor* is shown in Figure 1 for two experimental periods, 7 and 14 days. Over the shorter time span, there was no evidence that relative growth rate was affected by variation in night temperatures, although relative leaf growth rate was considerably reduced at the lowest temperature (4 C). A second experiment also confirmed that relative growth rate was unaffected by night temperature over a period of 7 days, and, in neither case, was there any measurable change of Chl away from the control values of 1.6 mg Chl g fresh weight. Thus, it would appear that the photosynthetic activity of the leaves was not greatly reduced by low night temperatures (*cf.* Taylor and Rowley [16]). However, with the longer time interval of 14 days, the effect of low night temperature on leaf expansion was also reflected in the values for dry matter growth rate.

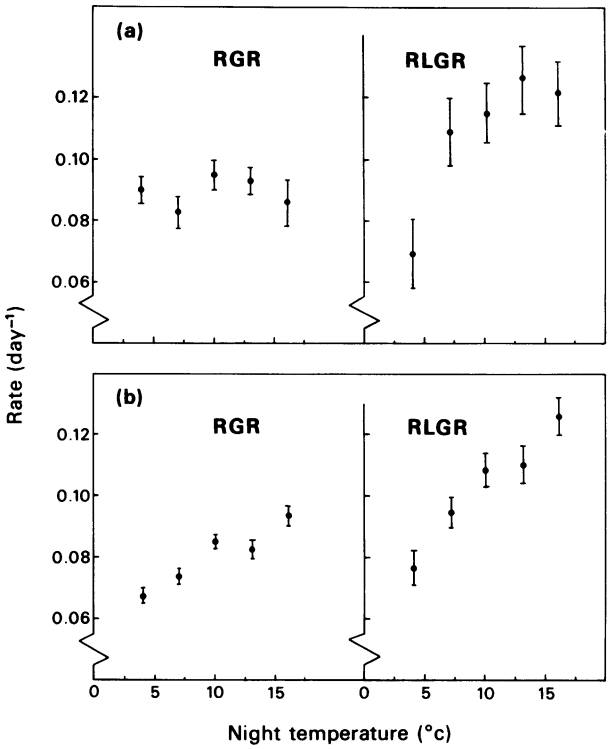


FIG. 1. The effect of night temperature on relative growth rate (RGR) and relative leaf growth rate (RLGR) of *S. bicolor* with day temperatures of 21 C. a, Period of measurement, 7 days; b, period of measurement, 14 days. Each point is the mean of 25 replicates, and the vertical bars indicate 2 × SE.

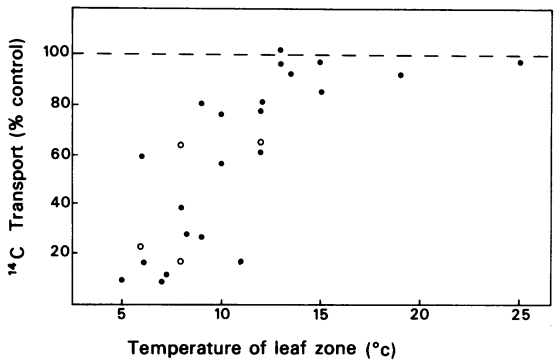


FIG. 2. The effect of pathway temperature on the transport of $^{14}C$-labeled photosynthate out of the leaf of *S. bicolor*. Each point is based on a comparison of the mean $^{14}C$ activity reaching the growing leaf tissue at the base of the shoot in four control plants and in four plants in which a 2-cm zone of leaf at the base of the blade was cooled to a set temperature below ambient. $^{14}CO_2$ was supplied to the distal part of the leaf 4 h prior to harvest. (●), Values obtained for $^{14}C$ movement in the 4 h immediately following the adjustment of jacket temperature; (○), values obtained for $^{14}C$ movement in a 4-h period following 3 days' equilibration at low temperature. Only when treatment values were less than 75% of the controls were these differences statistically significant.

**Effect of Pathway Temperature on the Export of $^{14}C$-Photosynthate.** Although the data (Fig. 2) show considerable variation, due at least in part to difficulties in obtaining uniform $^{14}CO_2$ uptake and variation between plants in the growing tissue, there was an obvious reduction in $^{14}C$ movement below 10 C, with considerably reduced transport at 5 C. The open circles on this figure refer to experiments where the specified jacket temperatures were held for

3 days prior to the uptake of $^{14}CO_2$ and the determination of translocation. These data points do not stand out from the remaining values; therefore, from this very limited analysis, there was no evidence of adaptation and recovery of translocation at low temperature.

