Photobiology of Diagravitropic Maize Roots[1]

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ABSTRACT

Light-induced modification of gravitropism in etiolated roots of *Zea mays* cv Bear × W38 is a low fluence response mediated by phytochrome. This cultivar has a threshold of 10^{-6} mol m^{-2} and becomes saturated with 10^{-2} mol m^{-2} of red light. The maximum light-mediated response of 32 degrees downward from horizontal occurs in roots 10 to 30 millimeters in length, 120 to 165 minutes after irradiation. Reciprocity is valid from 2 to at least 9,000 seconds and the response can be about 90% reversed by far red light. Photoreversibility is lost ('escape' occurs) about 20 minutes after red irradiation but appears to be regained 60 to 80 minutes later. A red light-induced (or synchronized) nutation in the apparent curvature rather than unusual escape characteristics may explain these results.

Many roots are diagravitropic in darkness and change their response to gravity, becoming more orthogravitropic when exposed to light (2; review 24). The root cap is apparently the site of photoperception both for this change in gravitropism (14, 15, 25, 26) and a general growth inhibition (16, 26). Despite the important role light plays in potentiating positive orthogravitropism in diagravitropic roots (2, 8), a complete characterization of the photobiology of any cultivar is lacking.

The photobiological data available are incomplete for several reasons. First, none of the available studies (cress: 4; maize: 19, 20) presents fluence-response curves which show both threshold and saturation values. Second, since experiments concerning light-mediated alteration in gravitropism have employed untested safelights (e.g. 17) which may cause a response themselves (review: 3; maize: 20, 23; bindweed: 22) analysis of the results is difficult. Third, the use of continuous irradiation without a test of the effects of varying duration and fluence rate of the light applied (Bunsen-Roscoe reciprocity) (cress: 4; maize: 14) limits the usefulness of some of the data. Finally, assessment of curvature long after the response may have reached a maximum (maize: 19-21; cress: 4) could obscure differences in rates of curvature development caused by various light treatments.

We have investigated whether light-mediated changes in the gravitropic responses of the roots of one maize hybrid (Bear × W38) is a very-low (photoirreversible) or a low fluence (photoreversible) response (3, 10), or both, delineated the kinetics of curvature development and decay in response to brief pulses of

broad band R,[4] and determined the reciprocity characteristics and FR reversibility for this phenomenon. The ultimate aim is to elucidate the photobiology that governs directional growth of roots underground.

MATERIALS AND METHODS

Zea mays (Bear × W38) caryopses were planted, imbibed, and grown in rows. Two rolls of modeling clay (Little Sculptor, 3M Co.) were positioned along the edges of a 1.5 × 17 cm black Plexiglas strip and about 20 maize caryopses were pressed into the clay so that all the embryos faced upward and in one direction. Each row was partially covered with water and imbibed in absolute darkness for at least 4 h. Styrofoam blocks, covered with absorbent paper (Kimpac, Kimberley-Clark) were placed inside opaque plastic boxes and the bottom of the boxes filled with deionized H_2O. The paper acted as a water wick for the duration of growth and treatment of the roots. Individual rows of imbibed seeds were placed into a rectangular groove which had been precut from a long edge of the styrofoam block, and the seedlings allowed to grow in the dark (95% humidity, 26°C) for 2 d so that the roots grew out over the edge of the styrofoam block and above the water soaked paper. All manipulations were done in the absence of any light.

Two-d-old roots were irradiated from above with broad band R or FR according to Mandoli and Briggs (10). After irradiation, seeds were grown for an additional time in the dark and then harvested in white light. Excised roots were arranged on lucite sheets and photocopied. Root lengths and angles were measured with a computerized digitizer (10). All experiments were done three times with 20 to 40 roots per datum point. SE per datum point rarely exceeded ±4° (e.g. Fig. 1).

RESULTS

Response Optimization. In the dark, maize (*Zea mays* cv Bear × W38) roots grew at an angle within about 10° of horizontal. They attained this orientation whether the embryo axis was oriented horizontally or with the primary root pointed 45 or 90° above or below horizontal. Hence, in total darkness, they are truly diagravitropic (e.g. Aegopodium: 2). The angle of growth established after exposure to a given fluence of light is dependent on root length (Fig. 1). Horizontal roots from 10 to 40 mm in length developed a downward curvature of 32 ± 2.5° with longer roots attaining smaller curvature. The longest roots tested, those from 60 to 70 mm in length grew at an angle of about one-half that of the shortest roots, about 14° below horizontal, given the same fluence (Fig. 1). These data do not indicate whether longer roots are less sensitive to R or are less capable of response. All subsequent analyses used only roots from 10 to 30 mm in length at the time of harvest.

