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Role of Heat Girdling in Early Seedling Death of Sorghum

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ABSTRACT

High soil temperatures (>45 °C) can inhibit the field establishment of sorghum [*Sorghum bicolor* (L.) Moench.] seedlings in the semi-arid tropics even when adequate soil moisture is present. The objective of this study was to demonstrate an apparatus for simulating this situation under controlled conditions. The apparatus applies a high temperature localized near the leaf intercalary meristem while maintaining an adequately moist root environment. With this technique, we tested the hypothesis that seedling death may be due to a heat-girdling, leading to restricted translocation of carbohydrates to the root. Exposure of 10-d-old sorghum seedlings to heat-girdling temperatures of 52 ± 2 °C caused complete cessation of leaf elongation. At the same time, shoot carbohydrate concentrations increased while root carbohydrates declined, indicating that phloem transport from the shoot to the root may have been blocked or restricted. During the first 173 h of stress, turgor of both expanded leaves and leaf growing regions (basal 1 cm of leaf tissue) increased, indicating a favorable water balance in the seedlings. After 198 h of heat-girdling, seedlings died. The apparatus we developed should prove useful for the large scale screening of seedlings for resistance to high soil surface temperatures.

POOR STAND ESTABLISHMENT is a major problem limiting the stable production of sorghum in India and the eastern and southern regions of Africa (Peacock, 1982; O'Neill and Diaby, 1987). Abiotic factors limiting crop establishment in these areas include low moisture availability, soil compaction and crusting, and high soil temperatures.

Failure to establish an adequate crop stand might be due to either failed germination, inhibited epicotyl emergence from the soil, or death of seedlings after emergence. Abdullahi and Vanderlip (1972) reported a positive correlation between sorghum seedling vigor in the laboratory and field emergence. Seed of enhanced quality, however, did not consistently improve stand establishment in farmers' fields in Botswana, Africa (Mortlock and Vanderlip, 1988). Genetic variation in the ability of sorghum and pearl millet [*Pennisetum glaucum* (L.) R.Br.] to emerge has been shown (Peacock, 1982). In sorghum, Ougham and Stoddart (1985) demonstrated that emergence is positively related to protein synthesis in germinating embryos, and this has been suggested as a selection criterion for heat tolerance at the germination and emergence stages (Ougham and Stoddart, 1985; Ougham et al., 1988). Soil crusting can obstruct plumule growth, and field techniques have been developed to screen for seedling emergence through such soils (Dasberg et al., 1966; Soman et al., 1984).

Sustained seedling growth following emergence depends not only on the physiological processes for ger-

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mination but also on the capacity of the seedling to elongate, produce leaves, and become autotrophic. The rate of leaf growth in many of the *Poaceae* species is largely determined by the temperature of the stem apex (Watts, 1974; Peacock, 1975b). In southern Africa, soil surface temperatures in fields commonly exceed 45 °C and temperatures as high as 60 °C have been recorded (Soman and Peacock, 1985). In sorghum, leaf growth stops at air temperatures above 47 °C (Peacock, 1982). Unpublished data show that high soil surface temperatures, and not root zone moisture stress, may be the primary cause of seedling death (Soman and Peacock, unpublished data, 1984). To date, the mechanisms associated with seedling death in sorghum are not understood.

A laboratory technique was developed that controls the temperature of a localized region of seedlings in order to simulate the elevated soil surface temperatures that can occur in the field. This apparatus was used to examine the water relations and carbohydrate levels within seedlings exposed to high temperatures near the leaf intercalary meristem but supplied with adequate root zone moisture. The apparatus allowed an evaluation of response to high soil surface temperature independent of soil moisture content, and allowed us to test the hypothesis that seedling death is due to heat-girdling and, presumably, restricted phloem transport into the roots.

MATERIALS AND METHODS

Plant Materials

In all experiments, sorghum (cv. RS626) seeds were germinated in vermiculite at 25 ± 2 °C using a 13-h photoperiod from 0700 to 2000 h. Irradiance (approximately 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$) was supplied from cool-white fluorescent tubes. After a 7-d germination period, vermiculite was carefully washed from roots and seedlings were transferred to wooden racks as described previously by Matsuda and Riazi (1981). The racks were held together with a rubber band, and a strip of soft foam on each internal surface provided firm, nondamaging support for the seedlings. Seedlings were grown in plastic trays, using an aerated Hoaglands' solution (Hoagland and Arnon, 1938) supplemented with an iron chelate. Each tray contained 90 plants (six racks of 15 plants each), and heat treatments were initiated when the plants were 10-d old.

