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Assimilate transport in maize (*Zea mays* L.) seedlings at vertical low temperature gradients in the root zone

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Abstract

Even moderate chilling temperatures may cause important modifications in assimilate movement in maize seedlings from the shoot to the roots, but there is no information on long-distance transport of assimilates in plants subjected to vertical gradients of moderately low temperatures in the root zone. Seedlings of a chilling-tolerant (KW1074) and a chilling-sensitive inbred line (CM109) of maize were grown in a system that allowed the maintenance of temperature gradients between the topsoil (0–10 cm) and the subsoil (10–50 cm). After pregrowth at 24 °C until the third-leaf stage, plants were subjected to chilling-stress regimes for 6 d (17/17/17 °C, 17/17/12 °C, 12/12/12 °C, 12/12/17 °C, air/topsoil/subsoil). The time taken for the assimilates to enter the phloem from the second leaf increased at low temperatures for both lines, but to a much greater extent in CM109. Although mainly influenced by air and topsoil temperature, low temperature in the subsoil also affected this trait in CM109. The speed of assimilate transport between the second leaf and the mesocotyl in KW1074 was strongly reduced by cool temperatures in the shoot and topsoil as well as by 12 °C in the subsoil in CM109, because the latter line had a larger portion of its root system in the subsoil as compared to KW1074. The portion of assimilates allocated to the root decreased at low temperatures in both lines, but to a greater extent in CM109, and was controlled mostly by the subsoil temperature. After rewarming, values of all measured parameters of assimilate transport returned to near pregrowth levels within a few days.

Key words: Assimilate transport, low temperature stress, root growth, vertical soil temperature gradients, *Zea mays* L.

Introduction

Cultivation of maize in areas at high latitudes or altitudes is limited mostly by its cold-sensitivity, as manifested by a strong retardation of growth at temperatures from 10 to 15 °C (Stamp, 1984; Verheul *et al*., 1996) as well as by leaf necrosis and plant death at temperatures below 10 °C (Janowiak and Markowski, 1994).

The sensitivity of maize photosynthesis to low temperatures seems the main cause of low chilling-tolerance (Long *et al*., 1983; Stirling *et al*., 1993), as indicated by a reduction of the photosynthetic rate, disturbances in the photosynthetic electron transport (Dolstra *et al*., 1994), and changes in the composition of photosynthetic pigments (Haldimann *et al*., 1995). However, photosynthesis may not be the only limiting factor for maize growth at moderately low temperatures of 10–15 °C (Crèvecoeur and Ledent, 1984), the usual temperature situation at early maize sowing in Central Europe. Co-ordinated growth processes at the whole-plant level (Engels and Marschner, 1992; Richner *et al*., 1996), in combination with a functional morphology of both shoot and root (Stamp, 1984), are regarded as crucial for the adaptation of maize to moderately low temperature. Therefore, a temperature-stable transport of assimilates from source to sink is essential.

Earlier studies on assimilate transport from leaves to roots in chilling-treated maize seedlings show that even moderate chilling temperatures cause important modifications in the movement of assimilates to the roots (Sowinski and Maleszewski, 1990; Sowinski, 1993;

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Sowinski and Królikowski, 1995). These studies were done on a wide spectrum of maize inbred lines and hybrids used in Polish breeding programmes. Observed modifications of long-distance transport by low temperatures were strongly correlated with the level of chilling-tolerance of the genotypes. Furthermore, it was shown that moderately low temperatures at the whole-plant level decreased the amount of assimilates transported to the roots in chilling-sensitive, but not in chilling-tolerant genotypes. Another, more detailed study (Sowinski, 1995) with six inbred lines, including KW1074 and CM109, confirmed these observations and showed further differences in processes of assimilate transport among genotypes with different chilling-tolerance, indicated by (i) an increase in the time at which assimilates appear in the phloem, which was much greater in chilling-sensitive inbred lines than in chilling-tolerant ones; (ii) a decrease in the speed of transport, although there were no distinct differences among genotypes with different chilling-tolerance; and (iii) changes in the proportion of assimilates allocated to roots—increasing slightly in chilling-tolerant genotypes and markedly decreasing in chilling-sensitive ones.