The development of curvature after a brief pulse of R has a

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[4] Abbreviations: R, red light; FR, far red light; VLF, very-low fluence; LF, low fluence; HIR, high irradiance response.
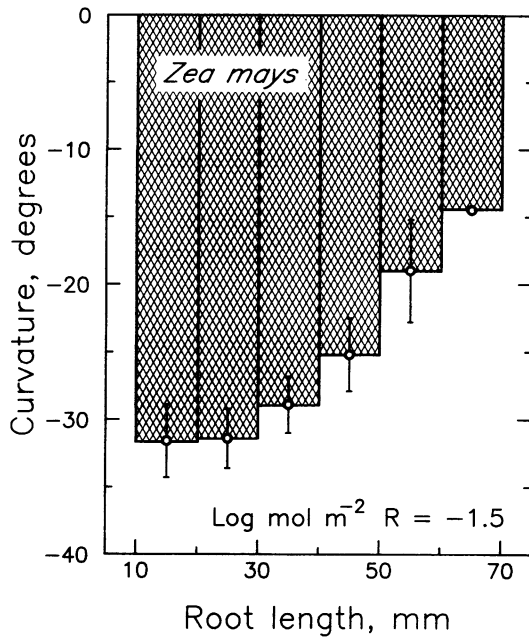
FIG. 1. Root photosensitivity as a function of root length. Etiolated 2-d-old *Zea mays* roots were irradiated with a saturating fluence of broad band R ($10^{-1.5}$ mol m^{-2}; see Fig. 3). Root lengths and angles were measured when curvature was maximum, 150 min after irradiation (see Fig. 2) from photocopies of excised roots with a computerized digitizer (10). Zero represents curvature in dark-grown, mock-irradiated roots. Curvature of irradiated roots is expressed in degrees below horizontal.
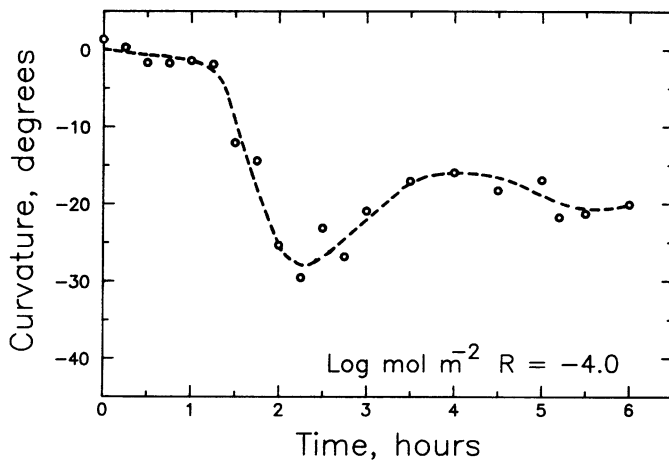

FIG. 2. Kinetics for light-altered gravitropism in maize. A saturating fluence of R ($10^{-4.0}$ mol m^{-2} in 26 min) was given from above to groups of etiolated 2-d-old maize roots. These treated roots were grown in the dark for the additional period of time specified and then harvested in the light, photocopied, and analyzed (10). Downward, 'positive gravitropic curvature' is presented with respect to the curvature at time zero (0 to −3 degrees).

lag of about 75 min and reached a maximum between about 120 and 165 min after irradiation (Fig. 2). The amount of curvature at longer times after irradiation decreased to about two-thirds of the maximum and fluctuated about that level for at least 6 h (Fig. 2). In each of the 3 replicates used (average shown in Fig. 2), a sharp maximum in curvature was found about 165 min after irradiation. Hence, this time was selected for harvest of all subsequent experiments except where noted.

