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# Nastic response of maize (*Zea mays* L.) coleoptiles during clinostat rotation

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**Abstract.** Rotation of unstimulated maize (*Zea mays* L.) seedlings on a horizontal clinostat is accompanied by a strong bending response of the coleoptiles towards the caryopsis, yielding curvatures exceding 100°. The corresponding azimuthal distribution shows two peaks, each of which is displayed by 30° from the symmetry axis connecting the shortest coleoptile and caryopsis cross sections. It is argued that this spatial pattern is not the result of two independent bending preferences, but caused by a one-peaked distribution encountering an obstacle in its central part and thus being split into the two subpeaks. The existence of one preferential direction justifies considering this response to be a nastic movement. Its time course consists of an early negative phase (coleoptiles bend away from the caryopsis) followed 2 h later by a long-lasting positive bending towards the caryopsis. In light-interaction experiments, fluence-response curves for different angles between blue light and the direction of the nastic response were measured. These experiments indicate that blue light interacts with the nastic response at two levels: (i) phototonic inhibition, and (ii) addition of nastic and phototropic curvatures. It is concluded that phototropic and phototonic transduction bifurcate before the formation of phototropic transverse polarity. The additivity of nastic and phototropic responses was followed at the population level. At the level of the individual seedling, one observes, in the case of phototropic induction opposing nastic movement, three distinct responses: either strong phototropism, or nastic bending, or an "avoidance" response which involves strong curvature perpendicular to the stimulation plane. With time the nastic bending becomes increasingly stable against opposing phototropic stimulation. This can be seen from a growing proportion of seedlings exhibiting nastic bending when light is applied at variable intervals after the onset of clinostat rotation. At the transition from instability to stability, this type of experiment produces a high percentage of seedlings displaying the "avoidance" response. However, no cancelling resulting in zero curvature can be observed. It is concluded that the endogenous polarity underlying the nastic response is different in its very nature from the blue-light-elicited stable transverse polarity described earlier (Nick and Schäfer 1988b).

**Key words:** Blue light and nastic response – Clinostat (coleoptiles) – Coleoptile (nastic response) – Nastic response (coleoptile) – Polarity (transversal, coleoptile) – *Zea* (nastic response)

## Introduction

Quantitative investigations of photo- and gravitropism often implied usage of horizontal clinostats in order to evade distortions due to gravitropic counterstimulation, which increase linearily with the gravity component perpendicular to the longitudinal axis of the coleoptile (Pickard 1972; Nick and Schäfer 1988a). The basic idea is to eliminate gravitropic perception and, possibly, transduction, such that the observed response can be interpreted exclusively as a consequence of stimulations applied before the onset of clinostat rotation. Whether the desired effect of clinostat rotation, sensory and – or transductory graviadaptation, is the only outcome, however, remains unclear in most cases.

In fact, considerable evidence casts doubt upon this supposition. Even a century ago it was noted that petioles of dicotyledonous plants performed strong movements without any directional stimulation when they were attached to a horizontal clinostat (Krabbe 1889). But also apparently radial organs such as roots (Talts 1932; Larsen 1962; Hestnes and Iversen 1978) and shoots (Hoshizaki

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*Abbreviation:* BL = blue light (449 nm) and Hamner 1962; Clifford 1979) curved to a remarkable degree if rotated on a horizontal clinostat for several hours, although no tropistically effective stimulus was administered. In those cases where it has been tested, this response was enhanced with increasing rotational speed (Talts 1932), i.e. decreasing overall gravitonic stimulation (the latter is supposed to grow linearily with the period of rotation (Larsen 1962)). Except for light-grown stems of *Xanthium* plants (Hoshizaki and Hamner 1962), no case of clinostat-elicited curvature reported so far allows the data to be explained in terms of centrifugal-force-governed gravitropism, since the direction of the response is not defined by the vector of centrifugal force. These clinostat responses rather appear to be favoured by the absence of any directional cues. It is consistent with this view that azimuthal analyses of clinostat-elicited curvature do not reveal any obvious bending preferences (Talts 1932; Larsen 1962; Hestnes and Iversen 1978; Clifford 1979). The behaviour of dorsoventral organs such as leaves, however, is clearly different, showing an unequivocal preferential direction (Doposcheg-Uhlár 1913; Rawitscher 1932).

Whereas the early studies of photo- and gravitropism (in those days still referred to as helio- and geotropism) considered coleoptiles as physiologically radial organs (Rothert 1894; Blaauw 1909; Pekelharing 1909), it was argued soon afterwards that they, being homologous to leaves, possibly exhibit dorsoventral behaviour. In fact, experiments on coleoptiles of oat (*Avena sativa* L.) clearly demonstrated a so-called dorsoventral or autonomous movement when unstimulated coleoptiles were rotated on a horizontal clinostat (Bremekamp 1925; Lange 1925; Beyer 1927), resulting in a conspicuous curvature away from the caryopsis. It was further shown that this autonomous movement interacted with phototropism (Lange 1925).

Unfortunately, however, these observations remained on a qualitative level and were therefore thoroughly neglected in the following years. Pfeffer (1904) defined nastic bending as movement whose direction is determined by dorsoventrality and not by the direction of the incoming stimulation. Thus, it seems justified to interpret the autonomous bending of oat coleoptiles as epinasty elicited by the removal of gravitropic counterstimulation.

