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Inversion of gravitropism by symmetric blue light on the clinostat

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Abstract. Gravitropic stimulation of maize (*Zea mays* L.) seedlings resulted in a continuous curvature of the coleoptiles in a direction opposing the vector of gravity when the seedlings were rotated on a horizontal clinostat. The orientation of this response, however, was reversed when the gravitropic stimulation was preceeded by symmetric preirradiation with blue light (12.7 μmol photons$\cdot$m^{-2}). The fluence-response curve of this blue light exhibited a lower threshold at 0.5 μmol$\cdot$m^{-2}, and could be separated into two parts: fluences exceeding 5 μmol$\cdot$m^{-2} reversed the direction of the gravitropic response, whereas for a range between the threshold and 4 μmol$\cdot$m^{-2} a split population was obtained. In all cases a very strong curvature resulted either in the direction of gravity or in the opposite orientation. A minor fraction of seedlings, however, curved towards the caryopsis. Furthermore, the capacity of blue light to reverse the direction of the gravitropic response disappeared with the duration of gravitropic stimulation and it depended on the delay time between both stimulations. This *tonic* blue-light influence appears to be transient, which is in contrast to the stability observed for *tropistic* blue-light effects.

Key words: Blue light (gravitropism) – Gravitropism (blue light) – *Zea* (gravitropism)

Introduction

Graminean coleoptiles have been studied in great detail with respect to phototropic and gravitropic induction (Dennison 1979; Volkmann and Sievers 1979; Pohl and Russo 1984). Recently, Nick and Schäfer (1988a) described a complex interaction of gravi- and phototropic stimulation in the response of maize coleoptiles. But also tonic blue-light effects upon gravitropism after symmetric irradiation, either bilaterally or from above, have been reported. Blaauw (1961) observed that a pretreatment with symmetric blue light slowed down the rate of the upward movement induced by gravitropic stimulation. Moreover, the initial, downward movement of maize coleoptiles, usually found in the early phase of the gravitropic response (Hild and Hertel 1972), is abolished by preirradiation with blue light (Hild 1977). These results have been interpreted in terms of a blue-light-elicited decrease of gravitropic sensitivity.

Phototropic stimulation induces stable curvature and a stable directional memory, if the seedlings are rotated on a horizontal clinostat (Nick and Schäfer 1988b). The memory can be detected as increasing stability towards counterstimulation of equal strength. In contrast, gravitropic stimulation can induce only a transient response on the clinostat (Nick and Schäfer 1988a). Surprisingly, it is nevertheless able to elicit the formation of a stable *memory,* i.e. stable curvature, whose direction cannot be reversed by opposing blue-light stimulation of equivalent strength (Nick and Schäfer 1988b). In order to explain this discrepancy it is necessary to test whether this switch from transient to stable gravitropic curvature on the clinostat might be due to the tonic, stabilising effects of the blue-light counterstimulation. Therefore, in this investigation, the influence of symmetric blue-light upon the gravitropic response on the clinostat will be analysed in more detail.

Material and methods

Plant material and light sources. Maize (*Zea mays* L., cv. BRIO 42.HT; Asgrow, Bruchsal, FRG, harvest 1987; stored at 3° C in the dark) seeds were grown in a phytochamber and prepared for experimental use as described by Nick and Schäfer (1988a). A description of the red-light source used in the growth chamber and as symmetric background illumination during curvature development on the clinostat is given in Mohr et al. (1964). Symmetric blue light was obtained by means of a mirror. For details on light sources, light measurement and filters see Nick and Schäfer (1989).

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Stimulation treatments. For stimulation by gravity the seedling tray was tilted such that the gravity vector was parallel with the longer coleoptile cross-section. This stimulation treatment lasted for 30 min, if not stated otherwise. Thereafter the seedlings were positioned on a horizontal clinostat and rotated at 0.5 rpm under symmetric red light (2.3 $W \cdot m^{-2}$) until excision (24 h after the onset of stimulation). Alternatively, seedlings were irradiated with varying fluences of blue light from above at different time intervals with respect to the onset of gravitropic stimulation and then brought onto the clinostat. The irradiation time never exceeded 90 s so as to avoid distortions caused by sensory adaptation (Iino 1987).