Control of Meristem Temperature and Measurement of Leaf Elongation

Opaque plexiglass racks identical in size to those described above were modified allowing temperature control of the leaf intercalary meristem. A 30-cm-length of square (0.50 by 0.50 cm) brass tube was inserted into a longitudinal groove cut into each piece of plexiglass (Fig. 1). Soft urethane foam was placed over the two brass surfaces on the inside of the rack to hold the seedlings in place and to prevent physical damage to the shoot and meristem. Meristem temperature modification was achieved by circulating water from a water bath (Haake, Type FE). Meristem temperatures were determined with a SensorTek (SensorTek, Inc., Lifton, NJ) IT-23 thermocouple microprobe coupled with a BAT-12 meter. The probe was inserted into the foam adjacent to the seedlings. For these studies, six racks were placed in each tray of Hoagland's solution. A total of 12 racks was used in the experiments. Six racks were used as controls and meristems were exposed to room temperature. The remaining six racks were connected in series by plastic tubing through which the heated water was circulated. There were three heated and three control racks in each tray, ensuring that the root temperature and shoot temperature were the same for both treatments. In heat-girdling experiments, meristem temperatures were set to 52 ± 2 °C, the temperature at which leaf elongation ceased. Elongation of the youngest leaf was measured with a plastic ruler using the method described by Peacock (1975a). Measurements were made at the beginning and end of each daily light period (0700 and 2000 h).

Water Potential (ψw), Solute Potential ($\psi \pi$), and Turgor (P)

Water and osmotic potentials were determined psychrometrically and turgor was calculated by difference. Psychrometric determinations of water potential and solute potential were made with a Merrill 75-13 chamber psychrometer (Merrill Specialty Equipment, Logan, UT) and a WESCOR MJ55 meter (WESCOR, Inc., Logan, UT) using sections of leaf tissue from three plants for each replicate determination. Two types of leaf tissue were sampled: expanded leaf blade tissues were 1-cm sections obtained from the mid-blade region of the two youngest leaves, and basal tissue was the 1-cm leaf segment that was nearest the seed. Although not specifically determined in this study, this basal segment was expected to contain the leaf intercalary meristem and associated elongating cells (Matsuda and Riazi, 1981). Five replicates of each tissue type were allowed to equilibrate for 4 h in an insulated water bath at 26 °C. Following the measurement of water potential, the chambers were immersed in

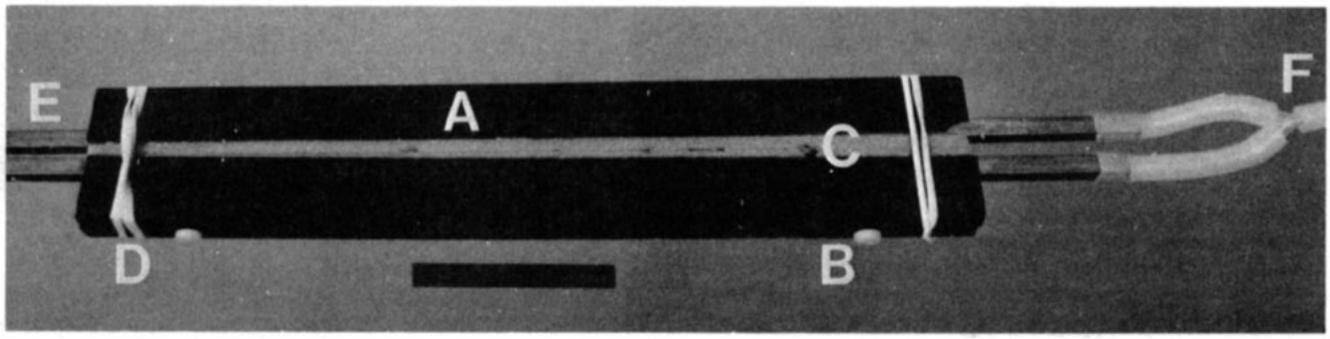

Fig. 1. The apparatus used to control meristematic and elongation zone temperature of sorghum seedlings. Components are: A, opaque plexiglass frames; B, adjusting screws to minimize and maintain uniform pressure on seedlings; C, foam strips to cushion seedlings; D, rubber bands to hold unit together; E, brass tubes through which heated water is pumped; F, distribution lines connected to a controlled temperature water bath. Bar indicates 5.0 cm.

liquid N_2 for 20 s, allowed to come to room temperature, then returned to the insulated bath. Values of osmotic potential were determined after 4 h. Measurements were made at the middle of the light period after 5, 29, 53, 101, and 173 h of heat girdling.

