Stem Extension Rate in Light-Grown Plants[1]

EFFECTS OF PHOTO- AND THERMOPERIODIC TREATMENTS ON THE ENDOGENOUS CIRCADIAN RHYTHM IN *CHENOPODIUM RUBRUM*

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ABSTRACT

Low temperature pulses have two effects on the circadian rhythm exhibited by stem extension rate of green *Chenopodium rubrum* plants. First, low temperature pulses have the same effect on the phasing of the rhythm as a dark period interrupting continuous light. Second, low temperature pulses stimulate stem extension rate during the 10 hours immediately following the end of the pulse. A difference in temperature between soil and air increases this effect. In any case, it is the change in temperature which is essential and not a specific temperature. Effects of light and temperature on phasing and amplitude of the rhythm explain why the maximal stem growth is observed under normal photo-thermoperiodic conditions, *i.e.* a high temperature during the photoperiod and a low temperature during the dark period.

In their natural environment, plants develop under photo- and thermoperiodic conditions which change with the seasons. Developmental and behavioral responses of plants clearly demonstrate their potential for measuring the duration and sequence of environmental conditions. It is now generally accepted that time measurement in eukaryotes depends on the interaction of an endogenous circadian rhythm or physiological clock with environmental signals (3). The most important environmental signals or 'Zeitgeber' are the light and temperature regimes.

Since controlled environment facilities became available, the influence of light and temperature on growth and development of plants has been studied intensively (25). From these early studies it became evident that a certain number of plants could not tolerate constant conditions of illumination and temperature. The detrimental effect of constant conditions was particularly noticeable during vegetative development (9, 11). In the case of the vegetative development of tomatoes, it could be demonstrated that a light-dark cycle or a temperature cycle of a few degrees was necessary for normal growth. A light-dark cycle with lower temperature at night seemed to be optimal (24). If cycle length differed significantly from 24 h, development was again seriously hampered (10). In terms of Bünning's hypothesis (3), these results were taken as an indication of the necessity of synchronization of physiological oscillations by external synchronizers like light and temperature. The experimental significance of Bünning's hypothesis for photo- and thermoperiodic control of vegetative growth, however, was limited by the lack of devices

for measuring growth kinetics with high enough precision and resolution.

With auxanometric methods using LVDT,[2] it is now possible to measure continuously the kinetics in growth rate of green plants for h or even d under different environmental conditions (13, 14). This technique has been used recently with *Chenopodium rubrum* to demonstrate a circadian rhythm in SER of internodes of green plants in constant conditions of light and temperature (15). This plant, therefore, seemed particularly suited to study the interaction of light and temperature signals with the physiological clock.

In this publication we report the effects of cycles of light-dark and high-low temperature on SER of *C. rubrum*. The basic kinetics of pure photo- and thermoperiodic effects on SER are taken as a system of reference to study the interaction of light and temperature signals. The results are discussed in relation to current hypotheses of the nature of the biological clock and light perception in green plants (22, 23).

MATERIALS AND METHODS

Plant Material and Growth Conditions. Seeds of *Chenopodium rubrum* L., ecotype 184 (50° 10' N; 150° 35' W) were sown directly on soil and germinated for 3 d under temperatures of 32.5°C for 12 h in light and 10°C for 12 h in darkness, following the procedures established by Cumming (5). After seeds were germinated, the temperature was kept constant at 24°C and plants were grown in continuous white light at 170 μmol m^{-2} s^{-1} (= 140 μmol m^{-2} s^{-1} between 400 and 700 nm) for 2 weeks. Plants were then transferred to continuous white light at 85 μmol m^{-2} s^{-1} for 4 d before onset of experimental treatments. The RH was kept constant at 50%. After this pretreatment, the 3rd internode above the cotyledons was about 5 mm long. White light at 85 μmol m^{-2} s^{-1} was supplied by xenon arc lamps (Osram, XQO, 10 KW; "Results"; Fig. 2) or mercury high pressure lamps (Osram, HQIL, 500 W; "Results"; Figs. 1, 3–6) and filtered through heat-absorbing glass (KG, 3 mm; Schott and Gen., Mainz, F.R.G.) and thermopane double glass, 6 mm.

