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COMMENTARY

PHYTOCHROME REGULATION OF MORPHOGENESIS IN GREEN PLANTS: FROM THE BELTSVILLE SPECTROGRAPH TO COLORED MULCH IN THE FIELD*

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Abstract—Development of the theory and use of photon ratios reflected from colored mulches to regulate plant morphogenesis in the field utilized information obtained on the Beltsville Spectrograph, in controlled environments and in field plant spacing studies. An upwardly reflected far-red to red (FR/R) ratio higher than the ratio in incoming sunlight resulted in larger shoots and a higher shoot/root biomass ratio. A reflected FR/R ratio lower than the ratio in incoming sunlight resulted in larger roots and a lower shoot/root biomass ratio.

INTRODUCTION

Photomorphogenesis in green plants is an important aspect in emerging strategies for crop improvement under field conditions, and observation of plant growth and development in natural surroundings is an important step in designing experiments that eventually lead to new crop production and management systems. Early studies in photomorphogenesis followed observations that plants such as soybean [*Glycine max* (L.) Merr.] would flower at about the same time each year, even though seeding dates (and plant size at flowering) differed. Sometimes a plant would grow larger and flower later than the majority of its kind. In one such example, W. W. Garner and H. A. Allard, who worked with the Maryland type of tobacco (*Nicotiana tabacum* L.), observed some "mammoth" plants that did not flower in the field during the growing season but did flower in the greenhouse during the shorter days of winter. This observation of Maryland Mammoth tobacco and awareness of the seasonal response of soybean led to the discovery of photoperiodism (Garner and Allard, 1920). The research suggested that adapted plants contain a season-sensing mechanism which signals them to flower early enough so that the next generation of seed can develop and ripen before freezing temperatures occur. The discovery of photoperiodism was a breakthrough in science. It was also of major importance to plant breeders who could then synchronize flowering time for critical genetic crosses that allowed incorporation of disease resistance and other desirable factors into new varieties of crop plants.

Subsequent studies by M. W. Parker, H. A. Borthwick, S. B. Hendricks and their colleagues concentrated on the control mechanism and led to the discovery that a R-FR† photoreversible pigment (phytochrome) was involved in photoperiodic control of flowering, as well as seed germination, hypocotyl elongation and hook opening, leaf expansion and accumulation of chemical compounds such as anthocyanin (Borthwick, 1972). These studies were facilitated by the design and construction of a spectrograph which allowed evaluation of the efficiency of various narrow wavebands of radiation in the control of seed germination, inhibition of flowering in short-day plants, promotion of flowering in long-day plants, *etc.* The major breakthrough occurred when Borthwick *et al.* (1952) discovered R-FR photoreversible control of seed germination. The Beltsville Spectrograph was a unique and useful tool in the study of photomorphogenesis. Results of most of the experiments conducted on the spectrograph supported the hypothesis that R was the driving force and Pfr was the biologically active form of phytochrome. Although the hypothesis seemed to fit the responses studied, some investigators suggested that it was too simplistic for all phytochrome-regulated responses.

Observations that different morphological characteristics develop in close-spaced *vs* wide-spaced plants have led to the realization that the phytochrome system is involved in the sensing of competition from other plants, and in the initiation of events within the growing plant that influence its

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†*Abbreviations*: FR, far-red light; Pfr, far-red absorbing form of phytochrome; Pr, red absorbing form of phytochrome; R, red light.

morphological adaptation according to the amount of competition. Much of my research over the last 30 years has concerned phytochrome regulation of various aspects of plant growth and development. The objective of this presentation is to bring together observations and experimental findings that have contributed to development of theory and use of FR/R photon ratios in light reflected from different colored mulches to regulate morphogenesis of field-grown plants.

PHOTOMORPHOGENESIS IN GREEN PLANTS

An early observation that contributed to our recent progress in phytochrome regulation of plant productivity under field conditions occurred on a farm in Iowa in the 1940s where I first became aware that pigweed (*Amaranthus retroflexus* L.) plants growing close together were taller, had fewer branches, and were easier to pull (due to smaller root systems) than isolated seedlings of the same species and age. These characteristics became apparent soon after emergence, before mutual shading became a factor. Also, the same general pattern was evident in oat (*Avena sativa* L.) fields. When plants were normally spaced within the fields they developed many tillers, but few tillers developed on plants that were closer together because rows overlapped. I wondered why such differences occurred but no one was able to provide a reasonable explanation at that time.