In investigations of the long-term effects of phototropically effective stimulation, a directional memory according to the inducing light gradient has been found in maize coleoptiles (Nick and Schäfer 1988b). This memory corresponds to a stable transverse polarity, presumably produced de-novo as a consequence of blue-light irradiation. This is consistent with the old idea of Sachs (1880) that polarization of organs in response to environmental cues occurs de-novo and not simply by reorientation of preexisting inherent polarities as held by Vöchting (1878). Nevertheless, the existence of such preexisting polarities can be expected from the dorsoventrality manifest in the autonomous bending of oat coleoptiles. At this point, two presumptions can be made and should be tested:

i) If dorsoventral polarity is present in maize coleoptiles, nastic bending should be observed when unstimulated coleoptiles are rotated on a horizontal clinostat.

ii) If it is true that blue-light-elicited stable transverse polarity is produced de-novo and not by reorientation of an internal polarity, one would expect there to be fundamental differences from the supposed dorsoventral polarity underlying a possible nastic response.

## Material and methods

*Plant material and light sources.* Maize seeds (*Zea mays* L. cv. BRIO 42.HT, stored in the dark at 3° C) were grown and prepared for experimental use according to the method described earlier (Nick and Schäfer 1988b). The red light used for raising the seedlings ($1.5 \cdot 10^{-2}$ $W \cdot m^{-2}$) and as symmetrical background light during the experiments (2.2 $W \cdot m^{-2}$) was obtained following the method of Mohr et al. (1964). Light for phototropic induction was delivered by a Prado Universal Projector (Leitz, Wetzlar, FRG) with blue light isolated by a DEPIL (Schott, Mainz, FRG) interference filter (maximal transmission at 449 nm, halfband width 10 nm, maximal transmission 10%). For irradiances exceding $2.5 \cdot 10^{-2}$ $W \cdot m^{-2}$ a Zeiss-Ikon Xenosol III projector with an Osram XBO 2500 W Xenon arc (Osram, München, FRG) was used instead. Irradiation with blue light from above involved using a mirror. Irradiation time was kept at 30 s by means of an automatic shutter system. Fluence rates were regulated using neutral-density filters (Schott). Irradiation time was kept constant in order to avoid distortions due to sensory adaptation (Iino 1988). Light measurements were performed with a digital photometer (J16/Option 2; Tectronix, Beaverton, Ore., USA).

*Stimulation treatments.* Unless stated otherwise, seedlings were rotated on a horizontal clinostat at 0.5 rpm without any photo- or gravitropic stimulations. In order to minimize unsystematic gravistimulation during the installation of the seedlings on the clinostat, vials were mounted on the already moving clinostat. Certainly no significant gravitropic stimulation can be ascribed to this procedure. The angle of the shortest transverse axis of the coleoptile to the direction of centrifugal force exerted by the rotation was carefully recorded and was 0°, 90°, 180°, 270°, respectively. For the blue-light interaction experiments, seedlings were irradiated for 30 s, in an upright position, with variable fluence rates either from above or unilaterally at different angles to the shortest axis of the coleoptile; they were put onto the clinostat immediately.

*Curvature measurements and data processing.* At the end of the experimental period (24 h after the onset of clinostat rotation) shoots were excised and final curvatures measured as defined in Nick and Schäfer (1988a). For time-course measurements, seedlings were removed from the clinostat and photographed standing upright for roughly 30 s, a treatment shown not to affect curvature development on the clinostat (Pickard 1972). The negatives were then enlarged and the angles of bending marked and measured as described in Nick and Schäfer (1988a). For the spatial characterization of curvature an azimuthal reference system was introduced, in which the azimuth was given by the angle between the vector connecting the shortest transverse axes of caryopsis and coleoptile (pointing from the caryopsis towards the coleoptile) and the direction of bending read in the clockwise direction. In this system an azimuth of 0° means bending away from the caryopsis, 180° bending towards the caryopsis, and 90° (or 270°, respectively) within the plane of the longest coleoptile transverse axis. In cases where the azimuth deviated from these four main directions, curvature was split into components so as to facilitate the analysis.

For the azimuth-distribution plot (Fig. 1) the data of 1200 seedlings were used and divided into azimuth classes of 15° width for the calculation of frequency. For the plots of time course and fluence-response curves, values from eight seedlings were averaged for each datum point, with bars indicating standard deviations. The stabilization time course (Fig. 7) and the angular-distribution plot of Fig. 6 involved 150–180 seedlings for each treatment.

## Results

*Occurrence of clinostat-elicited curvature and its preferential direction.* When coleoptiles were rotated on a horizontal clinostat without tropistic stimulation, a strong curvature, eventually of 120°, was nevertheless observed. Its azimuthal distribution 24 h after the onset of clinostat rotation (Fig. 1, black bars; for the calculated curves additionally shown see *Discussion*) revealed a markedly bimodal curve with a main peak at an azimuth of 210° and a somewhat minor peak at 150°, both separated by a conspicuous trough. Thus, the response is more or less directed towards the caryopsis with the peaks a little displaced from the symmetry axis connecting the shortest coleoptile and caryopsis cross sections. No significant differences due to positioning on the clinostat could be observed (data not shown), with all clinostat positions yielding the same two-peaked azimuthal pattern.