Extension Rate Recording. Stem extension rate was monitored continuously using linear voltage differential transformers, LVDT (series L, Ifelec, Chauvin Arnoux Paris, France) in combination with demodulators (type GDL, Ifelec). The sensor unit is composed of a fixed and a moving part. The latter is a magnetic iron core, the fixed part an assembly of electric coils. Depending upon the position of the magnetic core, an electric signal is transmitted to the demodulator producing a voltage linearly related to the core position. Plant internodes were attached to

[2] Abbreviations: LVDT, linear voltage differential transformer; SER, stem extension rate; D, darkness; L, light.

the transducer cores via a beam-balance system and a short cotton thread tied to the stem by means of a double loop. The whole assembly was kept under tension by a weight of 1.5 g. The cotton thread was tied to the upper part of the 3rd internode above the cotyledons. A maximum core displacement of 20 ± 0.01 mm could be registered. SERs in mm h⁻¹ were computed as the integral of 1-h intervals. Stem extension may be amplified up to 100-fold but a 20-fold amplification was used throughout the experiments, giving a good signal-to-noise ratio. The SER kinetics represent the mean value of three to five plants measured simultaneously.

Temperature Treatments. Temperatures between 10 and 32°C were chosen in view of the requirement of two to three cycles of 12 h at 10°C and 12 h at 32°C either in darkness or in light to induce 100% germination. At constant temperatures only about 1% of *C. rubrum* seeds germinate.

Effects of the different environmental conditions on the measuring device were recorded with a glass rod in place of a plant. Light-dark cycles at constant temperatures gave no response from the LVDT. Transitions from 32 to 10°C induced a response similar to an increase in stem length. The transition from 10 to 32°C induced just the opposite effect as the transition from 32 to 10°C with exactly the same amplitude and the same kinetics. After less than 1 h, a new stable base-line was reached: the sensitivity of the system was the same at 10°C as at 32°C and the values of SER at 10 and 32°C could be compared directly. The time required for a transition in air temperature from 10 to 32°C and vice versa was less than 30 min (Fig. 1) except for the experiments shown in Figure 2 where the transitions took 2 h.

The changes in soil temperature were much slower than those for air and took about 3 h when the air was changing from 32 to 10°C and 5 h for a transition from 10 to 32°C. Even after equilibration the soil temperature was always 4°C lower than the air temperature, due to cooling by evaporation of water from the soil.

In some experiments this difference in the kinetics of temperature transition between soil and air were eliminated through temperature control of the water wetting the soil. Under such conditions the soil could be brought from 10 to 28°C within 30 min.

Light Treatments. Continuous light or 12:12 h light:dark cycles were given with the light sources as specified above.

EXPERIMENTAL PROTOCOL

In all experiments shown in Figure 2 two successive treatments were imposed on the plants. First, there was a periodic treatment of light and temperature with the following program:

(a) 12L (32°C)-12D (32°C), light-dark cycle at constant 32°C; *i.e.* photoperiodic treatment only.

(b) 12L (32°C)-12L (10°C), temperature cycle at 32 and 10°C in constant illumination; *i.e.* thermoperiodic conditions only.
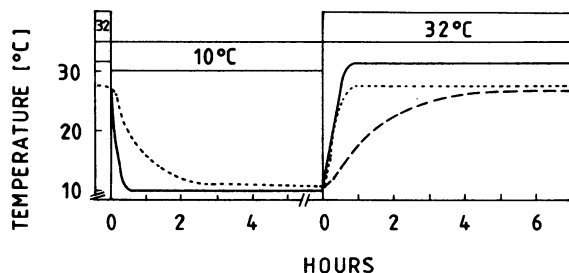
(c) 12L (32°C)-12D (10°C), light-dark and temperature cycle