Fluence Response Curve. This maize cultivar showed no significant deviation from its diagravitropic behavior until given a

fluence of greater than 10^{-6} mol m^{-2} of R (Fig. 3). The small but consistent downward curvature seen at lower fluences (10^{-12} to 10^{-6} mol m^{-2}) is within the biological variation of the organ (Fig. 1). Thus, this cultivar does not show the prominent VLF response which is evident in many other photoresponses from 10^{-11} to 10^{-9} mol m^{-2} (3).

At 10^{-6} mol m^{-2} R an altered gravitropic response that becomes saturated at about 10^{-2} mol m^{-2} R (Fig. 3) was apparent. Maximum inducible curvature for single, brief pulses of R is about 32° from horizontal. These threshold and saturation values are compatible with the LF class responses to R (3, 9, 10). This response is a 'graded' rather than a 'threshold' type LF response (11).

Reciprocity Characteristics. A subsaturating fluence of R ($10^{-4.5}$ mol m^{-2}) will evoke approximately the same magnitude of curvature whether given in 2 s or 9000 s (Fig. 4). For this experiment, all roots were harvested 166 min after the onset of irradiation, since this time was the duration of the treatment with the lowest fluence rate. Hence, the response is equivalent when the fluence rate and the time over which the fluence is delivered are varied reciprocally. The simplest interpretation of this result suggests that the response is limited by the first order
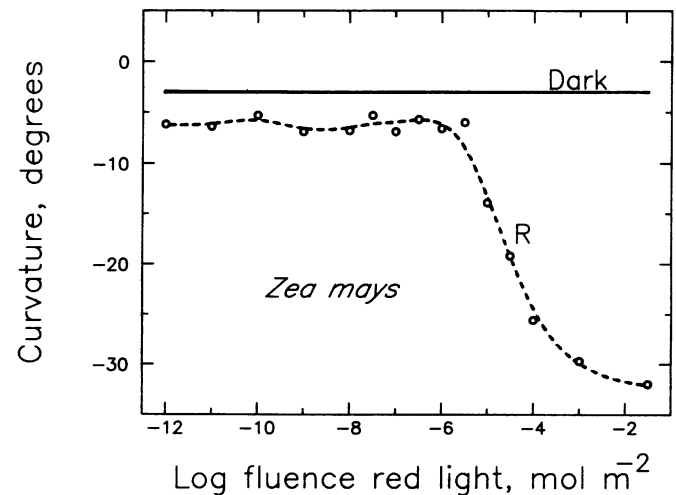

FIG. 3. Fluence response curve of etiolated maize roots. Duration of R pulses ranged from 1 s to 26 min. Fluence rate was varied with Balzers neutral density filters.
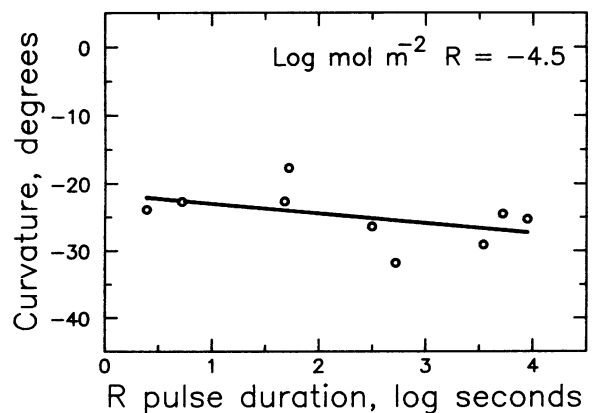

FIG. 4. Reciprocity characteristics of R-irradiated, maize roots. All roots were etiolated, 2 d old, and 10 to 30 mm in length at the time of irradiation. Fluence rate was varied with Balzers neutral density filters. Duration of the exposure was varied from 2 to 9000 s. Fluence was held constant at $10^{-4.5}$ mol m^{-2}. Harvest of treated and control roots occurred 9000 s after the start of illumination.

photochemistry of the phenomenon.

FR Photoreversibility. This gravitropic response is about 90% photoreversible with a fluence of FR 100-fold greater than the initial fluence of R (Fig. 5). The R/FR reversibility of the phenomenon implicates phytochrome as the photoreceptor that modifies the gravitropic response in this cultivar.