Carbohydrate Extraction and Analysis

All biochemicals, resins, and enzymes used for carbohydrate analyses were obtained through Sigma Chemical Co. (St. Louis, MO). Seedlings were harvested at 5, 29, 101, and 173 h after the beginning of the heat girdling and dissected into (entire) shoot and root components, and the seed remnant was discarded. Three randomly selected plants were used in each replicate. Tissues were freeze-dried and stored in a desiccator at room temperature. Tissue was ground, then extracted with three 3-mL volumes of 12 methanol : 5 chloroform : 3 water (by volume). Mannitol (1.0 mg) was added as an internal standard. Details of sample preparation for HPLC analysis have been previously described (Miller and Langhans, 1989). Samples were injected into an isocratic HPLC system consisting of an LKB 2150 pump (Pharmacia, Inc., Piscataway, NJ) and refractive index detector (Knauer, Model 198, Rainin Inst. Co., Inc., Woburn, MA), using a Rheodyne 7010 injector equipped with a 20-μL sample loop (Rheodyne Corp., Cotati, CA). The system was operated at 0.6 mL · min^{-1} using degassed water as the mobile phase. Soluble sugar separation was achieved on a stainless steel column (Bio-Rad HPX-87C, 300 by 7.8 mm i.d., Bio-Rad Laboratories, Richmond, CA) maintained at 85 °C. A guard column (Bio-Rad Micro-Guard) was used at room temperature between the injector and analytical column. Peak quantitation and retention times were determined using a Hewlett Packard 3390A integrator with peak identification based on retention times of authentic D-sugars. The water used as eluant was prepared from glass distilled water, using ion exchange cartridges (Norganic, Millipore Corp., Bedford, MA) followed by filtration through a 0.45 μm membrane under vacuum.

Starch was determined on the air-dried residue of shoot tissue left following soluble carbohydrate extraction. After hydrolysis by amyloglucosidase, the glucose released was determined using a coupled glucose oxidase-peroxidase method described in detail elsewhere (Miller and Langhans, 1989).

All experiments were conducted using a completely randomized design. Data were analyzed using CoStat (CoHort Software, Berkeley, CA) or SAS (SAS Inst. Inc., Cary, NC).

Table 1. Dry weight of sorghum shoot and root systems as a function of duration of 52 °C temperature localized at the leaf intercalary meristem. Data are the means of three replications of three seedlings each.

Duration of stress	Roots		Shoots	
	Heated	Control	Heated	Control
h	mg			
5	15.6	15.6	44.0	47.6
29	17.0	17.3	49.3	48.3
101	10.6	27.6	66.6	77.3
173	11.0	43.0	88.0	127.3
LSD*	3.8		11.3	

* LSD (least significant difference) at the $P = 0.05$ level across all time periods for each tissue type.

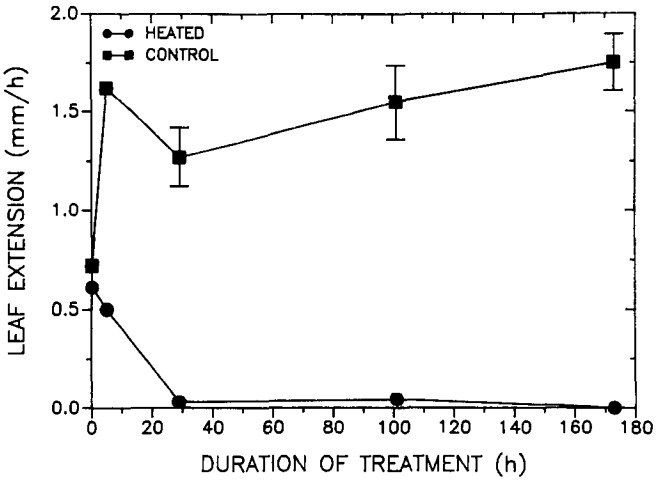

Fig. 2. Rate of leaf elongation in sorghum seedlings as affected by 52 °C (heat-girdled) or 31 °C (nongirdled) meristem temperatures. Data are the means of 10 plants. Bars indicate ± SE. If no bar is shown, the SE is within the dimension of the symbol.