**Effect of Temperature on the Speed of $^{32}P$ Movement.** Two groups of plants were used for these experiments. One group was grown at 30/25 C with measurements made at an ambient air temperature of 30 C. (Fig. 3A) and comparable with the $^{14}C$ data. A second group was grown at 21/16 C with measurements at an ambient air temperature of 21 C (Fig. 3B) which provide a better comparison with the growth studies.

At an ambient temperature of 30 C, there was a marked reduction in the speed of movement of $^{32}P$ down the leaf when the jacket temperature was below 20 C for short pretreatments (2-h equilibration) (Fig. 3A). In nine plants, leaf temperatures under the jackets were maintained at low temperature (1, 5, or 10 C) for 3 days prior to the application of $^{32}P$ to the distal part of the leaf. This equilibration time did allow recovery in the speed of movement of $^{32}P$, with the values reaching nearly 100 cm $h^{-1}$ (Fig. 3A). These were still only about one-third of those observed at the higher temperatures.

At an ambient temperature of 21 C (Fig. 3B) the maximum speed of movement observed of about 180 cm $h^{-1}$ was much less than that observed at 30 C. This speed was maintained until the jacket temperature was reduced to about 5 C and then dropped considerably as temperatures were reduced below this.

**The Effect of Temperature on Leaf Dry Weight.** The next approach was a simple one in which the temperature response of the transport system in plants held at either 21 C or 30 C was assessed by measuring changes in dry weight of leaf tissue above a temperature block placed across the base of the blade.

For each analysis shown in Figure 4, the mean change in dry weight with time for control plants is compared with the mean change in dry weight of leaf tissue above a temperature or steam block. Completely blocking transport by steam killing the tissue at the base of the leaf resulted in a marked increase in dry matter of the leaf, and this provides a base response when looking at the effect of temperature on translocation.

For plants grown at 30/25 C and examined at an air temperature of 30 C (Fig. 4A), considerable blockage of transport was evident at 0.5 C and 5 C; this was confirmed in two subsequent experiments, but no significant differences were evident at 8 C or above.

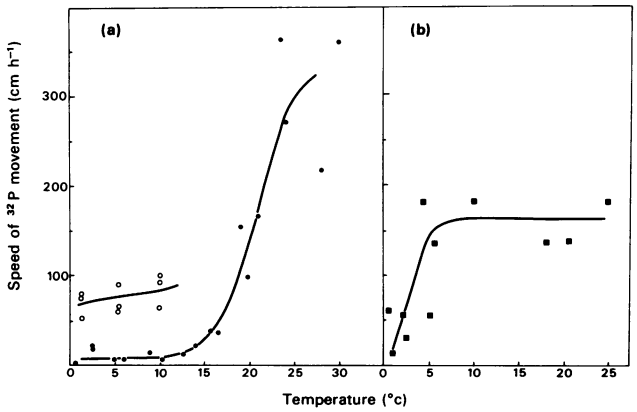


FIG. 3. The effect of pathway temperature on the speed of movement of $^{32}P$ through the leaf of *S. bicolor*. Each point is the estimate from a single plant. (●), Values obtained by measuring the movement of $^{32}P$ as soon as the jacket temperature had equilibrated (1 to 2 h); (○), values obtained 3 days after temperatures had equilibrated. a, Translocation at an ambient air temperature of 30 C; b, translocation at an ambient air temperature of 21 C.

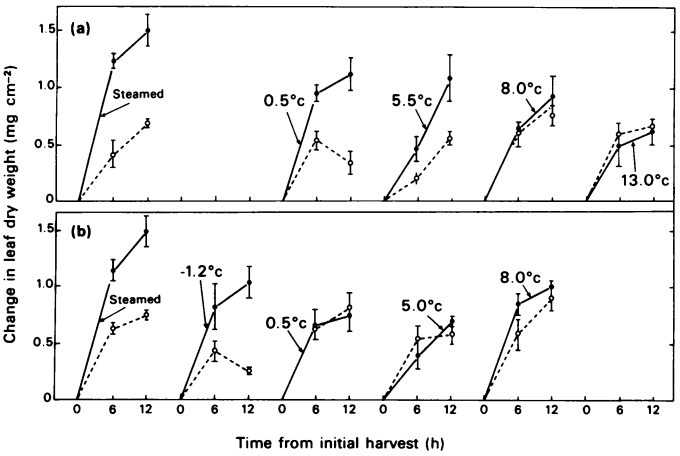


FIG. 4. The effect of pathway temperature on the accumulation of dry matter in the distal part of the leaf of *S. bicolor* under conditions of continuous light (650 $\mu E\ m^{-2}\ s^{-1}$). (——) and (●), values for leaves in which translocation was stopped by steaming or the temperature of a 10-cm zone at the base of the leaf was cooled below ambient. (- - -) and (○), comparable changes in ambient controls. Each point is the mean of 5 replicates, and vertical bars indicate 2 × SE. (a), Cabinet air temperatures 30 C during the experimental period; (b), cabinet air temperatures 21 C during the experimental period.