Curvature measurements and statistics. Time courses of curvature development and final curvatures 24 h after the onset of stimulations were measured as given elsewhere (Nick and Schäfer 1988a, b). As the direction of the response deviated from the direction of gravitropic stimulation it was necessary to regard not only the curvature component parallel to the stimulation vector but also the azimuth of curvature. For this the azimuthal system described in Nick and Schäfer (1989) was used. In this system, curving away from the caryopsis was indicated by an azimuth of 0°, whereas 180° characterised bending towards the caryopsis. Azimuthal angles of 90° and 270° stood for curvatures parallel to the longer coleoptile cross-section, i.e. within the plane of gravitropic stimulation. Frequency distributions over azimuthal classes of 30° width were measured for all major parts of the fluence-response curve of Fig. 3 and the gravity dose-response curve of Fig. 5, respectively. For the frequency-distribution plot of Fig. 2 the width of the classes was 15°. Note that in these curves error bars indicate standard deviations. The time courses of curvature development (Fig. 1) were obtained as means from 16 seedlings. The frequency-distribution plots (Figs. 2, 4, 6) cumulated the data from 50 to 100 individuals, whereas in the fluence-response curve of Fig. 3, the dose-response curve of Fig. 5 and Table 1 each value represents the data from 20 to 30 seedlings.

Results

Time course of gravitropic curvature after preirradiation with blue light from above. When maize seedlings on the clinostat were stimulated with gravity for 30 min without blue irradiation a more or less continuous gravitropically negative movement was observed (Fig. 1). It should be mentioned, however, that bending was not continuous, but appeared to be biphasic. After an initial increase of curvature the angle (about 20°) remained constant from 120 to 240 min after the onset of clinostat rotation, followed by a second increase of curvature saturated at about 70° in the plane of the gravity stimulus 24 h after the onset of clinostat rotation. Nick and Schäfer (1989) reported a nastic response of maize coleoptiles towards the caryopsis during rotation on the horizontal clinostat. In order to check the influence of this response, not only the curvature component parallel to the gravity vector (Fig. 1), but also the azimuth of final curvature was analysed. The corresponding frequency distribution of azimuth (Fig. 2) exhibits a clear peak at an azimuth 270° indicating the usual gravitropic response in the direction opposite to the gravity vector. However, a significant proportion of seedlings was oriented towards the caryopsis, as is shown by a second, smaller peak in the azimuth region around 180°, possibly due to the influence of the nastic component.

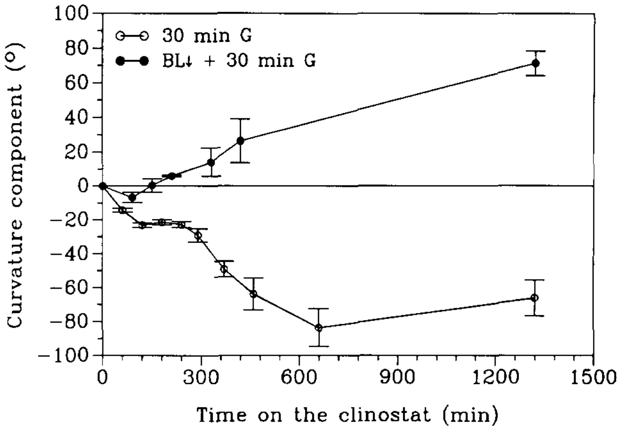

Fig. 1. Time course of gravitropic curvature of *Zea mays* coleoptiles on the horizontal clinostat with and without preirradiation (blue light, *BL*, from above) immediately before the onset of gravistimulation. *Open circles:* control (30 min gravistimulation without blue-light pretreatment). *Closed circles:* 12.7 μmol photons$\cdot m^{-2}$ of blue light followed by 30 min gravistimulation. Positive values indicate gravitropically positive curvature, i.e. bending in the direction of the gravity vector. The time axis starts with the end of gravity stimulation, which at the same time is the onset of clinostat rotation

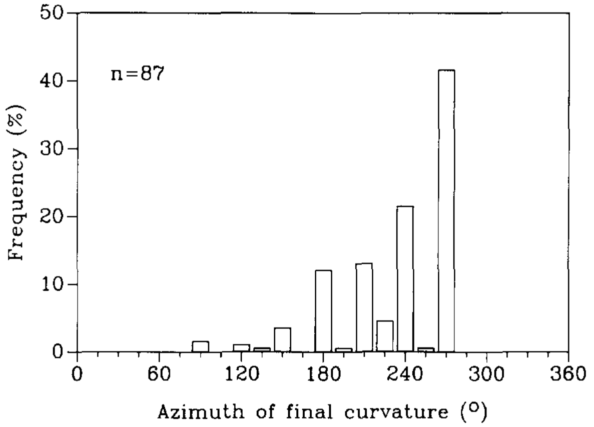

Fig. 2. Frequency distribution over the azimuth of final curvature of *Zea mays* coleoptiles after 30 min gravistimulation and subsequent rotation on the clinostat for 24 h. Gravitropically negative curvature (opposing the direction of the gravity vector) is indicated by an azimuth of 270°, whereas curvatures towards the caryopsis (perpendicular to the stimulation plane) are characterized by an azimuth value of 180°. An azimuth of 90° represents bending in direction of the gravity vector