RESULTS

Leaf Elongation and Plant Growth

When temperatures in the region of the seedling meristem were raised to 52 °C, elongation of the youngest leaf stopped within 29 h of exposure to elevated temperature (Fig. 2). In seedlings kept at the control temperature (31 °C), the leaf elongation rate increased from 0.7 to 1.5 mm h^{-1}. By the seventh day, control plants had developed two new leaves, while the heated plants had only the three original leaves. Throughout the duration of the experiments, leaves of plants in both treatments remained turgid and green, except that leaves of heated seedlings displayed an increasing purple pigmentation during the final 2 to 3 d of the experiments. In control seedlings, root and shoot dry mass nearly tripled (Table 1). In heated plants, shoot dry matter doubled, and root mass decreased significantly over the 7-d period. Root:shoot ratios remained stable for the control plants but declined more than 60% in the heated plants (calculated

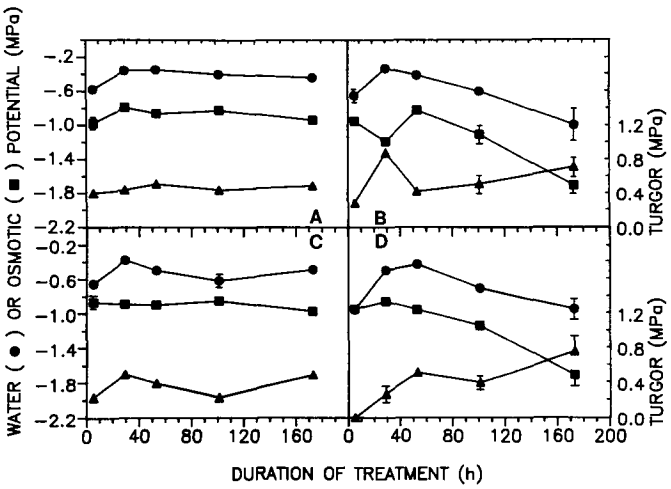

Fig. 3. Water (•), osmotic (■), and turgor (▲) potentials of (A) nonheat-girdled, expanded leaf blades, (B) heat-girdled, expanded blades, (C) nonheat-girdled basal leaf regions, and (D) heat-girdled, basal leaf regions over a 173-h time course. Seedlings were heat-girdled with 52 °C meristem temperatures or nongirdled with 31 °C. Each point is the mean of 5 replications of 3 seedlings each (±SE). If no bar is shown, the SE is within the dimension of the symbol.

from data in Table 1). At 198 h (Day 8), heated seedlings collapsed and died, whereas control seedlings remained healthy.

Water Potential, Solute Potential, and Turgor

Although some fluctuations in water status values were evident over the course of the study, water potential, osmotic potential, and turgor of both expanded midblade and basal regions of the control plants remained relatively constant over the 173-h study period (Fig. 3A, 3C). In contrast, leaf samples obtained from plants whose meristem regions were heated to 52 °C showed more variability, and after prolonged stress, osmotic potential and water potential of expanded midblade and basal regions of the leaf were both lower than values obtained before application of the heat treatment (Fig. 3B, 3D). The basal region of the leaf showed a temporary loss of turgor after 5 h exposure to high temperature, but positive turgor was regained by 29 h and turgor continued to remain positive through 173 h, even though there was discoloration of the basal tissues. Although not measured, we inferred that complete turgor loss occurred at 198 h, when seedlings collapsed and died.