Photoperiodic regulation of biomass allocation in a biennial legume

Some of the answers became more clear to me in the late 1950s when, as a graduate student of W. E. Loomis at Iowa State University, we studied effects of photoperiod and temperature on morphogenesis in biennial sweetclover (*Melilotus alba* L.). It became apparent that a number of environmental factors interacted in regulating how this biennial plant proceeded through its life cycle, from seed to the next generation of seed. The seedling shoots grew rapidly under the relatively long days of spring. Then, as days shortened in autumn, shoot growth rate slowed while size of storage roots increased and crown buds formed (Kasperbauer *et al.*, 1962, 1963b). The following spring, new shoots developed from the crown buds and they flowered, set seed and died, completing the life cycle. It was evident that photoperiod regulated the relative amounts of shoot and root growth during the seedling year, and that photoperiod interacted with temperature in floral induction. Awareness of photoperiodic regulation of increased root biomass in autumn was important in determining when to plow this legume into the soil in order to maximize incorporation of biologically-fixed nitrogen.

Another important observation was that stems of close-spaced sweetclover seedlings branched less and elongated more than isolated plants, just as was observed in pigweed during earlier years on the Iowa farm. This developmental effect of plant spacing suggested involvement of a competition-sensing and growth-regulating mechanism, possibly related to the mechanism involved in photoperiodic regulation of shoot/root biomass ratios.

Narrow wavebands on the Beltsville spectrograph

The paper announcing discovery of a R-FR photoreversible pigment in dark-grown seedlings (Butler *et al.*, 1959) in combination with theories of Hendricks and the earlier action spectra developed on the Beltsville Spectrograph (Parker *et al.*, 1945, 1946) led to the start of my postdoctoral research with Borthwick and Hendricks in 1961. From the very first day at Beltsville, my goal was to develop sufficient knowledge of phytochrome regulation of morphogenesis in green plants to allow utilization of the phytochrome system to improve quantity and quality of plant products.

The initial challenge was to develop a green plant bioassay that would allow determination of more precise action spectra for growth-regulatory effects of phytochrome. In the preliminary studies, an Iowa strain of pigweed was compared with a Canadian strain of *Chenopodium rubrum* L. (see Cumming, 1959) for early floral responsiveness to photoperiod. The *Chenopodium* seedlings were responsive at a slightly smaller size and were used in a series of experiments with Borthwick and Hendricks on effects of prolonged FR irradiations, and on the energy requirements for photoconversion of Pr to Pfr, and *vice versa*, at different narrow wavebands within the R and FR parts of the spectrum (Kasperbauer *et al.*, 1963a, 1964). *Chenopodium rubrum*, like soybean, is a short-day plant and treatment on the spectrograph was in the middle of 16 h nights following 8 h photosynthetic periods in a growth room. The *Chenopodium* seedlings were treated for five consecutive diurnal cycles and then grown in a noninductive greenhouse until they were evaluated. This approach allowed use of more plants in narrower wavebands on the spectrograph and, thus, more precise action spectra were obtained with *Chenopodium* than with the much larger soybean plants (see Parker *et al.*, 1945). Nevertheless, the information obtained with *Chenopodium* was relevant to soybean and other crop plants.

Red light action peak in green plants. The R action peak for inhibition of flowering occurred at about 645 nm. The shift in action peak from 660 nm (the approximate *in vitro* absorption peak for Pr) to about 645 nm in green plants was attributed to competitive absorption by chlorophyll at 660 nm (Kasperbauer *et al.*, 1964).

Red light effect of prolonged irradiation with far-red light. While a brief exposure to FR at about

735 nm (following a saturating exposure to R) reversed the effects of R, "prolonged" exposure at 735 nm (the approximate absorption peak for Pfr) produced a R effect (Kasperbauer *et al.*, 1963a). Similarly, prolonged exposure to FR at 735 nm without prior exposure to R also produced a R effect. This response was attributed to some overlapping absorption of Pr at 735 nm, which resulted in maintenance of a low level of Pfr for a "prolonged" period of time. Radiation at 755, 775 and even 795 nm reversed the effects of R, but progressively longer irradiation periods were required with the increasingly longer wavelengths. That is, wavelengths in the range of 755–795 nm resulted in a FR effect in green plants when prolonged irradiations were used. Therefore, while a short exposure to FR at 735 nm was more efficient in reversing the effects of R in photoperiodic control of flowering, the FR effects of radiation at 755 nm (and beyond) became more meaningful in interpretation of morphogenic patterns in field-grown plants in response to prolonged reflection from competing plants and from different colored soils, plant residues and colored mulches, as discussed below.