Escape from FR Reversibility. If one attempts to reverse the R-induced response at various times after R-irradiation with FR, one can define the time at which this photomorphogenic response escapes from photoreversibility. Escape occurred within about 20 min after the initial R fluence (Fig. 6). It should be noted that although the curvatures in this experiment (Fig. 6) were slightly greater than those in previous experiments, the dark control also shows a few degrees curvature and the variation is
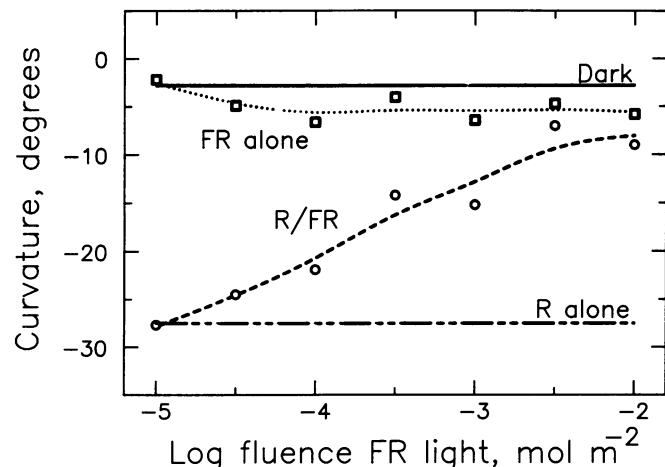

FIG. 5. FR reversibility of light-stimulated gravitropism of maize roots. Upper solid line represents curvature of 2-d-old etiolated roots in the dark. Lowest dashed line indicates mean response of etiolated roots to a fluence of R just below saturation for the LF response (10^{-4} mol m^{-2}). Responses to increasing fluences of FR alone are shown ($\square$). Responses to a pulse of R (10^{-4} mol m^{-2} R given in 2.6 s in all cases) followed within 5 min with a specified fluence of FR (given in from 0.5–500 s) are indicated (O).

not incompatible with the biological variation seen within a population (cf. Fig. 1). The intriguing observation is that after the initial escape from reversibility (from 20 to 55 min), sensitivity to the reversing effect of FR appears to be regained (from 60 to 80 min) and then lost again (80 to >100 min) (Fig. 6). An alternative interpretation of these data is that the R pulse induced or synchronized an oscillation (or nutation) in the roots (7) and that the response to a subsequent pulse of FR reflects an interaction with this nutation.

Gravitropic Nutations Apparently Induced by R. Completely dark grown roots show no apparent growth nutation (Fig. 7). These roots (■, Fig. 7) were handled as in the R/FR reversal experiment (O, Fig. 6) but were not irradiated. However, as mentioned above, roots of the same age given a single, brief pulse of R almost sufficient to saturate the light response (log mol m^{-2}; 26°; Fig. 3) showed nutational movements when they were harvested at later times (150–360 min postirradiation) with a mean curvature two-thirds that of the maximum curvature observed at 150 min postirradiation (Fig. 2).

A second R pulse given at various times after the first produced a variable final curvature at harvest, 150 min after the initial R pulse. If the second R pulse was given immediately after the first, the degree of curvature was that expected for the total R fluence given (Fig. 7 at $t = 0$; Fig. 3 cf. 2×10^{-4} versus 1×10^{-4} mol m^{-2}), because reciprocity does not fail over these pulse durations (Fig. 4). However, if the second R pulse is delayed (Fig. 7), it either slightly enhances or mitigates the effect of the initial R pulse creating an oscillation in the final curvature response with a periodicity of about 50 to 60 min and an amplitude of ±5° (Fig. 7). In addition, as the pulses are separated by increasing periods of time, the mean curvature about which the oscillations vary decreases at the rate of about 0.1°/min. These effects are evident if the two R fluences are equivalent (Fig. 7; $10^{-4.0}$:$10^{-4.0}$) or if the second fluence is less than the initial one ($10^{-4.0}$:$10^{-4.9}$, not shown).