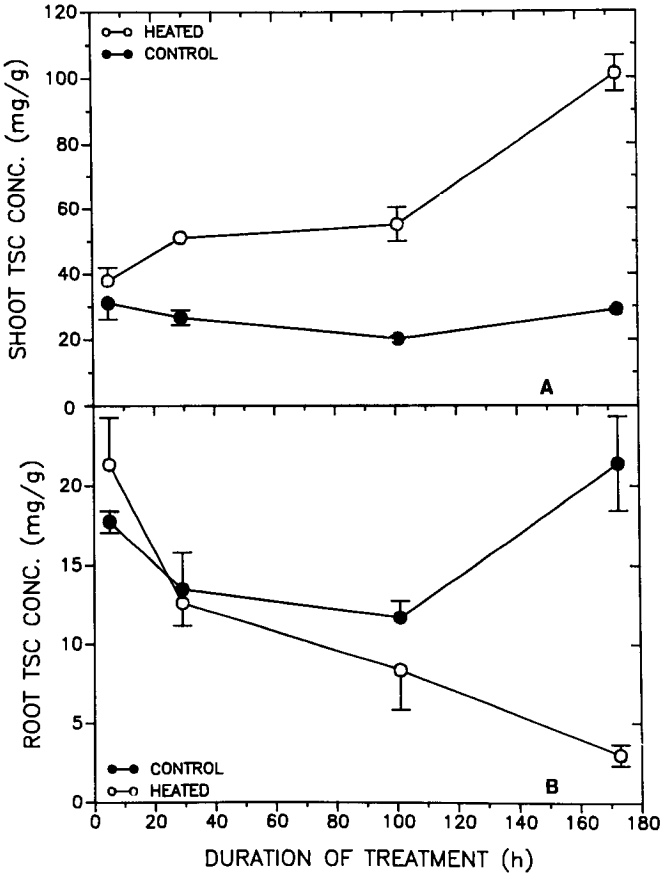

Fig. 4. Effects of meristem temperature on (A) shoot total soluble carbohydrate (TSC) concentration (sum of sucrose, glucose, and fructose, as determined by HPLC) and (B) root total soluble carbohydrate concentration, each on a dry weight basis. Seedlings were heat-girdled with 52 °C meristem temperatures or nongirdled with 31 °C. Data points are the means of 3 replications of 3 seedlings each (±SE). If no bar is shown, the SE is within the dimension of the symbol.

Shoot and Root Soluble Carbohydrate and Starch

In shoots of heated plants, the concentration of total soluble carbohydrate (TSC, primarily sucrose, glucose, and fructose) increased substantially during the experiment (Fig. 4A). After 173 h of heat, both TSC and starch concentration were at least 3.4-fold greater than in nonheated seedlings (Fig. 4A, Table 2). In contrast, meristem heating reduced root TSC to very low concentrations, with a 5.5-fold decrease seen in 173 h (Fig. 4B). Sucrose was the major soluble carbohydrate accumulating in the shoots of heated seedlings, with glucose and fructose present at lower levels (Table 2).

DISCUSSION

In the field, sorghum seedlings grown under dryland conditions can die after emergence, even though adequate moisture is present in the root zone (Soman and Peacock, unpublished data, 1984). Since temperatures of the soil surface can reach 60 °C as the upper soil layers dry, it seemed reasonable that seedling death may result from tissue damage near the soil surface. While it is recognized that heat and light intensity in the laboratory are far lower than in the field, the heat rack apparatus used in these studies appears to simulate the field situation of adequate moisture in the root zone and elevated soil surface temperatures. Studies with the apparatus have also given further insight into the possible sequence of events leading to seedling death.

Table 2. Effect of 52 °C meristematic temperatures on concentrations of sucrose, glucose, fructose, and starch in roots and shoots of sorghum seedlings. Data are the means of three replications of three seedlings each.

Duration of stress	Carbohydrate concentration			
	Roots		Shoots	
	Heated	Control	Heated	Control
h	mg g dry wt^{-1}			
	Sucrose			
5	7.8	7.6	11.6	7.2
29	6.6	6.7	24.8	6.9
101	5.1	8.5	29.7	10.0
173	2.2	13.7	71.3	15.9
LSD*	3.6		9.0	
	Glucose			
5	6.2	4.8	16.8	16.5
29	3.4	2.6	18.2	13.0
101	2.0	1.9	16.1	5.9
173	0.8	3.6	20.6	6.5
LSD	2.2		5.1	
	Fructose			
5	7.3	5.3	9.5	7.4
29	2.6	4.2	8.3	6.9
101	1.3	1.3	9.4	4.3
173	0.0	4.0	9.1	6.6
LSD	2.6		3.4	
	Starch			
5	–	–†	54.7	44.5
29	–	–	54.5	44.7
101	–	–	34.7	29.5
173	–	–	49.7	14.2
LSD	–		13.0	

* LSD (least significant difference) at the $P = 0.05$ level across all time periods for each carbohydrate and tissue type.
† Starch not detected in root tissue.