Studies were undertaken to follow the transport of assimilates in seedlings of maize subject to moderately low temperatures at the whole-plant level. Since the changes in assimilate allocation to the roots correspond to reduced growth and activity of roots at moderately low temperature (Sowinski and Maleszewski, 1989; Sowinski and Królikowski, 1995), the question arises as to whether a decreased demand of the roots for assimilates inhibits long-distance transport or whether root functioning is limited by a reduced supply of assimilates. In order to study the mechanisms involved in the reduction of assimilate transport to the roots by suboptimal temperatures, maize seedlings were subjected to moderately low temperatures, which are within the normal range of chilling events in spring.

Materials and methods

Plant growth

Two inbred lines of maize (*Zea mays* L.) were used in the experiments: chilling-tolerant KW1074 and chilling-sensitive CM109 (Verheul *et al.*, 1996). The inbreds were chosen from six genotypes studied earlier (Sowinski, 1995) on the basis of their similar growth patterns.

Plants were grown in a solid substrate, a mixture of 95% (w/w) fine sand (0.08–0.12 mm) and 5% vermiculite powder. A modified Hoagland solution containing 4.5 mM $Ca(NO_3)_2.4H_2O$, 2.5 mM NH_4NO_3, 2 mM $MgSO_4.7H_2O$, 1 mM KH_2PO_4, and 1 mM K_2SO_4 was added to the substrate (15%, w/w). Moist substrate (1.8 kg) was filled into aluminium tubes, 6 cm in diameter and 50 cm in length. The tubes were installed in a water bath system (Richner *et al.*, 1992) that allows the maintenance of different temperatures in the upper (0–10 cm) and lower part (10–50 cm) of the tubes by circulating controlled-temperature water through two water baths that are horizontally separated at a depth of 10 cm.

Seeds were germinated in the dark on wet filter paper at 24 °C for 2 d. Seedlings with primary roots about 10 mm long were transplanted, one plant per tube and covered by a 5 cm layer of substrate, thus forcing the plants to develop mesocotyls 2–3 cm long. These elongated mesocotyls were the place of radioactivity detection (see below). Plants were grown in a controlled environment chamber at temperatures of 24/24/24 °C (air/topsoil/subsoil) for 12 d up to the third-leaf stage. The photoperiod was 12 h, and 300 mmol m^{-2} s^{-1} were provided from a mixture of incandescent and cool-white fluorescent lamps (Sylvania 'Cool-White' VHO, 235 W). Relative air humidity was maintained at 70%.

At the third–leaf stage, air and root zone temperatures were lowered and the following regimes applied for 6 d: 17/17/17 °C; 17/17/12 °C; 12/12/12 °C; 12/12/17 °C (air/topsoil/subsoil). Simultaneously, a control regime with uniform warm temperatures (24/24/24 °C) was investigated. Due to faster growth at 24 °C and limiting tube length, control plants could be monitored only up to the fifth day of the stress period. After stress, growth temperature was raised to the prestress level, and the recovery of the plants was studied for 5 d.

Five seedlings per treatment and replication were harvested at the third-leaf stage before the beginning of the temperature treatments. Shoots and roots from the two separate root zones were dried at 65 °C for 2 d.

Transport studies

The time taken for assimilates to enter the phloem, the speed of transport and the proportion of assimilates distributed to the roots were studied using the ^{14}C technique, developed for *in vivo* transport studies (Sowinski *et al.*, 1990; Sowinski, 1995).

$^{14}CO_2$ was obtained by injecting $NaH^{14}CO_3$ with a total activity of 5 MBq (74 MBq ml^{-1}) into 10 ml of 2.5 M H_2SO_4. $^{14}CO_2$ was fed into the middle part of the second leaf via photosynthesis during 10–15 min for chilled plants and during 5–10 min for control plants, using a feeding chamber ($30 \times 30 \times 10$ mm). The movement of ^{14}C-labelled assimilates was monitored by two counters (Sowinski *et al.*, 1990) fitted tightly to the leaf below the feeding area and to the mesocotyl. The width and the height of the counter windows were 40 mm and 10 mm, respectively.