DISCUSSION

The magnitudes of the gravitropic responses seen here with Bear × W38 (32 + 2.5°) are compatible with those obtained with other cultivars. Shen-Miller (20) obtained only 10° curvature with *Zea mays* Wisconsin hybrid 64A × 22R but she employed
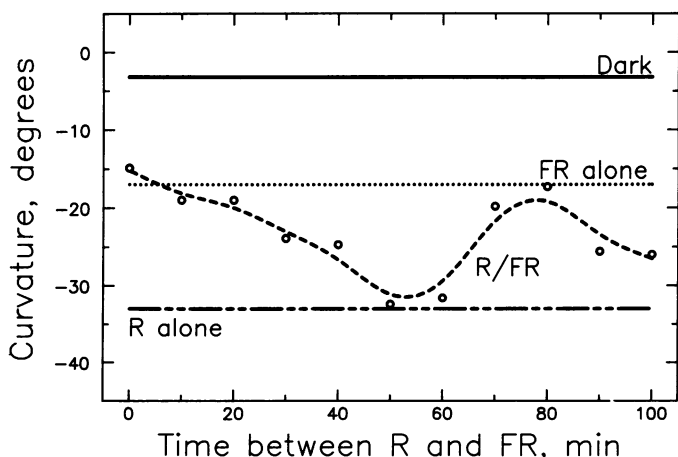

FIG. 6. Escape from R/FR reversibility of light-stimulated gravitropic response in etiolated maize roots. Solid line represents mean response of mock-irradiated roots. Lowest dashed line and the dotted line represent mean responses to fluences of R alone ($10^{-4.0}$ mol m^{-2} given in 2.6 s) and FR alone ($10^{-2.0}$ mol m^{-2} given is 500 s), respectively. Responses to R ($10^{-4.0}$ mol m^{-2}) followed by FR ($10^{-2.0}$ mol m^{-2}) at a time from 0 to 100 min after the end of the R pulse were as indicated. Harvest of all roots occurred 150 min after the start of the R irradiation.
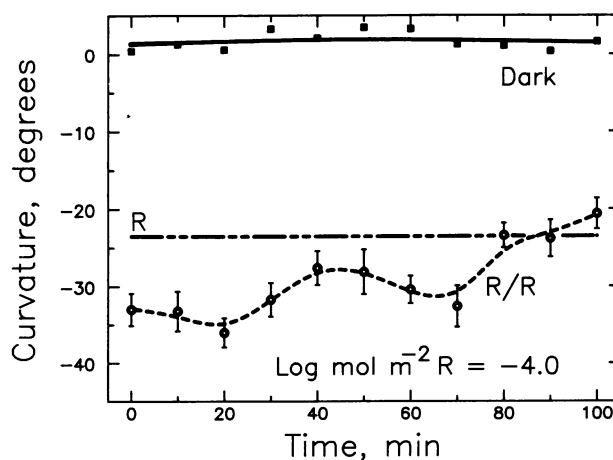

FIG. 7. Gravitropic responses of roots which were either handled as were illuminated roots but not irradiated (D) or given two nearly saturating pulses of R (R/R) are shown. R represents a fluence of $10^{-4.0}$ mol m^{-2} given in 2.6 s. Means and SE represent 20 to 40 roots per datum point from 1 experiment. Similar results were obtained in 2 replicates (not shown). Results were not averaged since the phase relationships differed slightly between experiments.

green working lights and nonsaturating fluences (Fig. 2 in 20). Suzuki and Fujii (21) obtained curvatures of 35° with *Zea mays* Golden × Bantam 70 and Pilet (14) observed curvatures from 20 to 50° in excised roots from several maize cultivars illuminated with continuous white light. Clearly, the response magnitude in maize depends not only on illumination conditions (*e.g.* fluence rate, fluence, safelights, wavelength, and exposure duration) but on root length, or physiological age (Fig. 1), the variety used, and the time of assay (Fig. 2).