If, immediately before the onset of gravistimulation, a blue-light irradiation from above (12.7 μmol photons$\cdot m^{-2}$) was administered, this behaviour changed dramatically (Fig. 1). On the clinostat, now, curving became gravitropically positive throughout the experimental period, after a slight initial bending in the opposite direction. Here, again, the response reached 70° in the plane of the gravity stimulus within 24 h. The vast majority of coleoptiles were oriented in the direction of the gravitational force (data not shown, but the azimuthal distribution corresponds to that in Fig. 4c).

Fluence dependence of the tonic blue-light effect upon gravitropic curvature. To test the fluence dependence of this

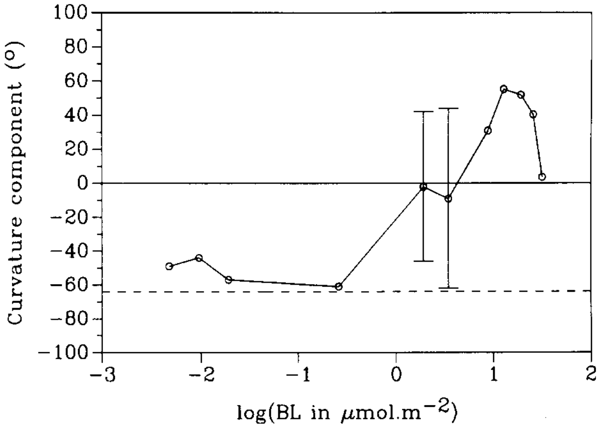

Fig. 3. Fluence-response curve of the tonic reversion of gravitropic bending of maize coleoptiles by blue-light (*BL*) preirradiation from above immediately before the onset of a gravistimulation of 30 min duration (curvature development 24 h on the clinostat). Negative values indicate components of final curvature opposing the stimulation vector, positive values the reversion of the gravitropic response by the light treatment. *Dashed line:* final gravitropic curvature without blue irradiation. Note the extraordinarily large standard deviations in the transition from the negative to the positive branch of the curve (standard deviations in the other parts of the curve amounted to about ±30°)

blue-light effect, varying fluences of blue light from above were given immediately before the onset of gravistimulation and the response was allowed to develop on the clinostat. Then, the component of final curvature oriented within the plane of gravistimulation was plotted versus the fluence of blue light (Fig. 3). For fluences up to 0.5 μmol·m^{-2} no significant effect of blue-light irradiation was observed, indicated by a curvature component of 50–70° in a direction opposite to that of gravitational force (Fig. 3, compare the dashed line). When fluences increased, the curvature component approached zero, and, for fluences exceeding 4 μmol·m^{-2}, became positive, which indicates gravitropically positive bending. This positive branch of the curve peaked at about 10 μmol·m^{-2}, but fell back to zero, when fluences were further raised. It should be noted that the standard deviations in the transition from the negative towards the positive branch of the curve (between 1 and 4 μmol·m^{-2}) were extraordinarily large (Fig. 3, bars). In order to analyse this in more detail, frequency distributions of azimuthal orientation were plotted for the three major ranges of the fluence-response curve (Fig. 4a–c). For fluences up to 5 μmol·m^{-2} the majority of seedlings were oriented in the direction opposite to the gravity vector, and only minor portions in the direction of gravity or towards the caryopsis (Fig. 4a). In contrast, at fluences between 8 and 25 μmol·m^{-2} (Fig. 4c) almost a mirror image of the previous distribution is obtained. Here, less than 10% of the seedlings curved in a direction opposite to the gravity vector. In the transition range between 1 and 4 μmol·m^{-2} (Fig. 4b) a split population was observed: some seedlings curved into the direction of gravitational force, some opposite to this direction, and a conspicuous portion in the latter orientation showed a deviation towards the caryopsis. This causes the large standard deviations found in Fig. 3.