Transport kinetics were described by means of radioactivity curves. The time at which radioactivity appeared in the transport path was calculated as the time difference between the time after half of the feeding period and the time when the radioactivity registered by the first detector had reached half of its maximum value. Speed of transport was determined as the speed of the movement of radioactivity between the first and the second radioactivity detector, by defining the movement time as the period between the times at which the radioactivity values registered by the radioactivity detectors at the second leaf and at the mesocotyl had reached half of their maximum values, and the movement distance being defined as the distance between the detectors.

For measuring the proportion of ^{14}C-labelled assimilates distributed to the roots, plants were harvested when the radioactivity measured by the detector at the mesocotyl had decreased to a minimum, which indicated that the transport of ^{14}C assimilates to the roots was completed. Plants were dried at 65 °C for 48 h. The dried shoots and roots were ground separately, and their radioactivity was measured in a scintillation cocktail.

Statistics

The experiment was conducted with four replications. Data shown represent means ±SE.

Results

Growth

Shoot and root growth were measured before the onset of chilling at the third–leaf stage (Table 1). The shoot and root biomass did not differ significantly between the two lines, but great differences existed in the distribution of root biomass between topsoil and subsoil. Contrary to KW1074, a significantly larger part of the root system of CM109 was located in the subsoil.

Entrance time

KW1074 needed 3.5±0.5 min (Fig. 1A) and CM109 4.4±1.1 min (Fig. 1B) for the assimilates to enter the phloem at 24 °C. The time needed increased in response to chilling in both lines, but the increase in time was usually much greater for the chilling–sensitive CM109 than for KW1074. The time taken was longer at a shoot and topsoil temperature of 12 °C than at 17 °C. At a moderately cool temperature of 17 °C in the air and topsoil, varying the temperature in the subsoil had no impact on entrance time, whereas at 12 °C air and topsoil temperatures, entrance time was almost comparable to the less rigid temperature regimes at least for KW1074 when the subsoil was kept at the warmer 17 °C. Under the most severe chilling treatment, entrance time remarkably increased towards the end of the chilling period for the chilling-sensitive CM109. After rewarming, entrance time decreased to the pregrowth levels in both lines under all temperature regimes.

Transport speed

The speed of movement of ^{14}C assimilates between the leaf and the mesocotyl continued to increase until the third–leaf stage. It was 86.5±6.0 cm h^{-1} in KW1074 (Fig. 2A) and 105.1±10.4 cm h^{-1} in CM109 (Fig. 2B) before chilling. The speed of transport was strongly reduced at low temperatures, and in KW1074, little influenced by the temperature of the subsoil. The speed of transport in this line was twice as high at a shoot and topsoil temperature of 17 °C than at 12 °C. In CM109, the speed of transport was least decreased at 17/17/17 °C and most at 12/12/12 °C; plants grown at 17/17/12 °C and 12/12/17 °C maintained intermediate values. An alteration of the temperature of the subsoil had no clear effect on transport speed in KW1074 during chilling, whereas in CM109 transport speed was much lower at a subsoil temperature of 12 °C compared with 17 °C at both 17 °C and 12 °C in the air and the topsoil. After rewarming, the speed of transport recovered within 2 d to the level observed in control plants of the same physiological age.

Table 1. *Shoot and root dry weight per plant (mg) of two maize inbred lines at the third-leaf stage after growth at 24 °C*

Means followed by different letters are significantly different among genotypes at the 0.05 probability level according to LSD.

	KW1074	CM109
Shoot	167.2 a	177.9 a
Root		
0–10 cm depth	74.6 a	48.6 b
10–50 cm depth	51.1 a	63.7 b
Total	125.7 a	112.3 a