FIG. 1. Kinetics of the change in air (——) and soil temperature with (···) and without (– – –) additional control of the soil temperature during the transition of air temperature from 32 to 10°C and from 10 to 32°C.
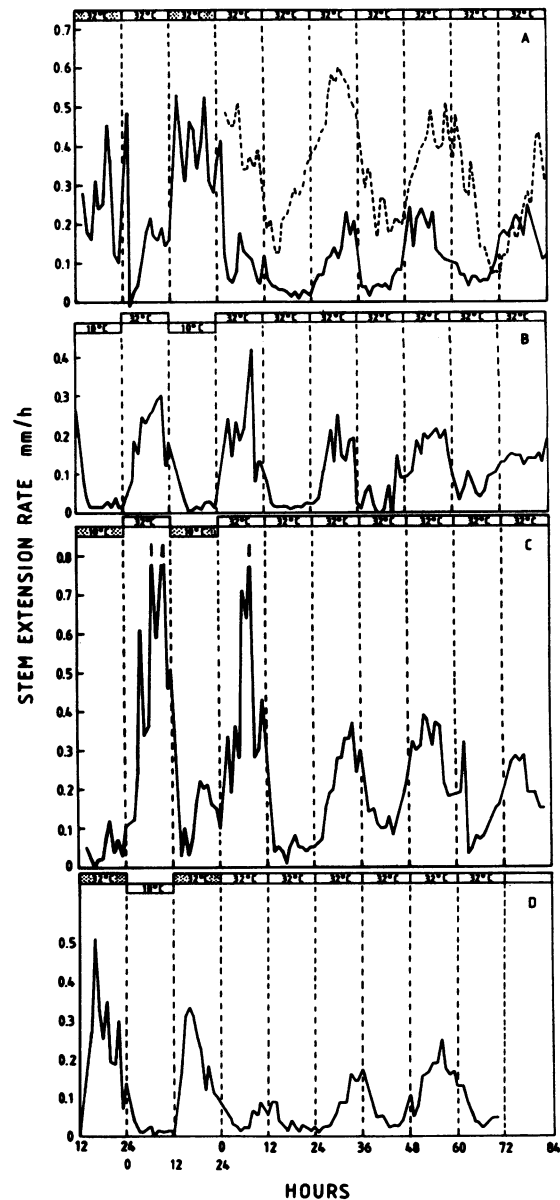

FIG. 2. Changes in mean stem extension rate of *C. rubrum* under different photo- and thermoperiodic conditions before transfer to 84 h of white light at 32°C (LL 32°C). The periodic conditions were given for three 24-h cycles but are only shown for the last 36 h before transition to the constant conditions. ▭, white light; ■, darkness. A, solid line, 12D (32°)-12L (32°)-12D (32°) → LL (32°); broken line, data from (15) = LL (24°) after 12D (24°). B, 12L (10°)-12L (32°)-12L (10°) → LL (32°). C, 12D (10°)-12L (32°)-12D (10°) → LL (32°). D, 12D (32°)-12L (10°)-12D (32°) → LL (32°). The values are the mean of four plants measured simultaneously.

with the high temperature in light and the low temperature in dark; *i.e.* normal photothermoperiodic conditions.

(d) 12L (10°C)-12D (32°C), light-dark and temperature cycle with the low temperature in light and the high temperature in dark; *i.e.* reversed photo-thermoperiodic conditions.

Second, the periodic treatment was always followed by constant conditions, *i.e.* in continuous light at 32°C, LL (32°C), and the SER recorded for at least 72 h.

RESULTS

A. Periodic Conditions. With photoperiodic cycles under constant temperature of 32°C, 12L (32°C)-12D (32°C) (Fig. 2A),

SER was high during darkness and low during light. The transitions between these two conditions were very rapid. After a dark-light transition, SER showed a rapid stimulation for about 20 min followed by a rapid decline to almost 0 SER at about 3 h after the change of conditions. Thereafter, SER increased again to reach a maximum at the 6th h of light.

With thermoperiodic cycles in constant light, 12L (32°C)-12L (10°C) (Fig. 2B), SER was high at the high temperature and practically 0 during the low temperature treatment.

In normal photo-thermoperiodic cycles, 12L (32°C)-12D (10°C) (Fig. 2C), SER was high during light at high temperature and low during darkness at low temperature. However, SER was not 0 in D10°C and after a minimum at the 2nd to 3rd h of darkness, SER increased to reach a maximum at the 7th h of darkness.

In inverse photo-thermoperiodic conditions, 12L (10°C)-12D (32°C) (Fig. 2D), SER was high during darkness at 32°C and almost 0 during the whole light span at 10°C in contrast to the SER at 10°C in darkness (Fig. 2C).