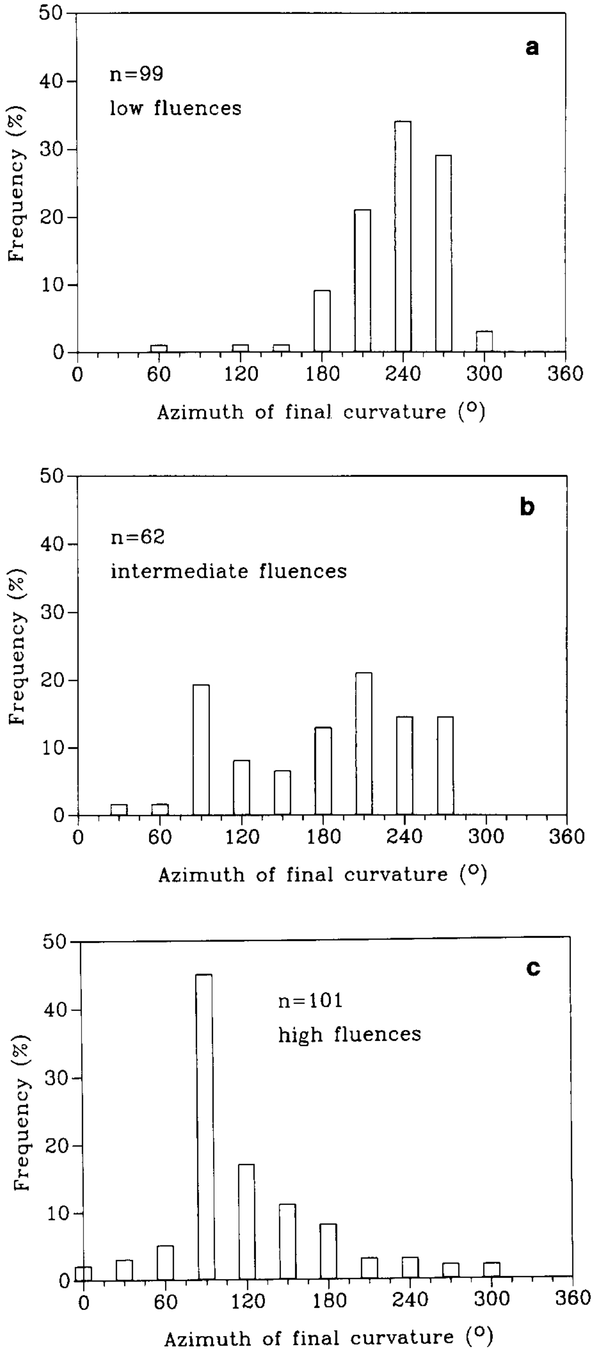

Fig. 4a–c. Frequency distribution over the azimuth for the different fluence ranges of Fig. 3. For the azimuth system compare Fig. 2. **a** Less than 0.5 μmol·m^{-2} blue light; **b** 1–4 μmol·m^{-2} blue light; **c** 8–25 μmol·m^{-2} blue light

The dependence of the tonic blue-light effect on the duration of gravitropic stimulation. Based upon the observation that a symmetric blue-light pretreatment can reverse the direction of the gravitropic curvature, it was tested whether or not this effect is dependent on the duration of gravitropic stimulation. For this, seedlings were irra-

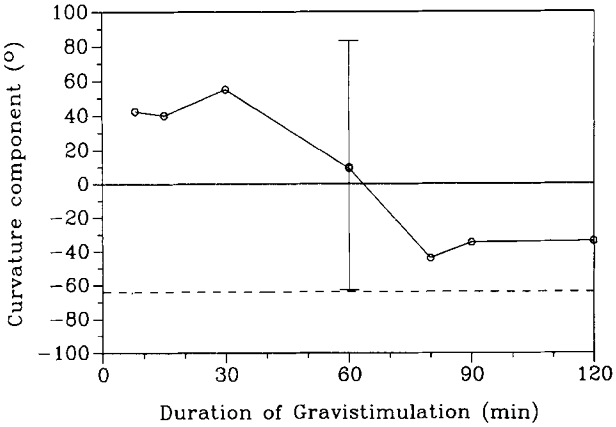

Fig. 5. Gravistimulation dose-response curve of gravitropic reversion by preirradiation of maize coleoptiles with 12.7 μmol·m^{-2} blue light from above immediately before the onset of gravistimulation (of the duration given on the abscissa). Positive values indicate reversion of the gravitropic response. For comparison, the value for 30 min gravistimulation without blue preirradiation is shown (*dashed line*). Note the large standard deviation in the transition from the positive to the negative branch of the curve (standard deviations in the other parts of the curve were around ±35°)