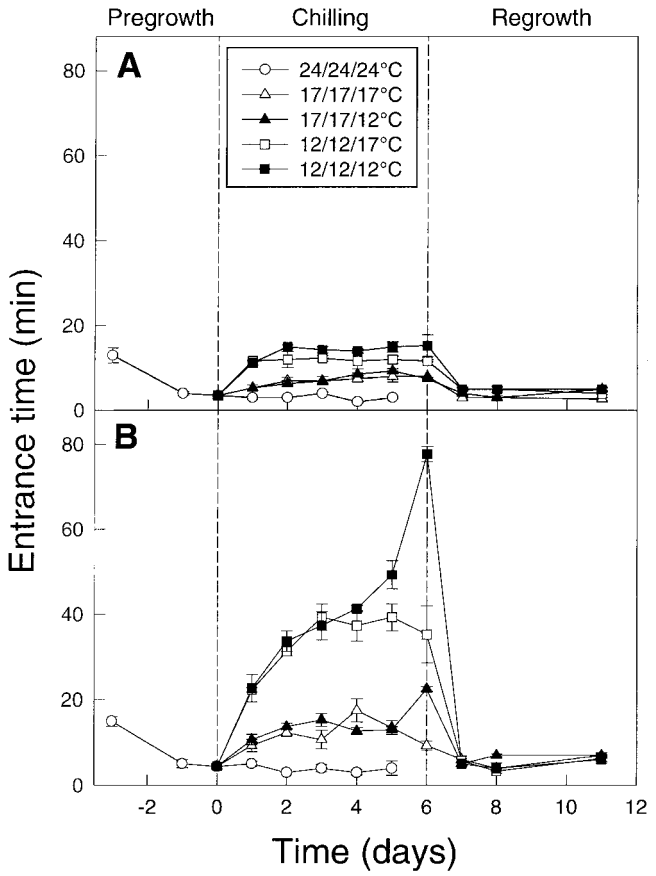

Fig. 1. Time taken for assimilates to enter the phloem [min] in seedlings of the inbred lines KW1074 (A) and CM109 (B) of maize, grown at temperatures of 24/24/24 °C (air/topsoil/subsoil) during pregrowth and regrowth periods, and 24/24/24 °C, 17/17/17 °C, 17/17/12 °C, 12/12/12 °C, 12/12/17 °C during the chilling period. Means are averages of a minimum of three replications ±SE (indicated by vertical bars if larger than symbols).

Proportion of assimilates allocated to the roots

By the end of the pregrowth period at 24 °C, both inbred lines had allocated a similar percentage of total applied ^{14}C assimilates to the roots. Root radioactivity in KW1074 (Fig. 3A) was 26.1±2.6% of the total plant radioactivity, and in CM109 (Fig. 3B) it was 28.6±3.5%. At low temperature, the proportion of assimilates alloc-

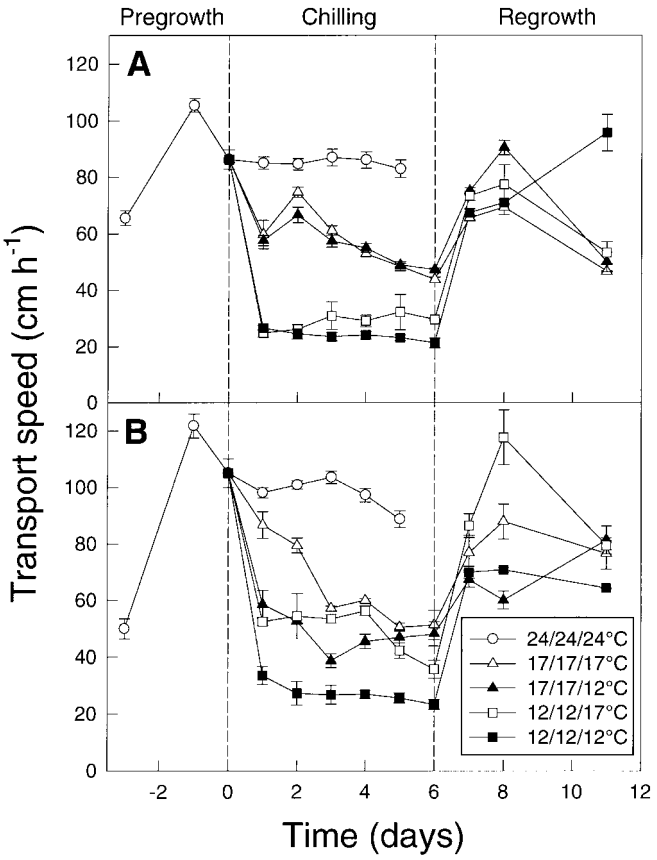

Fig. 2. Speed of transport of assimilates between the second leaf and the mesocotyl (cm h^{-1}) in seedlings of inbred lines KW1074 (A) and CM109 (B) of maize. Otherwise as for Fig. 1.