*Kinetics of clinostat-elicited curvature.* When the time course of clinostat-elicited curvature (Fig. 2) is measured it turns out that the strong final curvature towards the caryopsis is preceded by a smaller, but nevertheless distinct bending in the opposite direction, reaching a maximum of 20° about 2 h after the onset of clinostat rotation. Then, curving towards the caryopsis sets in, first straightening the coleoptiles (zero curvature is obtained 3.5 h after the onset of clinostat rotation) and, eventual-

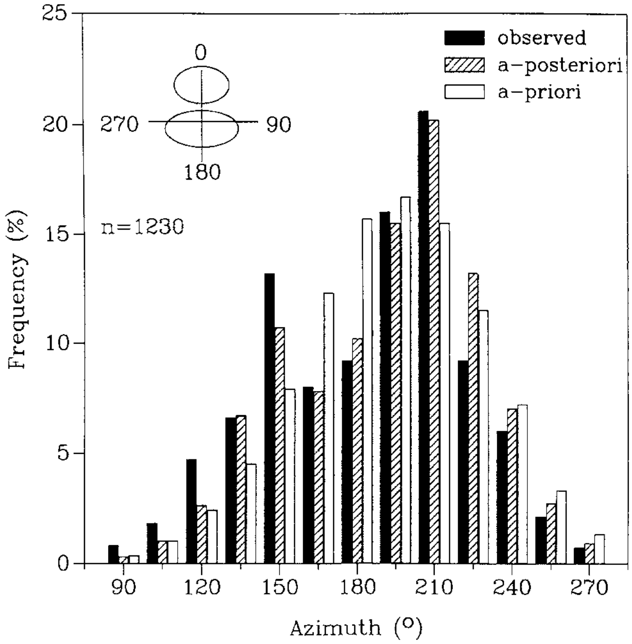

**Fig. 1.** Azimuthal distribution of final curvatures of maize coleoptiles 24 h after the onset of clinostat rotation. Azimuths were measured taking the symmetry axis connecting the shortest caryopsis and coleoptile cross sections (pointing from the caryopsis towards the coleoptile) as reference, reading the angle in a clockwise direction. Thus 90° and 270° indicate curvature within the longest transverse axis of the coleoptile, 180° bending towards the caryopsis. *Inset:* The larger ellipse stands for the caryopsis, the smaller for the coleoptile. *Black bars*, measured distribution; *striped bars*, calculated best-fit distribution on the assumption that the bimodal distribution is produced a-posteriori; *white bars*, calculated best-fit distribution on the assumption that the bimodal distribution is given a-priori. Data from the four positions on the clinostat taken together (no significant differences between clinostat positions were found).

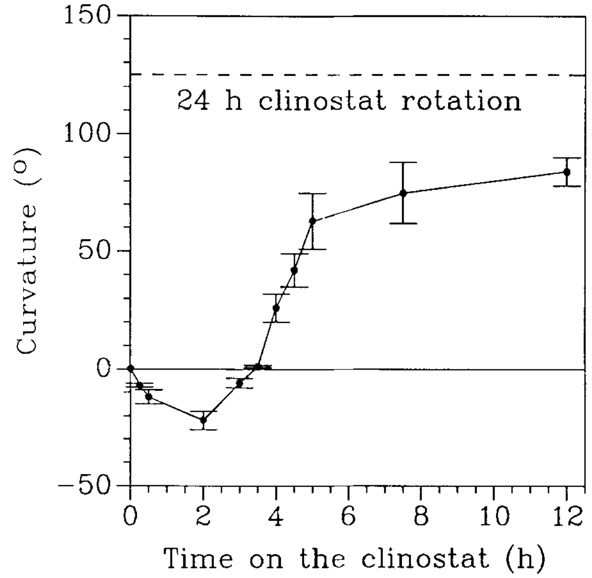

**Fig. 2.** Time course of clinostat-elicited curvature of maize coleoptiles. Negative values indicate bending away from the caryopsis, positive values bending towards the caryopsis. *Dashed line*, curvature 24 h after the onset of clinostat rotation

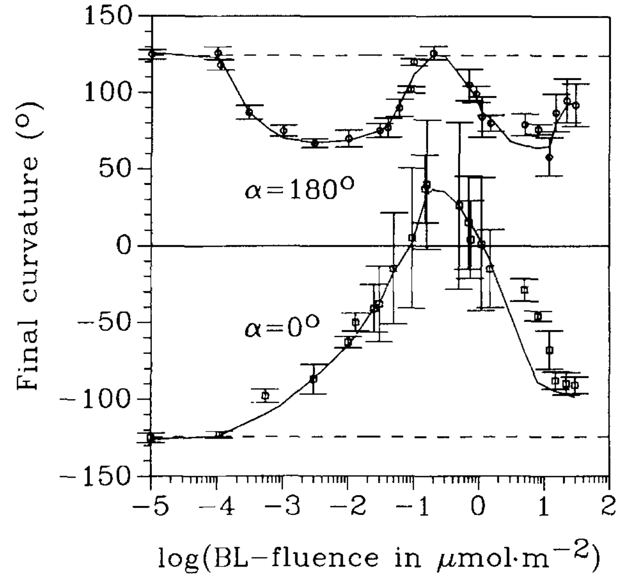

**Fig. 3.** Fluence-response curves of first positive phototropism for final curvature of maize coleoptiles 24 h after the onset of clinostat rotation. *Lower curve*, light irradiated in the azimuthal direction 0° (phototropic induction opposing clinostat-elicited curvature). *Upper curve*, light irradiated in the azimuthal direction 180°. Bars indicate standard deviations; positive values indicate bending towards the light. *Dashed line*, clinostat-elicited curvature without irradiation

ly, resulting in the strong final curvature towards the caryopsis. The major part of this response is completed within 7 h after the onset of clinostat rotation, although bending slowly continues for many hours.