The development of orthogravitropic curvature in intact maize roots has a lag of from 1 to 1.5 h (Fig. 2; 19, 20) after brief pulses of R or in excised roots given continuous white light (1, 13, 17). The shortest lag for gravitropic curvature was 8 min in excised roots under continuous fluorescent white light (17). However, these roots attained only 3 ± 0.5° of curvature in 28 min. Such curvatures could not be detected in dark grown roots in kinetic experiments with a sensitive position transducer (21). Although the difference in the cultivars used cannot be ignored, it seems more likely that this discrepancy in the kinetics of the development of curvature could result from the photographic technique used by Pilet and Ney (17) in which intact, vertically oriented roots were filmed under a background of continuous white fluorescent light with pulses of incandescent irradiation every minute for 3 h prior to horizontal presentation. It is probable that this treatment potentiated a subsequent response to additional light. Suzuki and Fujii (21) used intact R-irradiated roots and monitored gravitropic curvature with a position transducer. They state that their manipulations under green light did not induce gravitropic changes in Golden × Bantam 70 roots, consistent with the observation that this cultivar appears to lack a VLF response (20, 21). Hence, the longer lag and curvature seen by others and shown here (Fig. 2) is probably the more accurate representation of the LF phenomenon. This lag is much longer in other species given continuous irradiation (*Lepidium*: 4; *Aegopodium*: 2; *Vanilla*: 6). The responses obtained with continuous irradiation (*e.g.* 17) may well represent an high irradiance response (HIR) rather than an LF type response (4; see below).

Curvature reaches a maximum in intact maize roots 2 to 2.5 h after brief irradiation (Fig. 2; 20, 21) and much later (8–12 h) in excised maize roots given continuous white light (1, 13, 17) despite the fact that the degree of curvature is similar under these different conditions.

The change in the growth angle in intact, R-irradiated maize roots is apparently an LF response (3, 9, 10). *Convolvulus* roots apparently have a threshold in the VLF range (7.7 × 10⁻¹¹ mol m⁻²) although a complete fluence response curve was not done (23). There are indications that some maize cultivars are more light sensitive than others (Fig. 1 in 13). However, this may reflect differences in the magnitude of the VLF in a given cultivar rather than the absence of the VLF response on a molecular level.

Fluence rate and pulse duration can be varied reciprocally over extremely long periods of time without a dramatic failure of reciprocity (2–9000 s; Fig. 5). However, the slope of the line drawn through these data (Fig. 5) is slightly negative. Suzuki and Fujii (21) tested reciprocity at threshold for 1 to 65 s and found no reciprocity failure in Golden × Bantam 70 either. Reciprocity characteristics have not been explored in any of the papers which may deal with the HIR type gravitropic response (*e.g.* 4).

The capacity of roots to integrate a light fluence given over 9,000 s (2.78 h; Fig. 4) is somewhat surprising. The response to a brief light pulse begins after 1 h and is complete after about 2 h (Fig. 2). In analysis of reciprocity experiments for such LF responses, it is commonly assumed that the kinetics are the same in response to long, dim irradiations as they are to short, bright irradiations. If this is true here, then a significant portion of the light appears to have been administered not only during the

development of curvature, but after the roots have completed their response. At lower fluence rates and longer exposure times shown in Figure 4, curvature development may be much slower than after the briefer pulses. Whereas reciprocity holds for the final curvature achieved, it might well not hold for the rate at which the curvature develops. Alternatively, the onset of curvature could be later when fluence rates are lower. This problem clearly requires further investigation.

This LF response is clearly R/FR reversible (Fig. 6; 20, 22 but cf. 21). The spectral response curves for light-mediated gravitropism in *Zea mays* cultivars which employed a single, equal fluence at each wavelength used (20, 21) also suggest that phytochrome may be the pigment involved in photoperception.

A blue absorber as well as phytochrome may mediate the response in some species (*e.g.* cress: 4; maize: 20). Other evidence for interaction with a blue light-absorbing pigment comes from data on continuous light exposure (4, 18). These data and the lack of complete FR reversal (4) are compatible with a phytochrome-mediated HIR response that may also influence gravitropism in addition to the LF response studied here. Studies of light-induced root gravitropism which employ continuous irradiation all probably address this HIR, (1, 2, 4, 13, 17) and should be analyzed separately.

Escape from FR reversibility (Fig. 7) appears complex in this system. One explanation for the observed escape pattern is that the photosystem of escape itself is temporally dependent, being reversible from 0 to 20 and 80 to 100 min and irreversible from 20 to 55 min after the initial irradiation (Fig. 7). Photochemically this is hard to envision. A more plausible explanation is that the apparent escape characteristics reflect an interaction with an oscillatory system which also mediates the degree of gravitropic curvature in maize roots (Fig. 7).

Growth nutations in root have been studied for many years (reviewed by 7; see also 5, 12). Bennett-Clark and Ball (2) provided some evidence that the root nutations in *Aegopodium* were initiated by light but since they grew their roots in soil and dug them up in the light, the light-inducibility of this phenomenon is not entirely clear from their data. They did not attempt any rigorous photobiological characterizations.