Some relevant morphological side-effects of irradiations with the different narrow wavebands were noted at the beginning of the day following the fourth cycle of treatment of the *Chenopodium* seedlings on the spectrograph. Distinct leaf characteristics developed on seedlings that were treated on the spectrograph after a saturating irradiation with R. All durations of treatment at 640–660 nm resulted in dark-green horizontal leaves. Seedlings that received short irradiations at about 735 nm had more upright leaves that were lighter green in color. However, with prolonged irradiations, leaves on seedlings that were irradiated at 735 nm were more horizontal, whereas those on seedlings that were irradiated at 755 nm (and beyond) were upright. These observations were consistent with the floral responses, in that short exposures at 735 nm reversed the R-effect and prolonged exposures at 735 nm produced a R-effect. When irradiations were given at the beginning of the 16 h night (instead of in the middle of the night), plants that received R had horizontal leaves as described above, but responses to FR included some internode elongation in addition to the lighter green color and upright leaves. The morphological response to prolonged irradiations at 755 nm (and beyond) were puzzling in 1962 but now seem consistent with field plant morphological responses to FR reflected from competing plants.

Another very relevant observation toward interpretation of plant responses to FR was reported by Vogelmann and Bjorn (1984). They inserted fiber optic probes into fleshy green leaves and found that the measured amount of FR at about 750 nm was higher within the green leaf than at its exterior surface. This was attributed to photon scattering, internal reflection and little competitive absorption at 750 nm within the tissue. Thus, because of competitive absorption by chlorophyll at 660 nm and the enhancement of FR, the effective FR/R ratio within green tissue can be higher than the ratio at the exterior surface. The geometry of the plant part can influence light scatter and reflection within the tissue (Seyfried and Fukshansky, 1983), and likely helps to explain why green seedlings are extremely responsive to FR (Downs, 1955; Kasperbauer *et al.*, 1963a; Kasperbauer and Hiatt, 1966; Kasperbauer, 1971, 1972; Kasperbauer and Karlen, 1986; Ballare *et al.*, 1987, 1990).

Red light irradiation of upper versus *lower surfaces of leaves*

Another 1962 experiment (previously unreported) that had major relevance to the present use of FR/R ratios reflected from colored mulches in the field began with my question to H. A. Borthwick while we were awaiting completion of one of the prolonged irradiation periods on the spectrograph. I asked whether the response to R would be the same if received at the upper or lower surface of a leaf (due to internal leaf structure and possible localized differences in phytochrome concentration). After several days of discussing the apparent lack of ecological relevance of irradiating the lower surface of a leaf, curiosity prevailed and we performed the experiment using some surplus plants of a strain of cocklebur (*Xanthium pensylvanicum* L.) that could be induced to flower by one uninterrupted long night. The plants had been preconditioned under long days (noninductive) in a greenhouse as described by Downs (1956). For treatment, the plants were trimmed to a single fully-expanded leaf and placed in a growth room with an 8 h photosynthetic period. The R light treatments were applied to upper or lower surfaces of leaves in the middle of the subsequent 16 h night. Plants were positioned horizontally on a table below the R light source so that some leaves had the top side up while others had the lower side exposed to the light source. A panel of greenhouse glass was used to keep all leaves flat and at the same distance from and angle to the radiation source. Floral responses were evaluated according to the procedures and rating scale described by Downs (1956). An equal duration of R (0.5, 1, 2 or 5 min) applied to either the upper or the lower surface of a leaf in the middle of the night resulted in the same degree of floral inhibition. Response to R was reversed by irradiation with 5 min FR. Clearly, R received at the lower surface of a leaf could act through the phytochrome system within that leaf. Although light treatment of the lower surface of the leaf did not seem important in 1962, it became highly relevant in 1984 when we began to measure spectra of and plant responses to upwardly reflected light over different colored soils, plant residues and other mulches (Kasperbauer and Hunt, 1987).

Morphological responses to far-red light and red light in controlled environments

In 1963, I was hired by J. E. McMurtrey, Jr. (then head of tobacco physiology research for the US Department of Agriculture) to extend phytochrome research to its involvement in regulation of growth, development and chemical composition of tobacco. More than 40 years earlier, McMurtrey was hired by W. W. Garner and he was a junior colleague of Garner and Allard during the early years of research on photoperiodism. McMurtrey frequently observed our studies on the spectrograph while I was at the Pioneering Research Laboratory with Borthwick and Hendricks from mid-1961 to mid-1963. He was excited about the possibility of having tobacco [the species that led to the discovery of photoperiodism by Garner and Allard (1920)] involved in study of phytochrome regulation of plant developmental aspects beyond the well-documented photoperiodic control of flowering. Tobacco was an ideal plant in which to study phytochrome regulation of morphogenesis because stem elongation of seedlings as well as the physical and chemical characteristics of mature leaves were important to growers and could be altered by cultural practices including plant spacing in the field (Garner, 1951). My research on morphogenesis in green tobacco plants continued in the 1960s and 1970s through a combination of controlled environment and field plant spacing studies in which FR/R ratio effects were compared.