*Interaction of clinostat-elicited curvature with blue-light irradiation from different directions.* In order to assess possible influences of clinostat-elicited curvature upon first positive phototropism, fluence-response curves for average final curvatures 24 h after the onset of clinostat rotation were measured following the administration of blue light from azimuthal directions of 0°, 90°, 180°, 270°, and from above.

For an anzimuth of 0° (phototropic induction opposing clinostat-elicited curvature) a bell-shaped curve (Fig. 3, bottom) was obtained, with mean curvatures of −100° in relation to the direction of the phototropic stimulus for fluences lower than a threshold of $10^{-3}$ μmol·m$^{-2}$. For increasing fluences the curve becomes less and less negative, crossing the zero line at 0.1 μmol·m$^{-2}$. It reaches a maximum of 30° at 0.25 μmol·m$^{-2}$ and then drops back to −100° at 20 μmol·m$^{-2}$, this time intersecting the zero line at 1 μmol·m$^{-2}$. It should be mentioned here that standard deviations in the range of the maximum are extraordinarily large, a point which will be enlarged upon below.

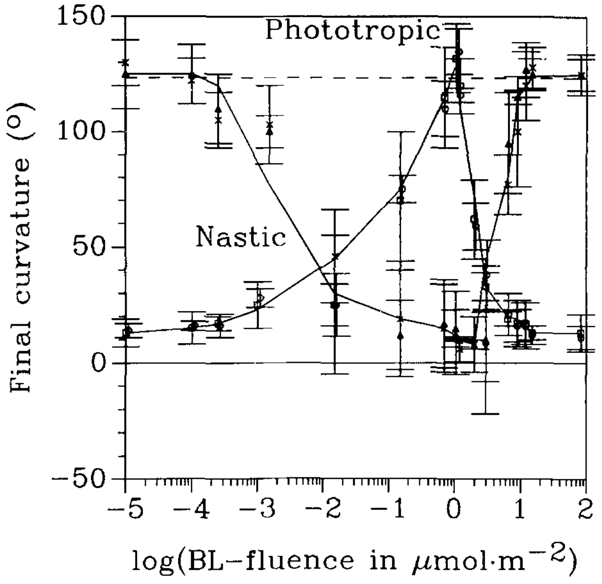

**Fig. 4.** Fluence-response curves for maize coleoptiles under the same conditions as Fig. 3 but for light irradiated in azimuthal directions of 90° and 270° (phototropic induction perpendicular to clinostat-elicited curvature). The *phototropic* curve shows the curvature component directed towards the light, the *nastic* curve the component directed towards the caryopsis. *Squares, triangles*, data for an azimuth of 90°; *circles, crosses*, data for an azimuth of 270°. Curves were drawn without differentiating between the two azimuths. *Dashed line*, clinostat-elicited curvature without irradiation

The curve for an azimuth of 180° (phototropic induction supporting clinostat-elicited curvature) is more complex (Fig. 3, top), having a central peak of 130° at the same fluence as the peak of the curve for an azimuth of 0°, i.e. 0.25 μmol·m$^{-2}$, flanked by two troughs with minima of 70° at $2.5 \cdot 10^{-3}$ μmol·m$^{-2}$, and 50° at 7 μmol·m$^{-2}$. For fluences between $10^{-5}$ μmol·m$^{-2}$ and the first minimum at $2.5 \cdot 10^{-3}$ μmol·m$^{-2}$, curvature falls from 125° (the level of the dark control) to 70°. For fluences exceeding the position of the second minimum at 7 μmol·m$^{-2}$ it grows steeply from 50° to 95° at 25 μmol·m$^{-2}$.

For azimuths of 90° and 270° (phototropic induction acting perpendicular to the direction of clinostat-elicited curving) many seedlings curved neither in the direction of the light nor followed the usual direction of the clinostat-elicited response. This necessitated a component analysis, splitting the observed curvature into a phototropic (azimuthal direction 90° or 270°, respectively, i.e. oriented within the stimulation plane) and a nastic component (azimuthal direction 180°, i.e. curvature directed towards the caryopsis). As the curves for an azimuth of 90° are almost identical to those for 270° it is feasible to treat them together. The fluence-response plots for the phototropic compo-