B. Constant Conditions, LL (32°C) Figure 2. *Phase of Rhythm in SER.* Irrespective of the preceding periodic conditions, SER always displayed a circadian rhythm in LL (32°C) with the same phasing and a first maximum around the 10th h of light, a second maximum at the 31st h, and a third at the 54th h. The phasing of the rhythm after the dark-to-light transition at 32°C was essentially the same as the one already observed at 24°C (15) (dotted line, Fig. 2A).

Amplitude of Rhythm in SER. The amplitude of the first peak (about 10 h) in SER after the transition to light at 32°C depended very much on the preceding periodic conditions. Clearly, the amplitude in SER depended only on the temperature preceding the transition, either in the light or in the dark. After a pretreatment at 32°C in darkness, the first peak in SER at the 10th h had only about half the value of the subsequent ones (Fig. 2, A and D). However, after a pretreatment at 10°C either in darkness (Fig. 2C) or in light (Fig. 2B), the first peak in SER was of much higher amplitude than the subsequent ones.

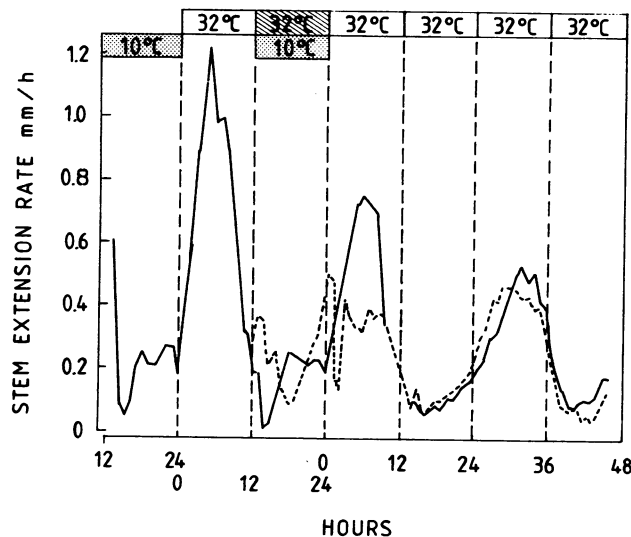**C. Effects of Different Temperature Treatments of Roots and**

FIG. 3. Changes in mean stem extension rate of *C. rubrum* in normal photo- and thermoperiodic conditions followed by 48 h of constant conditions, LL (32°). ▭, white light, ■ and ▨, darkness at 10 and 32°C, respectively. Solid line, 12D (10°)-12L (32°)-12D (10°) → LL (32°); the temperature of the soil was directly controlled during the second transition from 10 to 32°C but not during the first one (cf. Fig. 1). Broken line, LL (24°)-12D (32°) → LL (32°); SER is only shown for LL (32°). Mean of four plants measured simultaneously in both conditions.
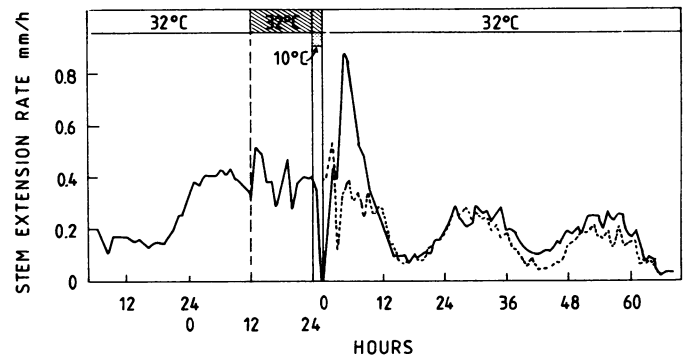

FIG. 4. Mean stem extension rate of *C. rubrum* under constant conditions of light and temperature (32°C) interrupted by 12 h of darkness at 32°C. Solid line, darkness at 32°C (▨) was followed by 2 h of darkness at 10°C (■) before transferring plants to LL (32°), ▭. The temperature of the soil was directly controlled at the 10 to 32°C transition (cf. Fig. 1). Broken line, control plants directly transferred to LL (32°) after 14D (32°).
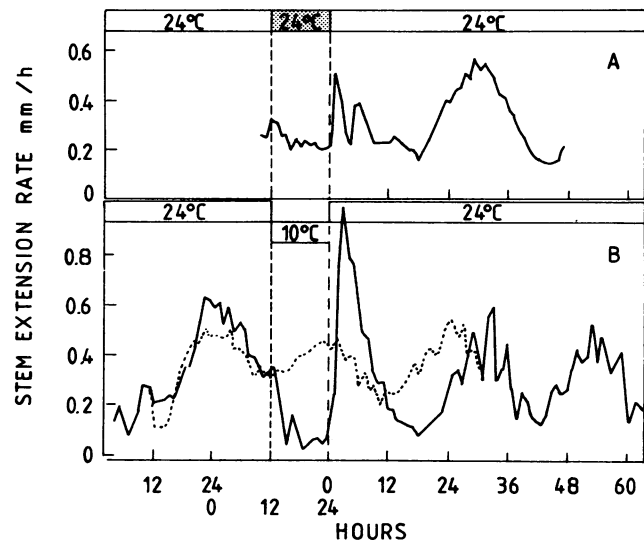