The effectiveness of end-of-day phytochrome form on morphogenesis in tobacco seedlings was demonstrated in a 1964 controlled environment study (unpublished) that involved exposure of seedlings to 5 min R, 5 min FR, or 5 min FR followed immediately by 5 min of R after either 0, 4 or 8 h of darkness had elapsed following an 8 h photosynthetic period. The stem elongation response was greatest when FR (a high FR/R ratio) was applied at the beginning of the uninterrupted night; and, although the response to FR was still evident after 8 h, it was less pronounced than after 4 h (Fig. 1). Stems of seedlings that received only 5 min R, or 5 min R after 5 min FR, were about the same length regardless of whether 0, 4 or 8 h of darkness had elapsed before the treatment. These morphological responses in tobacco seedlings were consistent with those observed in *Chenopodium* seedlings that were treated on the spectrograph, as discussed above.

When 5 min irradiations with R or FR were applied to tobacco seedlings at the end of the daily photosynthetic period, FR resulted in dramatically longer internodes, longer leaf midveins, less biomass per area of leaf, higher chlorophyll *a*/*b* ratio, more CO_2 fixation per mass of leaf and higher concentrations of sugar, as summarized in Table 1. In addition, chloroplasts in leaves from FR-treated plants had more, but smaller, grana and smaller starch grains (Kasperbauer and Hamilton, 1984), suggesting phytochrome involvement in development of the photosynthetic apparatus and in carbon partitioning at the cellular level.

The R-FR photoreversible control of these morphogenic and chemical responses (see Fig. 1 and Table 1) suggested that a low level of Pfr at the beginning of the night was involved in metabolic events that led to prioritization of photoassimilate partitioning which resulted in growth of longer stems, leaving less of the photoassimilate available for new root growth (Kasperbauer, 1971). The results, however, did not indicate whether the low level of Pfr initiated a chain of events leading to "competition-adapted" development or whether the events occurred because the level of Pfr was too low to signal a chain of events leading to "sun-adapted" plant characteristics (Kasperbauer and Peaslee, 1973). It is possible, however, that some factor other than Pfr level associated with FR/R photon ratio might be the initiator of events that affect morphogenesis in growing plants. Nevertheless, it was apparent that FR was a dominant factor in initiating morphological responses that might have survival value among close-spaced plants.

The working hypothesis that evolved from the controlled environment experiments was that the amount of FR relative to R could act through the phytochrome system and result in morphological features that might favor survival of a plant among other close-spaced plants under field conditions. That is, partitioning of more photosynthate to development of a longer stem would increase the probability that a plant could keep some leaves in sunlight above competing plants. Also, leaves that were photosynthetically more efficient might be advantageous when the amount of photosynthetic light received in the plant canopy is decreased by shade from competing plants. Field plant spacing studies were done concurrently with more controlled environment studies to explore the concept.

Responses to plant spacing in the field

In field plant spacing studies of the late 1960s and 1970s, close-spaced tobacco began developing longer internodes and narrower leaves even before mutual shading occurred. These responses were apparent during the pretransplant period (Kasperbauer, 1973), as well as after transplanting to the field. Seedlings that were grown close together during the pretransplant period developed longer internodes. However, after being transplanted to the field, the lengthening of new internodes responded to the nearness of other plants

Figure 1. *Nicotiana tabacum* seedlings that received (left to right): 5 min R at the end of day, 5 min FR at the end of day, 5 min FR after 4 h of darkness, or 5 min FR after 8 h of darkness (middle of night) following 8 h photosynthetic periods in a controlled environment. Photographed after 3 weeks of daily treatment.

in the field, indicating that a competition-sensing mechanism operated constantly and influenced the morphology of the developing plant part. The stem elongation and leaf shape patterns among close-spaced plants were similar to those of FR-treated plants in controlled environments. Plants photographed after 6 weeks of growth in close-spaced *vs* wide-spaced field populations are shown in Fig. 2. Note that the close-spaced plants developed narrower leaves, as well as stems that were longer and had smaller diameters. Comparison with controlled environment responses to FR (see Fig. 1 and Table 1) suggested that the close-spaced field plants may have received more FR and a higher FR/R ratio. Measurements of light spectra within, below and near tobacco canopies were initiated in 1967 with a 16-waveband portable spectroradiometer (designed by K. H. Norris of the Instrumentation Research Laboratory before I left Beltsville, and constructed by Agricultural Specialities Company, Hyattsville, MD)†. The approach was extended to other crops in 1983 with a new portable spectroradiometer (LiCor-1800, LiCor Inc, Lincoln, NE) capable of measuring at 2 nm intervals from 300 to 1100 nm.