In the present case, a single R pulse might induce such nutations, expressed as an apparent oscillation in the sensitivity of the roots to a subsequent pulse of FR (Fig. 6) or R (Fig. 7). Alternatively, the initial R pulse might simply synchronize existing oscillations in the several roots in a given row. Further experimentation, especially on individual roots, is clearly required to unravel this complex phenomenology.

LITERATURE CITED

1. BAEHLER W, PE PILET 1981 Kinetic analysis of root growth and georeaction. Physiol Plant 51: 27–32
2. BENNETT-CLARK TA, NG BALL 1951 The digeotropic behavior of rhizomes. J Exp Bot 2: 169–203
3. BLAAUW G 1983 Thoughts on the possible role of phytochrome destruction in phytochrome controlled responses. Plant Cell Environ 6: 173–179
4. HART JW, IR MACDONALD 1980 The influences of light on geotropism in cress roots. J Exp Bot 31: 903–911
5. HEATHCOTE D 1982 Nutation in georeacting roots. Plant Cell Environ 5: 343–346
6. IRVING JR, RH FREYE 1961 Diageotropism in *Vanilla* roots. Science 134: 56–57
7. JOHNSSON A, D HEATHCOTE 1973 Experimental evidence and models on circumnutation. Z Pflanzenphysiol 70: 371–405
8. LAKE JV, G SLACK 1961 Dependence on light of geotropism in plant roots. Nature 191: 300–302
9. MANDOLI DF 1983 Photomorphogenesis: Some cell and organ responses and the role of tissue light piping. PhD thesis. Stanford University, Stanford CA
10. MANDOLI DF, WR BRIGGS 1981 Phytochrome controls two low-irradiance responses in etiolated oat seedlings. Plant Physiol 67: 733–739
11. MOHR H 1972 Lectures in Photomorphogenesis. Springer-Verlag, Berlin
12. NEY D, PE PILET 1981 Nutation of growing and georeacting roots. Plant Cell Environ 4: 339–343
13. PILET PE 1975 Effects of light on the georeaction and growth inhibitor content

of roots. Physiol Plant 33: 94–97

14. Pilet PE 1978 The role of the cap in the geotropism of roots exposed to light. Z Pflanzenphysiol 89: 411–426

15. Pilet PE 1982 Importance of the cap cells in maize root gravireaction. Planta 156: 95–96

16. Pilet PE, D Ney 1978 Rapid, localised light effect on root growth in maize. Planta 44: 109–110

17. Pilet PE, D Ney 1981 Differential growth of georeacting maize roots. Planta 151: 146–150

18. Quail PH, WR Briggs 1978 Light-enhanced geotropic sensitivity in maize roots: possible involvement of phytochrome. Carnegie Inst Wash Year Book 77: 336–339

19. Scott TR, MB Wilkins 1969 Auxin transport in roots. IV. Effects of light on IAA movement and geotropic responsiveness in Zea roots. Planta 87: 249–258

20. Shen-Miller J 1978 Spectral response of corn (Zea mays) in root geotropism. Plant Cell Physiol 19: 445–452

21. Suzuki T, T Fujii 1978 Spectral dependence of the light-induced geotropic response in Zea roots. Planta 142: 275–279

22. Suzuki T, M Tanaka, T Fujii 1981 Function of light in the light-induced geotropic response in Zea roots. Plant Physiol 67: 225–228

23. Tepfer DA, HT Bonnett 1972 The role of phytochrome in the geotropic behavior of roots of Convolvulus arvensis. Planta 106: 311–324

24. Wilkins MB 1979 Growth-control mechanisms in gravitropism. In Encyclopedia of Plant Physiol 7: Physiology of Movements. W Haupt, ME Feinleib, eds, Springer-Verlag, Berlin, pp 601–626

25. Wilkins MB, RL Wain 1974 The root cap and control of root elongation in Zea mays L. seedlings exposed to white light. Planta 121: 1–8

26. Wilkins MB, RL Wain 1975 The role of the root cap in the response of the primary roots of Zea mays L. seedlings to white light and to gravity. Planta 123: 217–222