For plants grown at 21/16 C and examined at an air temperature of 21 C (Fig. 4B), blockage of transport was not evident at 0.5 C, although it did occur at −1.5 C (without tissue freezing). Leaves of plants grown at 21/16 C did show a greater level of ethanol and $H_2O$-soluble material, and also starch, than did those grown at 30/25 C. When dry matter accumulation was examined in plants grown at 30/25 C, but held at 21 C for the duration of the experiment, there was no apparent difference between controls and plants in which the temperature of the jacket was lowered to 5 C. Accumulation after 12 h was 0.44 ± 0.05 mg $cm^{-2}$ with 5 C block and 0.50 ± 0.09 mg $cm^{-2}$ in the 21 C controls.

## DISCUSSION

In rapidly growing plants held at 30 C, translocation was inhibited by a reduction in pathway temperature, although the critical temperature appeared to vary with the nature of the measurement. Determination of change in dry weight of a leaf distal to a temperature block gives a measure of the effect of temperature on mass transfer, *i.e.* the end result of changes in the speed of movement and concentration of solute in the phloem. The apparent reduction in translocation below 8 C, based on dry weight changes, was similar to the response observed in the $^{14}C$ studies which can also be considered as a less direct measurement of mass transfer. In contrast, the speed of movement of $^{32}P$ along the leaf, which generally can be equated with that of photosynthate (10), was found to be far more sensitive to pathway temperature than were either the movement of $^{14}C$ or the accumulation of dry matter, with evidence for an initial response as high as 20 C. This difference between the types of measurement can be accommodated by assuming that the initial restriction imposed by low temperature results in a reduced speed of movement; this is compensated by a rise in concentration of the photosynthate moving through the phloem, thus minimizing the effect on mass transfer.

The cause of the reduction in translocation was not investigated specifically in these experiments. However, the very rapid fall off in speed of movement of $^{32}P$ over a 5 C range suggests that this is unlikely to have resulted from a simple change in viscosity of solute due to low temperature, but it could be related to physical blockage of the sieve plate pores due to callose formation (9), or

the displacement of cytoplasmic contents (7).

The incomplete recovery of translocation when the pathway was maintained at low temperatures for 3 days is difficult to relate to other work, as most studies have only involved a period of a few hours (18). Bean, another chilling-sensitive species, has shown a continuing inhibition of transport over a period of 6 h at low temperature (5), while sugar beet, a chilling-resistant species, showed almost complete recovery over the same period (15).

If we moved away from the optimum temperature for growth, in this instance from 30 C to 21 C, there was a greatly altered response to low temperature. The speed of movement of $^{32}P$ was not significantly reduced until pathway temperatures were less than 5 C, and mass transfer (based on dry weight changes) was not reduced until temperatures were less than 0.5 C. The difference in response did not appear to be associated with differences in development of the plant under the two temperature regimes, as leaf dry weight changes, in response to a temperature block of 5 C at an ambient temperature of 21 C, were similar in plants grown at 30/25 C and 21/16 C. This suggests that the response was related to the immediate growth conditions of the plant. The demand for photosynthate is known to affect the speed of translocation from source to sink (19), and the adaptation to change can be very rapid (4, 19). Leaf extension rates in *S. bicolor* at 21/16 C are about one-half of those at 30/25 C, and it is tempting to suggest that these differences in growth were responsible for the difference in the speed of movement of $^{32}P$ at 21 C and 30 C.

One fact has become very clear from these studies. Caution must be used in attempting to determine the critical temperature for any physiological process that can be said to be characteristic for a given species or cultivar. This applies particularly to a process such as translocation, which is closely dependent on two other processes, the supply and demand for photosynthate.

For day temperatures of 21 C, a night temperature of 5 C considerably reduced leaf expansion; yet, a temperature block of 5 C across the leaf of plants at 21 C had no apparent effect on the mass transfer of photosynthate. From these observations, it would appear unlikely that translocation was a limiting factor at low night temperatures, but rather that low temperature had a more direct effect on tissue expansion. However, a very different conclusion might have been reached based on the response to a temperature block placed across the leaf of plants held at 30 C, and, again, caution is needed in extrapolating from an isolated set of responses to those likely to operate in the whole plant.

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