FIG. 5. Mean stem extension rate of *C. rubrum* in constant light and temperature (24°C) conditions, ▭, interrupted by: A, 12 h of darkness at 24°C, ■ (data taken from Lecharny and Wagner [15]); and B, 12 h at 10°C in light, ▭. In B, the dotted line represents the stem extension rate of control plants which were kept in constant conditions of 24°C in light.

Shoots. In Figure 1 it is shown that a transition from 32°C to 10°C is much slower in the soil than in the air surrounding the plants. The difference is considerable during the first 4 to 5 h and could be of importance in relation to the water status of the shoot and could possibly influence the SER of the first peak after a transition from 10 to 32°C. This problem was analyzed by monitoring SER in photo-thermoperiodic cycles of 12D (10°C)-12L (32°C) and subsequent transitions to LL (32°C) (Fig. 2C), with and without additional temperature control of the soil water to obtain the same rate of change in temperature in the air and the soil.

As shown in Figure 3, after the first low temperature pulse without additional temperature control of the soil, there is the same kinetic in SER as in Figure 2C with a maximum at the 5th h of light. The second low temperature pulse during which the temperature of the soil received additional control by a thermostat, results again in a strong stimulation of SER as compared to a pure photoperiodic treatment 12D (32°C)-12L (32°C) (dotted line, Fig. 3). However, thermostating the soil obviously had no influence on phase of the rhythm in SER.
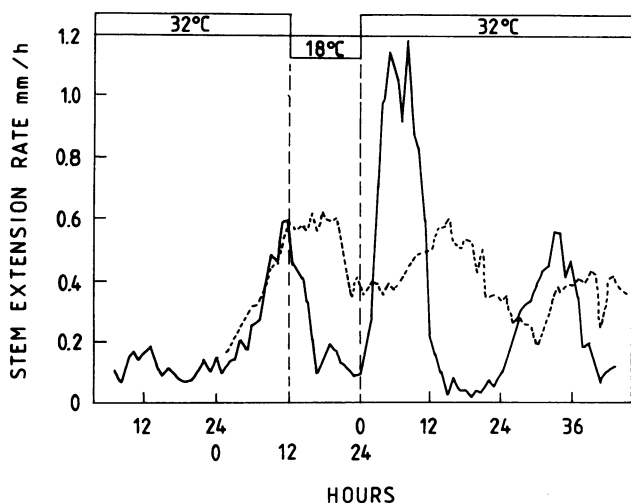
FIG. 6. Mean stem extension rate of *C. rubrum* under constant conditions of light and temperature (32°C) after an interruption by 12 h of 18°C in the light. The dotted line represents the stem extension rate of control plants which were kept at constant 32°C.

D. Short-Term Treatments at 10°C. In this series of experiments, one single 12 h dark-12D (32°C)—interrupted an otherwise continuous light treatment—LL (32°C). The dark span was either ended with 2 h at 10°C or remained constant at 32°C (Fig. 4). The low temperature was given with additional thermostating of the roots at the time of transfer from 10 to 32°C. The results in Figure 4 clearly demonstrate that 2h at low temperature are sufficient to induce a strong stimulation of the amplitude of the first maximum in SER after the dark-to-light transition. The effect was of the same order of magnitude as after 12 h of darkness at 10°C (Fig. 2, B and C; Fig. 3). Again, neither phase nor amplitude of the 2nd and 3rd maximum in SER was affected.