Measurements with the fixed-band spectroradiometer. Light measurements with the 16-waveband spectroradiometer within and near growing tobacco canopies were compared with incoming sunlight at each measured waveband (Kasperbauer, 1971). That spectroradiometer could measure at only 11 wavebands in the visible, and at 725 and 791 nm in the FR-response region. Nevertheless, it provided information that became very useful in interpretation of field responses to competition from other plants. Most of the blue and R were absorbed by the leaves, but 27 and 49% were transmitted at 725 and 791 nm, respectively. However, those percentages differed considerably if the canopy FR values

Table 1. Physical and chemical responses of *Nicotiana tabacum* seedlings to 5 min FR (high FR/R ratio) or 5 min R (low FR/R ratio) at the end of each 8 h day in a controlled environment for 3 weeks. Adapted in part from Kasperbauer *et al.*, 1970; Kasperbauer, 1971; Kasperbauer and Peaslee, 1973)

	End-of-day light and FR/R ratio		
Characteristic	FR (High)	R (Low)	Sig.[a]
Physical			
Stem length (mm)	126	37	*
Leaves/plant (no.)	8.0	8.2	NS
Leaf length/width (ratio)	2.13	1.83	*
Leaf thickness (mg/cm^2)	18.9	24.7	*
Shoot fresh wt. (g)			
Stem	8.1	2.8	*
Leaf midvein	13.9	11.6	*
Leaf lamina	20.8	32.6	*
Root fresh wt. (g)	6.1	10.6	*
Total fresh wt. (g)	48.9	57.6	*
Shoot/root (ratio)	7.02	4.43	*
Chemical			
Chlorophyll *a*/*b* (ratio)	1.97	1.87	*
CO_2 uptake (mg/g fresh wt/h)	8.3	6.9	*
Free sugars (mg/g dry wt.)			
Stems	97.5	37.6	*
Leaf midveins	37.8	11.5	*
Leaf lamina	13.3	9.8	*

[a] Statistical significance: *, values in the same row differ at the 5% level; NS, values are not significantly different at the 5% level.

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† Mention of a trademark or product does not constitute a guarantee or warranty of the product by the US Department of Agriculture and does not imply its approval to the exclusion of other products or vendors that may also be suitable.

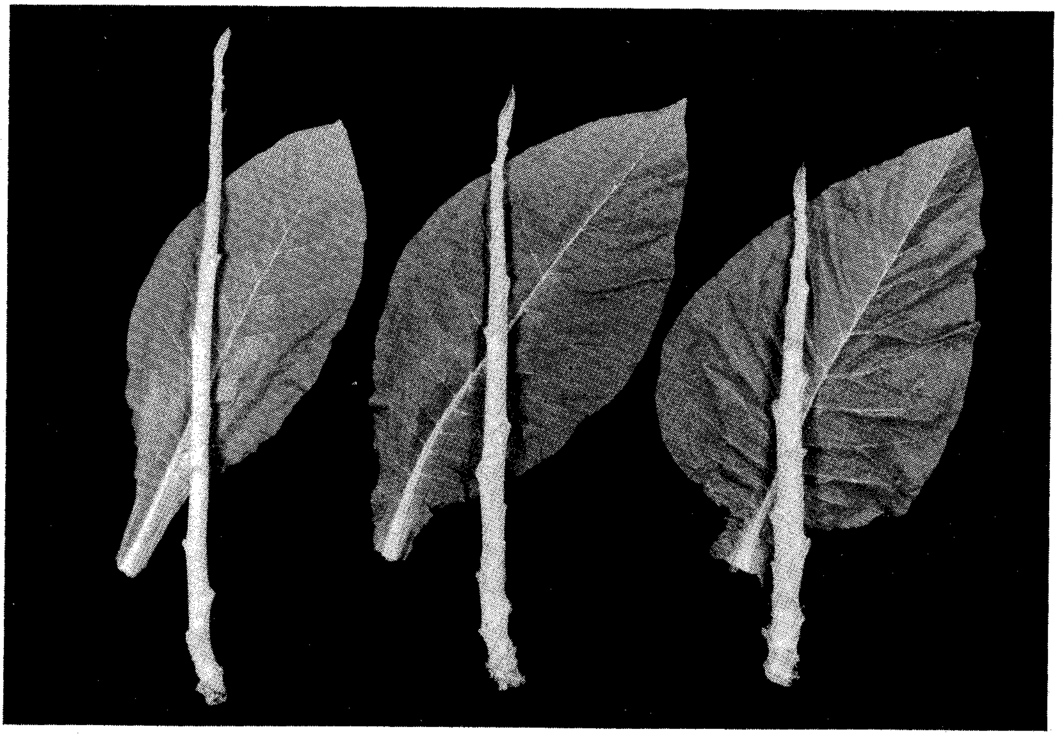

Figure 2. Stems and a representative leaf from *Nicotiana tabacum* seedlings 6 weeks after transplanting to close-, normal- and wide-spaced (left to right) populations in the field. Seedlings were started and grown to transplant size in a controlled environment that prevented floral induction (see Kasperbauer, 1973). Close-spaced seedlings were transplanted 30 cm apart in rows that were 30 cm apart, normal-spaced seedlings were 45 cm apart in rows that were 100 cm apart, and wide-spaced seedlings were 120 cm apart in rows that were 120 cm apart.