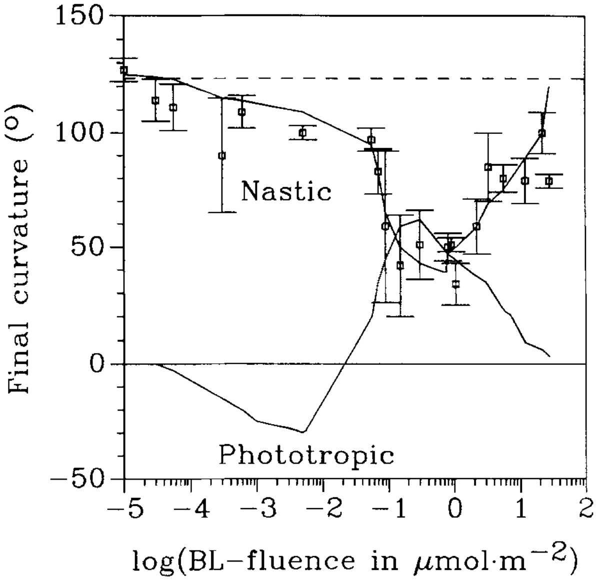

**Fig. 5.** Fluence-response curve of clinostat-elicited bending of maize coleoptiles measured 24 h after the onset of clinostat rotation with blue light irradiated from above immediately before the onset of clinostat movement. *Squares*, measured data; *upper solid curve*, curve for nastic bending calculated from the data shown in Fig. 3 (differences of both curves under the assumption of additivity of phototropic and clinostat-elicited curvatures); *lower solid curve*, curve for phototropic bending calculated from the data shown in Fig. 3; *dashed line*, nastic curvature without irradiation with blue light

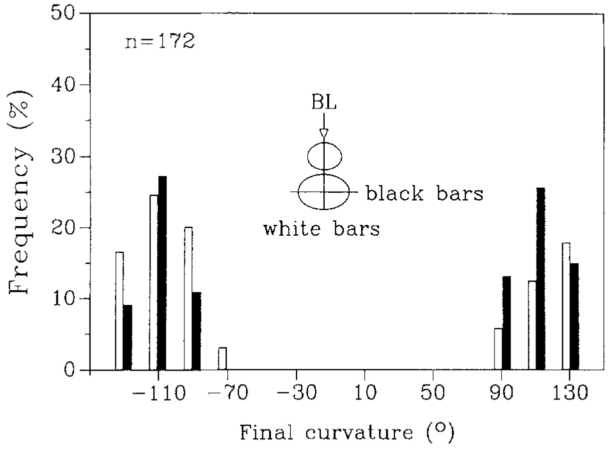

**Fig. 6.** Frequency plot of curvature of maize coleoptiles 24 h after the onset of clinostat rotation for phototropic induction with 0.3 µmol·m$^{-2}$ blue light in an azimuthal direction of 0° (opposing clinostat-elicited bending). *White bars*, curvature within the stimulation plane. Negative angles indicate curvature towards the caryopsis, positive angles bending towards the light. *Black bars*, curvature perpendicular to the stimulation plane (negative angles mean curvature in the azimuthal direction 270°, positive values bending in the azimuthal direction 90°). *Inset*, direction of blue light and direction of responses (parallel or perpendicular to the stimulation plane). The larger ellipse symbolizes the caryopsis, the smaller the coleoptile

nent (Fig. 4) resemble the well-known bell-shaped curve of first positive phototropism with a lower threshold at $10^{-4}$ µmol·m$^{-2}$ (angle 10°), maximum curvature of 135° at 1.5 µmol·m$^{-2}$ and a sharp drop to 10° at 8 µmol·m$^{-2}$. In contrast, the curves for the "nastic" components fall slowly from 105° at $10^{-4}$ µmol·m$^{-2}$ to 10° at 0.1 µmol·m$^{-2}$, remaining more or less constant for increasing fluences. As soon as a threshold of 3 µmol·m$^{-2}$ is crossed, however, they step up steeply to 110° at 10 µmol·m$^{-2}$. Thereafter, curvature does not change if the fluence is raised even further.

In order to follow the undirectional effects of blue light upon clinostat-elicited curvature, a fluence-response curve of final curvature for blue light irradiated from above was obtained with seedlings rotated for 24 h immediately after the application of the light pulse (Fig. 5). The data indicate a marked influence of blue light upon the observed expression of the clinostat-elicited response. Additionally, the large degree of congruence with the curve for the nastic component of Fig. 4 should be mentioned. For fluences lower than $10^{-4}$ µmol·m$^{-2}$, curvature (120°) is practivally identical to that for unirradiated seedlings. It then decreases to 40° at 0.1 µmol·m$^{-2}$. Between 0.1 µmol·m$^{-2}$ and 3 µmol·m$^{-2}$ one observes a broad trough with angles between 30° and 50°. Then the curve steps up again to 80–100° at 10 µmol·m$^{-2}$. An explanation for the calculated curves shown in Fig. 5 is given in the *Discussion.*