diated from above with blue light (12.7 μmol·m^{-2}) and, immediately afterwards, stimulated gravitropically for variable time intervals (8–120 min). After gravistimulation, the vials with the seedlings were mounted on a clinostat and rotated 24 h in red light (2.3 W·m^{-2}). The curvature component within the stimulation plane is given in Fig. 5. For gravitropic stimulations up to 30 min, values are positive, i.e. blue light reversed the direction of the gravitropic response. The frequency distribution over the azimuth (Fig. 6a) reveals a major peak in the direction of the gravity vector and a minor peak towards the caryopsis. For stimulation periods exceeding 80 min no reversion of the gravitropic response was observed. However, the curvature is slightly reduced as compared to gravitropic stimulation (lasting 30 min) without blue-light pretreatment (Fig. 5, dashed line). The correspondent frequency distribution (Fig. 6c) shows that still about 20% of the seedlings population show a reversion of the gravitropic response. Most seedlings, however, curve in a direction opposite to the gravity vector. A gravitropic stimulation lasting 60 min resulted in a split population (Fig. 6b) oriented either in the direction of the gravitational force or opposite to it. Averaging over both populations (Fig. 5) leads to a zero value with an extremely large standard deviation (Fig. 5, error bars).

Is the effect of the blue-light preirradiation stable? Blue-light irradiation (12.7 μmol·m^{-2}) immediately administered before or after a gravitropic stimulation of 30 min duration was able to reverse the direction of the gravitropic response on the clinostat (Table 1). An irradiation 30 min prior to gravitropic stimulation showed almost no effect compared with the controls, whereas a delay time of 15 min resulted in an intermediary behaviour.

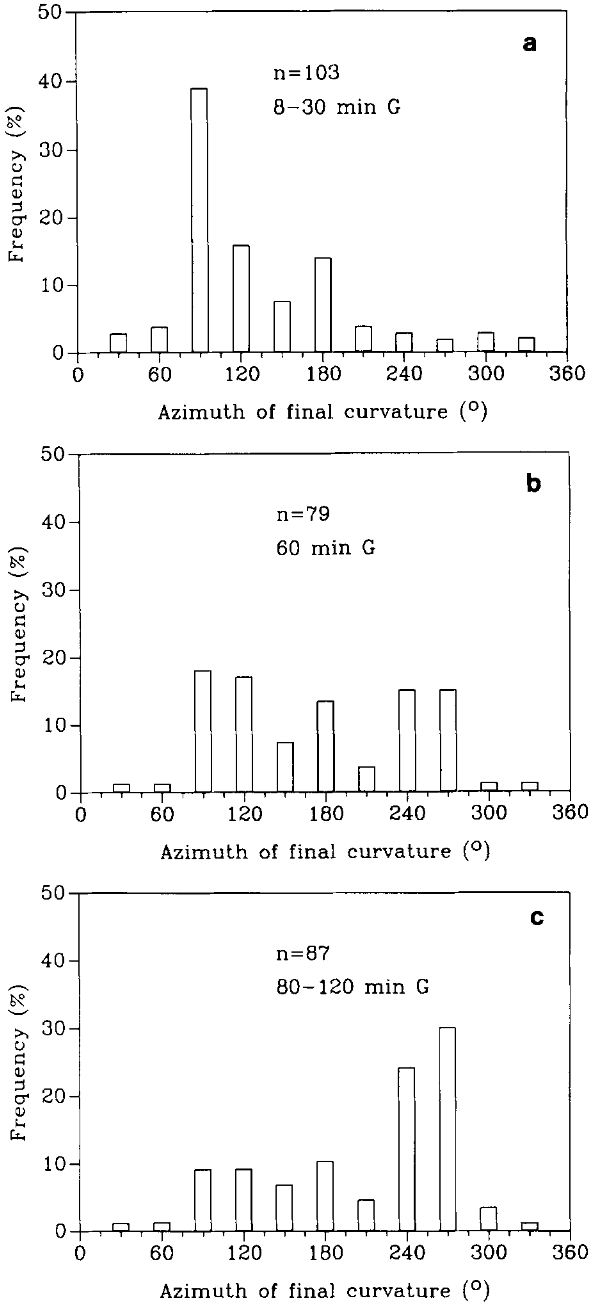

Fig. 6a–c. Frequency distribution over the azimuth for the different parts of the curve shown in Fig. 5. For the azimuth system compare Fig. 2. Duration of gravistimulation: **a** 8–30 min, **b** 60 min, **c**, 80–120 min

