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Ultraviolet-B Radiation-induced Inhibition of Leaf Expansion and Promotion of Anthocyanin Production

LACK OF INVOLVEMENT OF THE LOW IRRADIANCE PHYTOCHROME SYSTEM[1]

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ABSTRACT

Leaf discs from expanding leaves of *Rumex patientia* L. were exposed to 7 hours of visible plus different levels of ultraviolet radiation in the 290 to 315 nm waveband (UV-B) and then placed in darkness. Leaf disc expansion was reduced and anthocyanin production was increased in discs exposed to moderate or high levels of UV-B radiation when compared to control discs. The possibility that the inhibition of leaf expansion by UV-B radiation might be at least partially phytochrome-mediated was examined by giving discs brief red or far red irradiation following exposure to UV-B radiation. Brief red radiation (R) following treatment with moderate or high UV-B radiation did not alter the pattern of growth or anthocyanin production compared to discs placed in darkness following UV-B treatment. However, a posttreatment with far red radiation (FR) reduced the growth of discs subjected previously to either moderate UV-B or no UV-B irradiation to the level of growth of discs given high UV-B. FR posttreatment also decreased anthocyanin production in discs in moderate and high UV-B treatments. Effects of FR and UV-B radiation apparently do not involve the same mechanism. This was demonstrated by experiments in which FR following the UV-B treatments was in turn followed by R, which reversed the effects of the FR but did not alter the growth inhibition or increased anthocyanin production induced by moderate or high levels of UV-B radiation.

Recent work has shown that enhanced UV-B (280–320 nm) radiation corresponding to global solar UV irradiance which would occur with a substantial atmospheric ozone reduction resulted in inhibition of photosynthesis and leaf growth (23). The effect on photosynthesis was cumulative, but the inhibition of leaf expansion was most pronounced during the first days of leaf expansion. Fully expanded leaf dimensions were also less than those of control plants. Even UV-B irradiation corresponding to present-day levels of solar radiation was sufficient to effect some inhibition of leaf growth (24).

The mechanism by which UV-B radiation inhibits leaf expansion is not known. It appears that inhibition of expansion is not entirely due to reduction of photosynthetic rates (23). It is important to elucidate the physiological mechanisms involved in UV-B radiation inhibition of leaf expansion and how these may interact with mechanisms known to control leaf expansion. One mechanism operating in the control of leaf expansion is the low irradiance phytochrome system. Brief R[2] promotes expansion (18), and this can be reversed by brief FR (14), which can be given 8 to 48 hr after the R, showing persistent photoreversibility in control of leaf growth (4, 13). Growth, as measured by fresh weight increases, of bean seedlings grown in light can be depressed by exposure to brief FR (13).

Butler *et al.* (2) showed that monochromatic radiation in the near UV (down to 315 nm) was effective in transforming Pr and Pfr *in vitro* but concluded that this waveband had little significance in phytochrome-controlled responses in nature due to screening of near UV radiation by pigments such as carotenoids and because longer wavelength radiation was more effective in phototransformations. Like most proteins, however, both the Pr and Pfr forms of phytochrome absorb effectively in the UV-B waveband with a peak at 280 nm (21). Pratt and Butler (19) reported that energy from 280 nm radiation absorbed by the protein component of purified phytochrome was transferred to the chromophore and was effective in photoconversion. Therefore, an increase in UV-B radiation might affect the photostationary state of phytochrome *in vivo* and thus at least partially alter the expression of leaf expansion.

We have explored the possibility that inhibition of leaf expansion induced by UV-B radiation might be at least partially phytochrome-mediated by giving R or FR to *Rumex* leaf discs following irradiation with various levels of UV-B. We also examined the effect of such treatments on anthocyanin content in the leaf discs, since anthocyanin content may be controlled by the low irradiance phytochrome system (16) and UV radiation can also stimulate the formation of anthocyanins (17). Our study indicates that UV-B radiation and the low irradiance phytochrome system act independently on leaf expansion and anthocyanin formation.