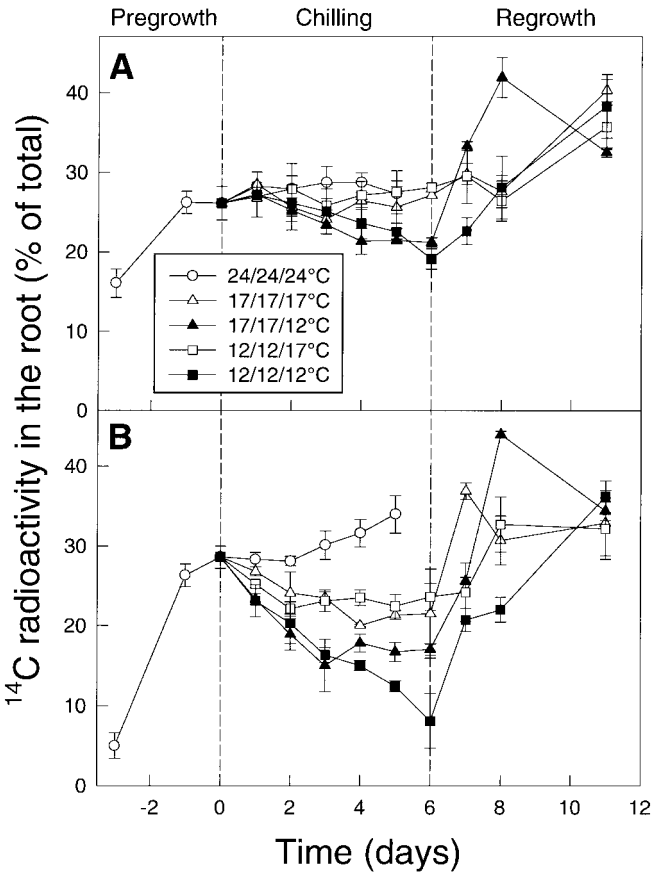

Fig. 3. Radioactivity of roots as a percentage of total plant radioactivity in seedlings of inbred lines KW1074 (A) and CM109 (B) of maize. Otherwise as for Fig. 1.

ated to the roots decreased much stronger in the chilling-sensitive CM109 as compared to KW1074. In both lines, a smaller proportion of ^{14}C-labelled assimilates was found at the cooler subsoil temperature (12 °C), but differences between the subsoil temperatures of 12 °C and 17 °C continued to increase in the chilling-sensitive CM109, and were generally greater for both lines at 12 °C compared with 17 °C in the air and the topsoil. After rewarming, the percentage of assimilates allocated to the roots increased again to about 35% of the total plant radioactivity in both lines, which is equal or larger than the values at the beginning of the stress period.

Discussion

Spatial temperature regimes were chosen that are representative of chilling events in a Central European spring, characterized by simultaneous changes in air and topsoil temperatures (Richner *et al.*, 1996). At this time of the year, subsoil temperatures are usually still lower than air and topsoil temperatures. After exceptionally long warm periods, somewhat higher subsoil temperatures may have a greater positive impact on chilling-sensitive genotypes like CM109, which have a larger portion of root biomass in the subsoil.

The time at which radioactivity first appeared in the transport path was strongly related to the ambient temperature of the upper plant part. This is not surprising, because the appearance of radioactivity is related to carbon assimilation and metabolism and to short distance transport and loading of the phloem with sucrose; processes that all take place in the leaf. On the other hand, the increase in the time taken for the assimilates to appear in the phloem, observed in chilling-sensitive CM109 towards the end of chilling regimes with subsoil temperatures of 12 °C as compared to 17 °C, shows that the initially exclusive impact of shoot temperature on the entrance time of assimilates is modified by root temperature later on. The results demonstrate that, at 17 °C in the subsoil and 12 °C in the air and topsoil, the sensitive line suffered a stronger retardation in time of assimilate entrance during the stress period as the tolerant line at 12 °C in all three compartments.

Underlying processes preceding the entrance of assimilates into the transport path do not seem to be strongly related to reduced photosynthesis (Sowinski, 1995) or

sucrose phosphate synthase activity at low temperature (unpublished data) because the differences among the inbreds of different chilling-tolerance level were rather small. For this reason, phloem loading initially seems to be the major sensitive process in chilling-treated maize seedlings.