were compared with incoming sunlight measured on a roadway or with the light measured in sunflecks near growing tobacco plants. Also, when light received in sunflecks near the growing tobacco was compared with that received on a road away from tall plants, values in the sunflecks near the tobacco were about 15% greater at 791 nm. Values at 725 nm were also higher in sunflecks near the tobacco plants. The only reasonable explanation was that the higher values at 725 and 791 nm in sunflecks near plants were due to reflection from the plants. Upwardly reflected FR from more numerous leaves could also explain why the upper leaves of close-spaced tobacco plants were narrower (see Fig. 2), more upright and showed other FR responses even before mutual shading occurred. Among variously-spaced tobacco plants, the amount of FR was always higher as the detector was placed closer to growing plants. In subsequent experiments during the 1970s, it became quite apparent that reflected FR and the resultant increase in FR/R photon ratios associated with closer plant spacings was involved in regulation of photosynthate partitioning which influenced morphological development of the growing plant.

In field experiments conducted in South Carolina during the late 1970s and early 1980s, Hunt *et al.* (1985) noted that soybean plants grown on sandy soil had higher seed yields in north–south (N–S) rows when irrigated, but yields were higher in east–west (E–W) rows if there was occasional water stress (not irrigated). We hypothesized that such a response could occur if some environmental variable associated with N–S rows caused the plants to partition more biomass to shoots and less to roots (which might not be critical if there were no water stress). Since end-of-day FR (a high FR/R ratio) in controlled environments had caused seedlings to develop larger shoots and higher shoot/root biomass ratios (see Table 1), we measured the spectra of light received by soybean plants at various times of day in N–S *vs* E–W rows.

Measurements with the computerized spectroradiometer. The spectrum of light reflected from a typical, recently-expanded soybean leaf is shown in Fig. 3. Note that the FR reflection curve begins to plateau at about 750 nm, well beyond the *in-vitro* Pfr absorption peak of about 735 nm. It should also be recalled that Vogelmann and Bjorn (1984) found the measured amount of FR at about 750 nm was enhanced through photon scattering and internal reflection within fleshy leaves, and the earlier spectrographic (Kasperbauer *et al.*, 1963a) studies showed FR responsiveness to "prolonged" irradiations at 755 through 795 nm.

Spectroradiometric measurements during the summer of 1983 quickly confirmed effects of

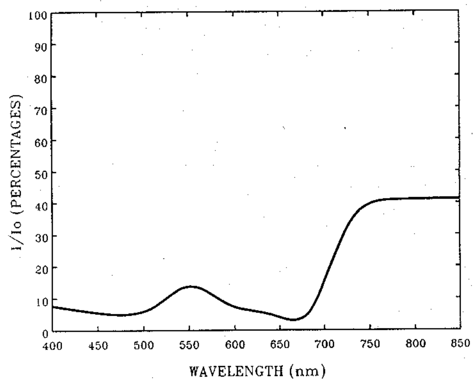

Figure 3. Spectrum of light reflected from the upper surface of a typical fully-expanded field-grown soybean leaf. The curve shows percentages of light reflected at each measured wavelength (at 5 nm intervals from 400 to 850 nm) relative to the quantity of incoming light at the same wavelengths. Measurements were made with a LiCor-1800-12 Integrating Sphere.

reflected FR on FR/R ratios and field growth patterns of soybean plants (Kasperbauer *et al.*, 1984; Kasperbauer, 1987). The amount of reflected FR and the FR/R ratio received by a plant were influenced by the nearness, number, size and directional orientation of leaves on competing plants. When light spectra were measured in N–S *vs* E–W rows numerous times during a day, the highest FR/R ratios were received by plants in N–S rows, especially near the beginning and end of the day. This was attributed to FR reflection from the heliotropic leaves such that each leaf became a directional FR reflector (Kasperbauer *et al.*, 1984). When 24 different scans were averaged, bean plants in both N–S and E–W row orientations received about the same amount of photosynthetically active light. However, plants in N–S rows received higher FR/R ratios than those in E–W rows (Table 2). The N–S plants grew taller and produced heavier shoots with greater fruit biomass per plant. In a similar experiment (unpublished) with southern pea (*Vigna unguiculata* L.), some seedlings in each row orientation were grown in irrigated soil in 10 cm (diam) × 45 cm tubes that were imbedded within the rows so that the tops of the tubes were at soil level. Within-row plant spacing was 5 cm in both row directions. The shoot/root biomass ratios were 3.07 and 2.75 for N–S and E–W rows, respectively, after about 3 weeks of growth. This response to FR/R ratio in the field was consistent with the earlier controlled environment experiments in which seedlings received a high FR/R ratio at the end of the day (Table 1). That is, seedlings that received higher FR/R ratios because of directional FR reflection from heliotropic leaves near the end of the day in the field responded essentially the same as those that received higher FR/R ratios in controlled environments, or because of nearness of competing plants.