*Phototropic induction opposing clinostat-elicited bending: behaviour of the single seedling.* To help understand the large standard deviations of the fluence-response curve for blue light opposing clinostat-elicited bending in Fig. 3, a curvature-frequency plot of final curvature 24 h after the onset of clinostat rotation for 0.3 µmol·m$^{-2}$ blue light (i.e. at the maximum of the fluence-response curve) was constructed (Fig. 6). It shows that seedlings either perform strong phototropic or clinostat-elicited bending. This yields two clearly distinct populations with peaks of −110° (indicating curvature towards the caryopsis) and +110° (curvature towards the light). Many seedlings, however, bent perpendicular to the plane of stimulation (Fig. 6, black bars). This curvature, too, is very large (the peaks of the corresponding distribution are at 110°). It should be emphasized that uncurved or only weakly curved seedlings were not observed. Thus, it seemed to be convenient to group the seedlings into three subsets. Set 1 contained seedlings performing phototropism, set 2 those bending towards the caryopsis (avoiding the light stimulus),

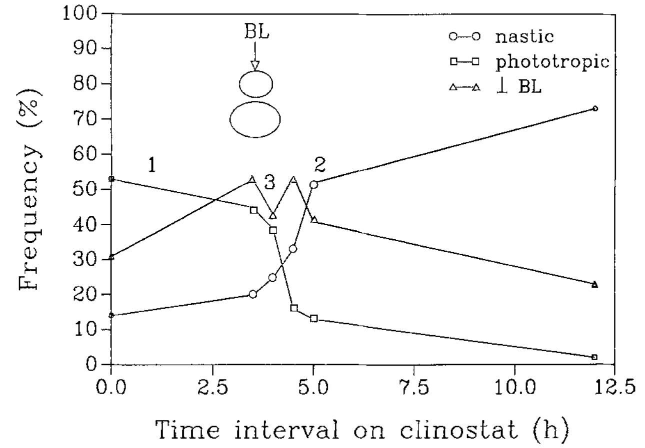

**Fig. 7.** Time course of frequency of different response types for the situation of Fig. 6 with blue light administered at varying time intervals after the onset of clinostat rotation. *Curve 1, squares*, occurrence of phototropic bending; *curve 2, circles*, occurrence of nastic bending; *curve 3, triangles*, occurrence of bending perpendicular to the stimulation plane. Each point represents four experiments (40–50 seedlings for each experiment). The average error between experimental groups was in the range of 5% of the whole population. *Inset* shows the direction of blue light (the larger ellipse indicates the caryopsis, the smaller the coleoptile)

and set 3 seedlings exhibiting neither phototropic nor clinostat-elicited bending but curving perpendicular to the stimulation plane with azimuth angles of 90° and 270°, respectively. The occurrence of these three response patterns was followed over time by administering blue light in an azimuth of 0° at variable time intervals after the onset of clinostat rotation and recording the frequency of the three sets 24 h after the onset of clinostat rotation (Fig. 7). In this experimental set-up the time for the development of the phototropic response was not constant. However, phototropic curvature is more or less completed within 10 h after induction (Nick and Schäfer 1988a). Thus, time for the expression of the phototropic response is not limiting. The frequency of phototropically curving seedlings (set 1) drops from 55% for blue light applied at the onset of clinostat rotation to less than 5% for blue light given only 12 h later with a marked step-down between 3 and 4 h after the onset of clinostat rotation. Set 2 (seedlings performing the clinostat-elicited response) on the other hand, increases with time from 15% (for a time interval of zero) to 75% (for a time interval of 12 h) again with a conspicuous step between 3 and 4 h. Seedlings curving perpendicular to the stimulation plane (set 3) follow an optimum curve rising from 30% at 0 h to a maximum of 50% at 3 h and then falling to 25% at 12 h.

## Discussion

*Clinostat-elicited curvature of maize coleoptiles is a nastic response.* The bimodal character of the azimuthal distribution of clinostat-elicited bending (Fig. 1) is unlikely to be the result of chance effects, because the data of more than 1200 seedlings were used and the same pattern was found for all four major orientations on the clinostat. Centrifugal-force-governed gravitropism can be ruled out, since different positioning of seedlings on the clinostat did not change the azimuthal distribution of clinostat-elicited curvature. Moreover, centrifugal acceleration for the given clinostat amounts to only 7% of the earth's gravity corresponding to a stimulation angle of 4°. Experiments with wheat (*Triticum aestivum* L.) seedlings under vertical rotation with similar centrifugal accelerations could not detect any significant effects of centrifugal force (Heathcote and Bircher 1987).

There are two possibilitie explanations of how a bimodal distribution can be brought about. Either there are two independent subpopulations, i.e. the two peaks are given a-priori, or a one-peaked distribution encounters an inhibiting effect in its center, disrupting it into two parts, i.e. the bimodal shape is produced a-posteriori. One can test which of the two models allows the best fit with the observed data (Pollard 1977). For this, one first has to determine the components involved under the assumption that in the periphery of the observed distribution interaction effects are negligible. For the a-priori case this approach yielded two normal distributions whose peaks were displaced by 30° from the symmetry axis connecting the shortest cross-sections of the coleoptile and caryopsis. The a-posteriori case, treating the ascents of the observed distribution as symmetrical, produced a positive normal distribution, whose peak was situated within the symmetry axis, interacting with a negative normal distribution, whose mean was almost identical.

A plot of the best-fit distribution under the suppositions of the a-priori and the a-posteriori cases, respectively (Fig. 1), indicates that the a-posteriori case producing a bimodal curve with the peaks at the correct positions mirrors the observed data better than the a-priori case, where one peak is visible only as a slight shoulder. A further advantage of the a-posteriori model is that the asymmetry of the peaks can be achieved although the coleoptile is fairly symmetrical within its longer cross section. Under the conditions of this model, minimal asymmetries are amplified and may suffice to cause the observed inequality of the peaks.