The speed of transport strongly depended on the temperature of the shoots and the topsoil in KW1074. However, in CM109 the speed of transport clearly depended also on the temperature of the lower root zone. This may have been due to its large portion of roots in the subsoil. Chilling-tolerant lines such as KW1074, on the other hand, may profit from moderately low topsoil and continuing lower subsoil temperatures by a delayed exploration of the subsoil.

In terms of Münch's hypothesis (Münch, 1930) on the mechanisms of long-distance transport of assimilates, the osmotic pressure gradient generated by loading of osmotically active assimilates in the source and unloading in the sink is driving the flow of assimilates through the phloem. Thus, a high sink activity results in a high speed of translocation. This relates to the present situation in which the speed of transport generally decreased to a lesser extent at warmer subsoil temperatures. In KW1074, whose roots developed mostly in the topsoil, it was observed that the speed of transport did not decrease to the same extent, even if the subsoil was cold. However, in CM109 seedlings, a larger portion of roots grew into the subsoil as compared with KW1074, and the initial decrease in transport speed was influenced by the temperature of the lower soil layer as well.

The percentage of assimilates allocated to the roots increasingly depended on the temperature of the lower root zone after the onset of chilling, especially in the chilling–sensitive CM109. The higher chilling-tolerance of KW1074 was clearly proven again by the fast recovery of the relative allocation of assimilates to the roots even before the end of the stress period when only the subsoil temperature was maintained at moderately cool 17 °C.

Generally, the observed changes in entrance time, together with earlier results of Sowinski (1995), indicate that moderately low temperatures cause a relatively stronger inhibition of export of assimilates from leaves of chilling-sensitive genotypes than from those of chilling-tolerant genotypes. This process should be further examined.

It seems that the temperature of the roots has an immediate influence (within 1 d in CM109) on the long-distance transport of assimilates. Changes in the speed of assimilate transport as a consequence of modifications of root activity by vertical gradients in soil temperature may be interpreted using Münch's hypothesis (Münch, 1930). The marked effect of 12 °C in the subsoil on assimilate allocation to the roots of the chilling-sensitive CM109 at moderately cool 17 °C in the air and topsoil suggests that a reduced sink activity, i.e. root activity, rather than an impeded assimilate transport within the shoot reduced the proportion of assimilates transported to the roots. This is supported by Giaquinta and Geiger (1973) who found that, after an initial inhibition, assimilate transport through petioles or stems of a large number of species recovered almost completely even at temperatures near 0 °C.

However, the observed changes in the proportion of assimilates transported to the roots do not yet allow causal conclusions. The possibility of changes in the synthesis of root-borne plant growth regulators due to low root zone temperatures in the subsoil should be looked at in more detail because of the strong effects of the subsoil temperature on long-term patterns of assimilate allocation to roots. The influence of hormones on assimilate distribution (Aloni *et al*., 1986; Jones *et al*., 1986) and even phloem loading (Lenton, 1984; Vreugdenhil and Kerckhoffs, 1992; Martinez-Cortina and Sanz, 1994) has been postulated. Nevertheless, the ecological significance of the influence of soil, especially subsoil temperature, on long-distance transport of assimilates should be considered. Intensive root growth of maize seedlings mostly in the upper soil layers may be advantageous during early growth in spring, when the air and topsoil are getting warmer, while the subsoil is still cold.

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References

Aloni B, Daie J, Wyse RE. 1986. Enhancement of [^{14}C]sucrose from source leaves of *Vicia faba* by gibberellic acid. *Plant Physiology* **82,** 962–6.

Crèvecoeur M, Ledent JF. 1984. Effets de basses températures (0–10 °C) sur la croissance juvénile du mais: quelques aspects de recherche fondamentale. *Bulletin van de Koninklijke Belgische Botanicske Vereninging* **117,** 382–412.

Dolstra O, Haalstra SR, van der Putten PEL, Schapendonk AHCM. 1994. Genetic variation for resistance to low-temperature photoinhibition of photosynthesis in maize (*Zea mays* L.). *Euphytica* **80,** 85–93.

Engels C, Marschner H. 1992. Root-to-shoot translocation of macronutrients in relation to shoot demand in maize (*Zea mays* L.) grown at different root zone temperatures. *Zeitschrift für Pflanzenernährung und Bodenkunde* **155,** 121–8.