Responses to light reflected from colored soils and painted panels

When it was apparent that field-grown plants respond morphologically to the amount of FR reflected from other green plants, we asked whether plants would also respond to spectral differences in light reflected from different colored soils and plant residues from a previous crop. This question was highly relevant to field crop production because agricultural soils vary widely in color, as do plant residues that are left on the soil surface in some conservation tillage procedures. Studies that were started at the Coastal Plains Soil and Water Conservation Research Center near Florence, SC in 1984 and 1985 revealed differences in spectra of upwardly reflected light 10 cm above different colored soils with and without a partial (about 80%) cover of dead plant residue (Kasperbauer and Hunt, 1987). This height above the soil was selected because it is in the seedling establishment zone, and green seedlings are extremely responsive to FR and the FR/R ratio (see Fig. 1 and Table 1). Reflection from brick-red soil was similar to that over black from 400 to 500 nm, and similar to that over white from 600 to 700 nm. Further, the red soil and straw residue reflected higher FR/R ratios than the other colors.

The next step was to determine whether soil surface color or plant residue could influence reflected light sufficiently to modify seedling growth. Soybean seeds were sown in 3 L pots of loamy sand on greenhouse benches. The pots were placed 60 cm apart in groups of four, and each group of four pots was covered with a 122 × 122 × 2 cm insulation panel with 2.5 cm holes centered over the emerging seedlings. The panels were covered with different colored soils, or with black soil covered with unweathered straw. Other insulation panels were painted to provide a range of reflected light spectra. This approach allowed evaluation of upward reflection from the different surface colors while minimizing differences in rhizosphere temperatures below the different surface colors. Soybean seedlings grown over bare brick-red soil and over the straw residue-covered soil received higher reflected FR/R light ratios than those grown over bare white soil; and they grew taller, had less massive root growth with fewer nitrogen-fixing nodules, and developed higher shoot/root biomass ratios (Hunt *et al.*, 1989). When the insulation panel surfaces were painted instead of being covered with different colored soils or plant residues, plants responded the same to either painted or soil-covered surfaces if they reflected the same spectrum of light.

Responses to light reflected from colored mulches in the field

Painted surfaces were used as reflecting surfaces for outdoor experiments in summer because soils

Table 2. Row orientation effects on the FR/R ratio and on plant characteristics of bush bean. Adapted from Kaul and Kasperbauer (1988)

	Row orientation		
Characteristic	N–S	E–W	Sig.[a]
Light[b]			
Photosynthetic (μmol m^{-2} s^{-1})	389	393	NS
Photomorphogenetic (FR/R ratio)	1.85	1.48	*
Green beans (g fresh wt./plant)	59	43	*

[a] Statistical significance: *, values in the same row differ at the 5% level; NS, values are not significantly different at the 5% level.

[b] Light coming to the shoot tips of two representative plants in each row orientation was measured from the north, south, east and west at 1100, 1330 and 1530 h on a cloudless day near Frankfort, KY. Each light value in the table is the mean for 24 individual measurements. Rows were 1 m apart and plants within rows were 10 cm apart.

Table 3. Characteristics of upwardly reflected light, shoot size and biomass distribution in plants grown in field plots over colored mulches in sunlight

	Mulch surface color		
Characteristic	Green	White	Orange
Upwardly reflected light* (relative to incoming sunlight)			
PPF (400–700 nm) (%)	11	44	21
Blue (400–500 nm) (%)	8	39	7
Red (645 nm) (%)	8	46	36
FR (735 nm) (%)	10	46	35
FR′ (755 nm) (%)	15	46	34
FR/R (ratio)	1.28	1.00	0.97
FR′/R (ratio)	1.83	1.00	0.95
Shoot crop† (soybean, seeded 24 June, measured 25 July)			
Stem length (mm)	226 a	193 b	199 b
Root crop† (radish, in field 18 April–12 May, 1988)			
Leaf wt. (g)	18.6 a	14.3 b	19.0 a
Root wt. (g)	65.9 b	54.6 c	79.6 a
Shoot/root (ratio)	0.282 a	0.262 b	0.239 c

*PPF, photosynthetic photon flux; FR/R, photon ratio at 735 nm relative to 645 nm; FR′/R, photon ratio at 755 nm relative to 645 nm. The FR/R and FR′/R ratios in upwardly reflected light are expressed relative to ratio in incoming sunlight, which was arbitrarily assigned a value of 1.00. Photon ratios were calculated before rounding off the mean values for R, FR and FR′.