Table 1. Dependence of the tonic blue-light effect (for 12.7 μmol·m^{-2} blue light from above) upon the time interval elapsed between irradiation and the onset of gravistimulation (lasting 30 min). −30 min means that the light was administered 30 min after the onset of clinostat rotation

Time interval	Curvature	Number of seedlings
+30 min	+55.6°	6
+15 min	−11.4°	7
0 min	−55.0°	59
−30 min	−61.2°	8
No blue light	+66.2°	15

Discussion

Following a 30-min gravitropic stimulation, maize coleoptiles exhibited a continuously increasing curvature in the direction opposing the gravity vector (Fig. 1), if they were rotated on a horizontal clinostat. This is in contrast to previous observations, where gravitropic curvature on a clinostat was reported to be transient (Nick and Schäfer 1988b). Using another seed batch (Harvest 1988), again only a transient curvature in a direction opposite to the gravity vector was observed (data not shown). A large proportion of those seedlings eventually bent towards the caryopsis, which can be interpreted to indicate that the clinostat-elicited nastic response found in maize coleoptiles has a dominating influence (Nick and Schäfer 1989). This indicates that the orientation of gravitropic curvature is not very stable and can be easily influenced by internal and external factors.

Therefore the influence of bilateral blue-light irradiation on the orientation of gravitropically induced curvature has been analysed. It was reported previously that symmetric blue light has an influence upon the gravitropic response of graminean coleoptiles (Blaauw 1961; Hild 1977). Those authors either investigated the effects on the rate of curvature or the initial phase of the response, when curving occurs in the direction of gravitational force. In contrast to those experiments, we analysed the long-term behaviour on the clinostat. Here, a reversion of the direction of the gravitropic response was observed (Fig. 1) when blue-light fluences were strong enough (Figs. 3, 4a–c), and when the gravitropic stimulation was not too long (Figs. 5, 6a–c). The corresponding curves mirror the behaviour of the mean, and on the population level the interaction between symmetric blue light and gravity appears to be graded. If individual seedlings are analysed instead of populations, however, one finds an all-or-none behaviour: the coleoptiles either curve according to the gravitropic stimulation or opposite to this direction (Fig. 4b, 6b). A minor portion exhibits nastic bending towards the caryopsis (Nick and Schäfer 1989). Graded behaviour of populations in combination with all-or-none behaviour of individual seedlings was found during analyses of counteracting phototropic stimulations (Nick and Schäfer 1988b) and interaction of nastic bending with phototropic counterinduction (Nick and Schäfer 1989). This all-or-none pattern of individual seedlings on the clinostat appears to be a very general phenomenon.

When delay times between 0 and 30 min were interposed between blue-light irradiation and gravitropic stimulation it was found that this tonic blue-light effect appears to be only transient (Table 1). In contrast, tropistic blue-light effects manifest as stable phototropic curving on the clinostat for many hours (Nick and Schäfer 1988a), seem to be of much longer duration. The same holds true for the directional memory induced by phototropic stimulation (Nick and Schäfer 1988b). It should be mentioned that the tonic blue-light influence upon the initial phase of the gravitropic response of coleoptiles (Hild and Hertel 1972) is also transient and exhibits a similar lifespan (Hild 1977). Additionally, the fluence-response relation of both phenomena is more or less identical. This indicates that both effects, inhibition of the initial positive phase of the gravitropic response (Hild 1977), and reversion of the direction of this response on the clinostat, are due to the same signal chain mediating the tonic blue-light effect. In this context it is worth mentioning that the tonic inhibition of clinostat-elicited nastic curving shows a very similar fluence-response curve (Nick and Schäfer 1989). Thus, besides the induction of phototropism by blue-light gradients (Kunzelmann and Schäfer 1988) one finds tonic effects upon differential growth. It is not unlikely that this effect uses a partially deviating signal transduction chain branching off phototropic transduction before the formation of phototropic transverse polarity (Nick and Schäfer 1989). The mechanisms mediating the effect of tonic blue light upon differential growth are not understood yet. A speculative hypothesis implies persistent differences in cell-wall elasticity of epidermal cells produced by stimulus-dependent reorientation of microtubules and subsequent reorientation of newly deposited cellulose microfibrils (data not shown).

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