Giaquinta RT, Geiger DR. 1973. Mechanism of inhibition of translocation by localized chilling. *Plant Physiology* **51,** 372–7.

Haldimann P, Fracheboud Y, Stamp P. 1995. Carotenoid composition in *Zea mays* developed at sub-optimal temperature and different light intensities. *Physiologia Plantarum* **95,** 409–14.

Janowiak F, Markowski A. 1994. Changes in leaf water relations

and injuries in maize seedlings induced by different chilling conditions. *Journal of Agronomy and Crop Science* **172,** 19–28.

Jones RJ, Griffith SM, Brenner ML. 1986. Sink regulation of source activity by hormonal control. In: Shannon JC, Knievel DP, Boyer CD, eds. *Regulation of carbon and nitrogen reduction and utilization in maize*. Rockwell, USA: American Society of Plant Physiologists, 233–46.

Lenton JR. 1984. Are plant growth substances involved in the partitioning of assimilate to developing reproductive sinks? *Plant Growth Regulation* **2,** 267–76.

Long SP, East TM, Baker NR. 1983. Chilling damage to photosynthesis in young *Zea mays*. *Journal of Experimental Botany* **34,** 177–88.

Martinez-Cortina C, Sanz A. 1994. Effect of hormones on sucrose uptake and on ATPase activity of *Citrus sinensis* L. Osbeck leaves. *Annals of Botany* **73,** 331–5.

Münch E. 1930. *Die Stoffbewegungen in der Pflanze*. Jena: Gustav Fischer.

Richner W, Soldati A, Stamp P. 1992. A system for studying vertical temperature gradients within the root zone on root growth. In: Kutschera L, Hübl E, Lichtenegger E, Persson H, Sobotik M, eds. *Root ecology and its practical application*. Klagenfurt: Verein für Wurzelforschung, 73–6.

Richner W, Soldati A, Stamp P. 1996. Shoot–to–root relations in field-grown maize seedlings. *Agronomy Journal* **88,** 56–61.

Sowinski P. 1993. A source–sink relationship in the cold-treated maize seedlings. In: Wilson D, Thomas H, Pithan K, eds. *Crop adaptation to cool*, *wet*, *climates*. Brussels: European Cooperation in the Field of Scientific and Technical Research (COST), 199–203.

Sowinski P. 1995. Transport of assimilates from leaves to roots in chilling- treated maize seedlings. *Acta Physiologia Plantarum* **17,** 341–8.

Sowinski P, Królikowski Z. 1995. Chilling sensitivity in maize seedlings. III. Relations between growth and functioning at low temperatures and during post-stress recovery. *Acta Physiologia Plantarum* **17,** 219–24.

Sowinski P, Maleszewski S. 1989. Chilling sensitivity in maize seedlings. 1. Growth and functioning of shoots and roots. *Acta Physiologia Plantarum* **11,** 165–71.

Sowinski P, Maleszewski S. 1990. Chilling sensitivity in maize seedlings. 2. Effect of low temperature on transport of ^{14}C assimilates from leaves to roots. *Acta Physiologia Plantarum* **12,** 35–40.

Sowinski P, Bednarek B, Jelen K, Kowalski T, Ostrowski K. 1990. An *in vivo* method for the transport study of assimilated substances using ^{14}C-isotope and X-ray proportional counters. *Acta Physiologia Plantarum* **12,** 139–48.

Stamp P. 1984. Chilling tolerance of young plants demonstrated on the example of maize (*Zea mays* L.). In: Geisler G, ed. *Advances in agronomy and crop science 7*. Berlin: Paul Parey.

Stirling CM, Rodrigo VH, Embrerru J. 1993. Chilling and photosynthetic productivity of field-grown maize (*Zea mays*): changes in the parameters of the light-response curve, canopy leaf CO_2 assimilation rate and crop radiation-use efficiency. *Photosynthesis Research* **38,** 125–33.

Verheul MJ, Picatto C, Stamp P. 1996. Growth and development of maize (*Zea mays* L.) seedlings under chilling conditions in the field. *European Journal of Agronomy* **5,** 31–43.

Vreugdenhil D, Kerckhoffs LHJ. 1992. Abscisic acid stimulates sucrose uptake into tobacco leaf discs. *Acta Botanica Neerlendica* **41,** 161–5.