That the coleoptile is symmetrical along its larger cross section can be concluded from the curves shown in Fig. 4. The a-priori model is not able to reconcile both observations.

Thus, there is one preferential bending direction towards the caryopsis, secondarily split by an obstacle of unknown nature situated in the main direction of the clinostat-elicited curvature. This preferential direction appears to be constant even with different positioning of the clinostat (angle of shorter coleoptile cross-section to the direction of the centrifugal force exerted by the rotation 0°, 90°, 180°, 270°, respectively). This justifies refering to this response as nastic a movement in the sense of Pfeffer (1904).

Like oat coleoptiles (Bremekamp 1925), maize coleoptiles show considerable dorsoventrality not only at the morphological level, as noted already during early studies of so-called heliotropism (Rothert 1894), but also at the physiological level. It should be emphasised, however, that the direction of this dorsoventral curving is opposite to that found in oat, a fact for which no explanation can be given at the present state of knowledge. On the other hand, both species also differ with regard to tropistic responses, with negative phototropism present only in oat coleoptiles, not in maize (Iino 1988). The fact that this nastic response was not mentioned (and possibly not noted) in earlier investigations might be partly due to the widespread practice of administering tropistic stimulations within the *longest* coleoptile cross-section, in order to maximize the light gradient (Iino 1987), a point made already by Bremekamp (1925). In the few experiments, however, where light was irradiated within the *shortest* coleoptile cross-section (Heathcote and Bircher 1987), nastic effects might well be relevant.

*The nastic and the phototropic response interact on two levels.* The partial parallelism in their central parts of the fluence-response curves for phototropic induction opposing or enhancing nastic bending (Fig. 3) indicates that curvatures are subtracted or added, respectively. However, this parallelism does not hold for fluences lower than $10^{-2}$ μmol·m$^{-2}$ or higher than 10 μmol·m$^{-2}$. Moreover, in the fluence range where parallelism is valid, the difference between the curves is much smaller than expected, taking into account the amount of nastic curvature in unirradiated coleoptiles (Fig. 3, dashed line). Thus, the simplest model, namely, that there are exclusively additive interactions without any further cross reactions must be replaced by a more sophisticated version.

This is supported by the fluence-response curves for blue light administered normal to the preferential direction of nastic curvature (Fig. 4). Here, the curve for the phototropic component resembles the usual fluence-response curves for first positive phototropism (Iino 1987, 1988). This allows the conclusion that clinostat rotation only magnifies phototropic curvature by excluding gravitropic counterstimulation (Nick and Schäfer 1988a). The corresponding curve for the nastic component, however, exhibits a remarkable fluence dependence with fluences inducing large phototropic curvatures diminishing the nastic component. This pattern can be explained in three ways:

i) Blue light inhibits nastic curvature in an undirectional, tonic manner.
ii) The blue-light gradient is inversely correlated with a gradient defining the expression of the nastic response.
iii) The growth gradient underlying the phototropic response limits the growth gradient determining nastic curvature.

To distinguish between the first possibility, and the second and third on the other hand, a fluence-response curve of nastic bending of blue-light irradiation from above (Fig. 5) was recorded. The resulting curve, similar to that for the endogenous component in Fig. 4, supports the idea of tonic inhibition of the nastic response by blue light. Regarding this fluence dependence it makes sense to reconsider the curves for blue light opposing and enhancing nastic curvature, respectively (Fig. 3). Under the assumption of additivity it is possible to calculate from Fig. 3 a curve reflecting the fluence dependence of nastic curvature for unilateral irradiation (Fig. 5, upper solid curve). This calculated curve represents strikingly well the measured data for nastic curvature under irradiation from above. From this, two conclusions can be drawn:
i) The fluence dependence of the nastic response is caused by tonic inhibition by blue light.
ii) Additionally, phototropic and nastic curvatures are added. Thus, both responses interact on two levels.

Under the supposition of additivity of phototropic and nastic curvatures (Fig. 3), and taking into account the tonic inhibition of the nastic response by blue light (Fig. 5, squares), it is possible to calculate a fluence-response curve of first positive phototropism for light irrdiated within the shortest cross-section of coleoptile and thus freed from the effects of dorsoventrality (Fig. 5, lower solid curve). Since this curve cannot be tested by direct measurement and therefore is of a more speculative character, it is not discussed here in further detail. However, it should be mentioned that for fluences between $10^{-4}$ and $10^{-2}$ $\mu mol \cdot m^{-2}$ this curve has negative values with a minimum of 30° at $10^{-3}$ $\mu mol \cdot m^{-2}$. Ignoring this feature, not yet understood, the calculated curve shows similarities with the measured curve of the phototropic component for light administered in the direction of the longest coleoptile cross-section (Fig. 4). The lower and upper thresholds of positive curvature ($10^{-2}$ and 10 $\mu mol \cdot m^{-2}$, respectively) are similar, but the peak is much lower, and shifted to lower fluences by almost an order of magnitude (65° at 0.3 $\mu mol \cdot m^{-2}$). Bearing in mind that the length of the shortest cross section is roughly one half of the longest cross section, this pattern is at least qualitatively consistent with the idea that the shape of the phototropic fluence-response curve results mainly from the light gradient (increasing with the length of the light path within the tissue), and can be explained by subtraction of two sigmoid curves. These curves describe the dependence of a putative light effect upon the irradiating fluence on the lighted and the shaded sides, respectively. The curves are shifted on the fluence axis by a factor proportional to the absorption gradient over the tissue (Kunzelmann et al. 1988). In this model, the shorter cross section should exhibit lower maximal curvature occurring at lower fluences, because the light path is shorter, and the shift between the curves describing the local light effect is smaller. It is possible to understand the calculated curve of first positive phototropism within the plane of the shortest coleoptile cross section in those terms.