†Values for both soybean and radish are means for 3 replicates of 20 plants each for each color of mulch. Radish seedlings were started in 5 cm (diam) pots of potting soil, selected for uniformity and transplanted to field plots at the two-leaf stage. Plants were spaced at least 30 cm apart. Trickle irrigation tubes were placed below the mulches to avoid water stress. Values in the same row followed by different letters differ at the 5% level of significance.

and plant residues would have been removed from the panels by wind and rain. This approach allowed growth of the plants outdoors in full summer sunlight for photosynthesis, as well as reflection of a FR/R ratio that acted through the phytochrome system to prioritize partitioning of the photosynthate among developing roots, shoots and fruits. The working hypothesis [based on many previous experiments that involved controlled environments (Fig. 1 and Table 1), reflection from other plants (Figs. 2 and 3), and upward reflection from colored soils and painted panels] for the use of colored mulches in the field was that an upwardly reflected FR/R ratio higher than the ratio in incoming sunlight would signal the growing plant to partition more of its new photosynthate to shoot (and fruit) growth, while a FR/R ratio lower than the ratio in incoming sunlight would favor partitioning to roots.

After observing the greenhouse experiments with colored panels, D. R. Decoteau (a horticulturalist) proposed that we test the concept with irrigated field-grown tomatoes (*Lycopersicon esculentum* L.) during the 1986 and 1987 seasons. Since black or white plastic mulches were widely used for soil and water conservation as well as for weed control in production of tomato and other high value food crops, we investigated the possibility that an altered surface color on the mulch could maintain those benefits and have an added favorable effect on plant productivity. Exterior paints applied to the surface of black plastic mulch provided an economical and effective method to obtain desired reflection spectra for small-plot field studies. This procedure allowed us to obtain desired reflection spectra and prevented weed growth below the mulches. Irrigated tomatoes grown in summer sunlight over mulches with red surfaces produced significantly greater fruit yield relative to those grown with conventional black or white mulches (Decoteau *et al.*, 1989). Similarly, field-grown irrigated cotton produced more bolls, fiber and seed per plant when grown over colors that reflected less blue and higher FR/R ratios (Kasperbauer and Hunt 1990). As hypothesized, the mulch surface colors that reflected FR/R ratios higher than the ratio in incoming sunlight resulted in plants that allocated more photoassimilate to shoots, including fruit.

Other experiments were conducted in field plots with many shoot and root crops over soil covers that were painted to provide a range of upwardly reflected FR/R ratios as well as differing quantities of blue and photosynthetically active light. Characteristics of upwardly reflected light and some field-grown plant responses are summarized in Table 3. Soybean seedlings developed longer stems over green and red surfaces, which reflected low amounts of blue and higher FR/R ratios than were present in incoming sunlight. On the other hand, the largest roots and the lowest shoot/root biomass ratio for the root crop (radish) developed over the orange mulch, which reflected a FR/R ratio lower than the ratio in incoming sunlight. All of these responses were as predicted from controlled environment experiments summarized in Table 1.

It may be of biological significance that seedlings grown over white surfaces received higher amounts of reflected photosynthetic light, but they developed shorter stems and accumulated the least total biomass (see radish, Table 3). This response was observed repeatedly with a number of shoot and root crops when grown outdoors in summer sunlight. Clearly, the greatest amount of photosynthetic light did not result in the greatest accumulation of biomass. One possible explanation is that the greater quantity of blue light reflected from the white surfaces might be able to affect the photosynthetic system of sun-grown plants. For example, cotton seedlings grown outdoors in summer sunlight over the white surfaces had lower concentrations of chlorophyll and light harvesting chlorophyll protein (LHC-II) (Bradburne *et al.*, 1989). Studies of CO_2 uptake, chloroplast structure and cytoplasmic streaming in seedlings grown in the field over different colored mulches are in progress.

CONCLUSIONS

Plants respond morphologically to reflected FR and FR/R ratio acting through the phytochrome system. Green leaves reflect FR and the amount of reflected FR received by a growing plant depends on the number, nearness, size and directional orientation of leaves on competing plants. Plants sense a higher FR/R ratio as an indicator of competition and they respond by allocating more photosynthate to the development of longer stems, leaving less for new root growth. That is, an increased FR/R ratio results in an increased shoot/root biomass ratio. The FR/R ratio reflected upward from different colored soils or soil covers (mulches) can also affect partitioning and morphological development. Mulches are frequently used to control weeds and conserve soil and water in production of high value crops. Use of alternate surface colors on mulches offers the potential to reflect pre-selected FR/R ratios that result in phytochrome regulation of quantity and quality of plant products in the field at little added cost to the grower.

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