E. Effect of 12 h at 10°C during Continuous Light at 24°C. After interrupting continuous light at 24°C with 12 h at 10°C, the phasing of the rhythm in SER (Fig. 5B) was the same as after an interruption by 12 h of dark at 24°C (Fig. 5A), both delayed about 10 h, as compared with controls in continuous light (Fig. 5B, dotted curve). However, the first peak in SER after the dark-to-light transition and after the 10 to 24°C transition were clearly different in amplitude. Growth rate increased much more after the low temperature than after the dark treatment.

F. Effect of 12 h at 18°C during Continuous Light at 32°C (Fig. 6). As in the previous experiments, the low temperature treatment again rephased the preexisting rhythm in SER and increased growth rate considerably in the first peak of SER after the low-to-high temperature transition. Both temperature treatments (Figs. 5 and 6) were given with additional control of the root temperature.

DISCUSSION

Low Temperature Treatments and Phasing of the Rhythm in SER. Previously it was demonstrated (15) that the endogenous circadian rhythm in SER of *C. rubrum* under constant conditions of temperature and illumination is reset by a dark span of at least 8 h and the phase is determined by the dark-to-light transition. Here we could demonstrate that a lowering of the temperature for 12 h had almost the same effect on the phasing of the rhythm in SER as a dark period interrupting continuous light. In constant conditions of light and temperature (24°C) peaks in SER are 5, 28, 51 h after the end of the dark period (15). Different low temperature treatments interrupting an otherwise constant higher temperature (32°-10°-32°C; 32°-18°-32°C; 24°-10°-24°C) all had the same rephasing effect on the rhythm in

SER (Figs. 2A, B; 3; 5). These results show that it is the difference in temperature which is resetting the rhythm and not a specific temperature level. The minimum difference in temperature for resetting the phase in SER in *C. rubrum* has not yet been determined. The equivalence of low-high temperature and dark-light treatments for the rephasing of circadian rhythmic phenomena has been shown in other plants in continuous light (12, 18) and continuous darkness (4, 7, 27).

Low Temperature and Amplitude Modulation of SER. The first maximum in growth after resetting the rhythm by 12 h at low temperature is strongly increased in comparison to that of plants kept at constant temperature. This stimulation cannot be explained by a trophic effect or by compensatory growth after the inhibition of stem elongation during the low temperature. Indeed, only 2 h at 10°C at the end of a 12-h dark treatment are sufficient to obtain the same effect as after 12 h dark at 10°C. The temperature treatments at different levels suggest that it is the difference in temperature which is essential for the effect on SER, as was the case for phase resetting.

The circadian rhythm in SER of *C. rubrum* is a so-called 'overt rhythm' and a manifestation of an intrinsic biological clock (3). The biological clock determines the sensitivity of a plant to external stimuli like light and temperature signals in alternating phases of low and high sensitivity on a circadian time scale. It has been proposed that rhythmic phenomena, in particular circadian oscillations and photoreceptor interactions, might be based on the membrane systems of the eukaryotic cell (6, 20–23). Membranes could also be the receptors for the temperature effects of certain photoresponses such as photo-inhibition of SER in *C. rubrum*. This view is supported by the observation of temperature-mediated changes in membrane fluidity and organization in other systems (8, 17). It seems likely that photoreceptors, in particular phytochrome (16, 26), involve interaction with cellular membranes, although the primary mechanisms of signal transduction are still unknown. The increase in amplitude of the rhythm in SER after thermal stimulation should therefore be due to a decrease in sensitivity to light promoted by changes in interactions between photoreceptors and membrane receptor sites (1, 19, 20) during the 10 h following the low temperature treatment.

Photo- and Thermoperiodism. The effects of temperature treatments on phase and amplitude of a rhythm in SER of *C. rubrum* emphasize the equivalence of photoperiodic and thermoperiodic treatments in their influence on vegetative growth of plants (see "Introduction"). Experiments with variation of only one of the two most important factors from the environment indicate light inhibition (14) and low temperature inhibition of SER (2). Taking these results as an indication for the independent action of these two factors, one should find very little growth of stems at low temperatures at night and at high temperatures during the d, *i.e.* in conditions which are close to the situation in the natural environment. The decrease in sensitivity to light by a low temperature pretreatment together with the different temperatures of soil and air in natural conditions most likely are the reasons why such conditions are very favorable and often are the most favorable conditions for vegetative growth of plants in controlled environments.

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