The optimum shape of the fluence-response curve of first positive phototropism was assumed to be due to gradient formation by subtraction of locally perceived light fluences (Kunzelmann et al. 1988). Such suppositions appear sound, as the relevance of such gradients is supported by classical experiments (Buder 1920). Therefore, it is worth scrutinizing the rather similar fluence dependence of the nastic response. Here, too, one observes a bell-shaped optimum dependence of phototonic inhibition (Fig. 5), although directional effects (such as gradient formation) cannot account for this shape. Two conclusions can be drawn from this:

i) The transduction chain of phototonic inhibition of nastic curvature branches from that of phototropic transduction before the integration of local perception over the coleoptile cross section, i.e. before the formation of phototropic polarity (Buder 1920).

ii) The optimum dependence of this phototropic inhibition is not produced by the transduction step supposed to account for the shape of the fluence-response curve of first positive phototropism (Kunzelmann et al. 1988). Such mechanisms of spatial differentiation can be ruled out, since the optimum shape is found even for symmetrical irradiation. It is presumed that the putative local fluence-absorbance curves are sigmoid (Kunzelmann et al. 1988). Thus, one must postulate the occurrence of a step within the transduction of phototonic inhibition with an optimum dependence upon the preceding step.

*Blue-light-elicited and dorsoventral transverse polarities are not identical.* The large standard deviations observed in the fluence-response curve for phototropic induction opposing nastic bending (Fig. 3) can be understood in terms of an ungraded behaviour of individual seedlings (Fig. 6) deciding between three distinct responses (phototropic curving, nastic response, bending perpendicular to the stimulation plane). Thus, the graded, "smooth" appearance of this fluence-response curve obtained by averaging over three different subpopulations reflects changes in the ratio of choice frequencies rather than graded responses of individual seedlings. It can be interpreted only by being aware that one is not dealing with individuals, but with a population. Taking into account that the ratio of choice frequencies changes with time, mirroring a stabilization of the dorsoventral polarity underlying nastic curvature, one is reminded of a similar phenomenon, namely, the time course of "solidification" of blue-light-elicited transverse polarity measured as increasing stability to counterstimulation of equal strength (Nick and Schäfer 1988b). There, too, the single seedling chooses between three clearly distinct responses: curving in the direction of the first stimulus (for time intervals exceeding 90 min), curving in the direction of the second stimulus (for time intervals shorter than 65 min), and zero curvature (for time intervals between 65 and 90 min). This pattern, analysed at the population level by averaging over the three responses, yielded a graded curve with extraordinarily high standard deviations. This curve was, actually, a cumulative probability (or better: frequency) distribution of the response expressing stability against counterstimulation.

In spite of these correspondences between both phenomena there is one decisive difference: in the experiments involving two opposing blue-light pulses, zero curvature was found for certain time intervals. This was interpreted as cancelling of both contradicting directional effects. However, no zero curvature of individual seedlings was observed in the experiments reported in this work (Fig. 6). Instead of zero curvature, strong bending perpendicular to the stimulation plane occurred, and this behaviour was most abundantly present at the transition towards stability of the nastic response against opposing phototropic stimulation (Fig. 7). This favours the view that blue-light-elicited polarity cannot cancel the dorsoventral polarity underlying the nastic response, but only reorients it. In other words: dorsoventral polarity and blue-light-elicited transverse polarity are different in their nature. This difference becomes manifest in the response to counteracting blue-light pulses. Blue-light-elicited transverse polarity can be cancelled (Nick and Schäfer 1988b); dorsoventral polarity can be only reoriented. This is consistent with the idea that dorsoventral polarity, unlike blue-light-elicited transverse polarity, is not induced de-novo as claimed for polarities in general by Sachs (1880), but is preexisting and only reoriented in the way suggested by Vöchting (1878).

There is a second interesting aspect of the zero-curvature response found in the experiments with two opposing blue-light pulses (Nick and Schäfer 1988b). Seedlings in which cancelling of both pulses occurred were straight and looked like dark-grown, unstimulated seedlings. However, they remained straight during rotation on a horizontal clinostat, whereas unstimulated seedlings under the same conditions exhibit strong nastic curvature (Fig. 2). Thus, if straightness is understood in terms of internal symmetry, there appears to exist two states of symmetry: an unstable initial state (corresponding to dark-grown unstimulated seedlings) permitting nastic bending on the clinostat, and a somehow "solidified" later state (resulting from the interaction of opposing blue-light transverse polarities cancelling each other), which is resistent to nastic curving.

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