Because turgidity of both the expanded blade and basal region (Fig. 3) of the leaf was maintained throughout the first 173 h of heat treatment, the shoot was maintained with an adequate supply of water. However, the data showing an increase in the concentration of total soluble sugar (Fig. 4), and especially sucrose (Table 2) in the shoot, provide evidence that phloem transport from the leaves to the roots was reduced or eliminated by heat girdling. This view is further supported by results that show that the osmotic potential of the expanded midblade region of young leaves decreases markedly after several days of heat treatment (Fig. 3B) as root dry matter (Table 2) and soluble sugar concentrations decrease (Fig. 4). A third line of evidence is the appearance of purple pigmentation, probably anthocyanin, in leaves of heat-girdled plants. Phosphorus deficiency often leads to anthocyanin synthesis in leaves and petioles. We feel, however, that P deficiency was not a factor, due to the relatively small dry weight changes of the seedlings during the course of the experiment. A more likely explanation is that the accumulation of soluble sugar in the shoot induced anthocyanin synthesis, a relatively common plant stress response (Harbone, 1976; Cobbina and Miller, 1987).

While the sugar analysis data for entire shoots and water status data obtained from blades show similar trends, the reductions in osmotic potential in the midblade region of the two young leaves are an order of magnitude greater than can be accounted for by changes in sucrose. In the interval from 5 to 173 h, the increase in the shoot concentration of sucrose (the major sugar component that was changed by the heat treatment) is almost 60 mg g dry wt^{-1}. Assuming a water content of 85%, this is about a 0.03-molal change and corresponds to a 0.08-MPa reduction in osmotic potential of the shoot. In contrast, data obtained from the midblade region of the young leaves show that an osmotic potential reduction of about −0.8 MPa occurred in the same interval (Fig. 3B). This difference may exist because of selectively higher concentrations of sucrose in the blades of young leaves or because there are other, presently unidentified factors contributing to the osmotic adjustment. The latter possibility is not unreasonable since a similar situation exists in barley (*Hordeum vulgare* L.) leaves when seedlings are subjected to osmotic stress (Riazi et al., 1985).

Interestingly, while application of heat to the area around seeds appeared to block or reduce translocation to roots, the water status data showing maintenance of turgor indicate that vital functions of cells in this region are maintained for a prolonged period under obviously unfavorable conditions. In fact, the close coincidence of seedling death on the eighth day and rapid turgor loss may mean this measure is a good indicator of viability. Additional work is necessary to determine if real-time measurements of leaf extension (e.g., within 1-2 d) may be used as a more rapid procedure for screening seedling resistance to elevated soil temperatures.

Studies on translocation and girdling are numerous, with experiments carried out by the Italian anatomist and microscopist, Marcello Malphighi, as early as 1675 and by Stephen Hales in 1727 (Salisbury and Ross, 1978). In these experiments, it became apparent that girdling of the stem had no immediate effects upon the growth of the shoot or loss of water by transpiration from the leaves. However, the root system was unable to survive without a continuous supply of photosynthate and other dissolved organic molecules. Our results are consistent with the occurrence of phloem girdling induced at the soil interface as a result of elevated soil surface temperatures. That the root system was unable to survive without a steady supply of current photosynthate from the leaves is indicated by the gradual loss of dry matter in the root system during the experiment (Table 2). During this time, the restricted supply of sugars undoubtedly contributed to the slow growth and later decline of the root system. In the field, this condition may cause a confinement of roots to the upper layers of the soil, where soil drying occurs faster, further accentuating stress on the seedling.

CONCLUSION

Death of juvenile sorghum plants in dryland situations may not be due to water stress per se, but to the inability of the root to maintain growth functions because of reduced assimilate import from the shoot. This finding has implications for developing countries in arid zones, where seedling death is a severe problem. Local changes in farming methods could be adapted to reduce the temperature of the soil surface microclimate. Because our thermal apparatus allows many plants to be studied under very closely controlled temperature conditions, it should prove useful for rapid screening of breeding lines for tolerance to high soil surface temperatures as well as for understanding mechanisms involved in plant growth and development under stress conditions.

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