MATERIALS AND METHODS

Plant Growth. *Rumex patientia* L. seeds were germinated in Petri dishes, and after 2 weeks the seedlings were planted in trays containing vermiculite and ¼ strength Hoagland solution. The Hoagland solution was changed every 6th day and the plants watered with ¼ strength Hoagland every 3rd day. The plants were grown in a controlled environment chamber equipped with a 6,000-w Osram Co. xenon arc lamp covered with a Mylar type A plastic film (5 mil). The UV spectral irradiance of the chamber was the same as the spectral irradiance of the no UV-B treatment illustrated in Figure 1. The chamber temperature regime was on a daily cycle with a maximum of 30 C and a minimum of 13 C.

Leaf Disc Assay. A cork borer (10 mm diam) was used to punch discs along the margin of the bottom half of expanding *Rumex* leaves (approximately 3 to 6 days after the leaf first emerged from the sheath). The discs were floated for 30 min on a solution of 25 units/ml nystatin, 0.25 mg/ml carbenicillin (Pyopen), and 50 μg/ml streptomycin sulfate and then were placed in

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[2] Abbreviations: R: red radiation; FR: far red radiation; UV-B_{BE}: biologically effective UV-B radiation; HIR: high irradiance reaction.

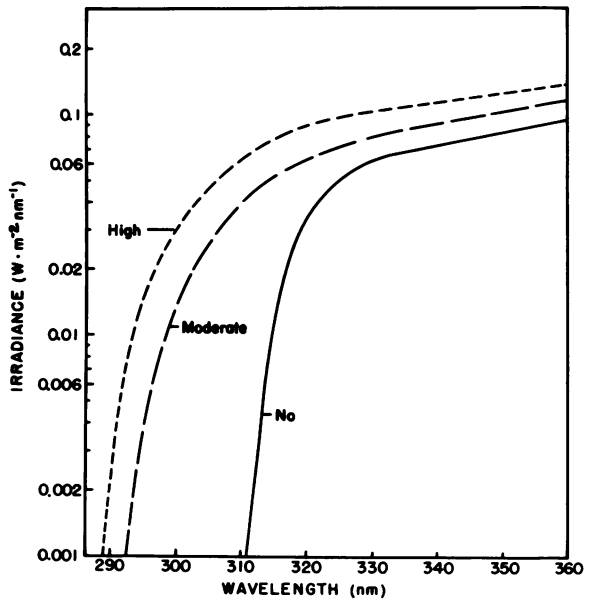

FIG. 1. Spectral irradiance for the three UV-B treatments. The growth chamber was equipped with a xenon arc lamp enclosed in 2-mm Schott glass filters and three FS-40 "sunlamps" covered with one layer of Kodacel TA-401 (5 mil) plastic film. No extra filters were used in the high UV-B treatment. The moderate UV-B treatment was achieved with two layers of Kodacel and the no UV-B treatment with one layer of Mylar type A (5 mil) plastic film.

9-cm Petri dishes with three pieces of Whatman No. 1 filter paper and 20 ml of a 2% sucrose and ½ strength Hoagland solution containing 10 units/ml nystatin, 0.05 mg/ml carbenicillin, and 0.01 mg/ml streptomycin sulfate. Generally 16 discs were used per dish. The uncovered Petri dishes were placed in a growth chamber, and the discs were exposed to visible and UV-B radiation for 7 hr. The chamber was kept at 24 C with a relative humidity of 55 to 75%. Three ml of water were added to each dish 2.5 and 4.5 hr after the treatment had begun. The chamber was equipped with a 6,000-w xenon arc lamp enclosed in 2-mm-thick Schott glass filters, and three Westinghouse FS-40 "sunlamps" arranged in a frame below the xenon burner and covered with one layer of Kodacel TA-401 (5 mil) plastic film which had been aged for 2 hr on the FS-40 lamps. A general description of the characteristics of the lamp and filter systems is found in Sisson and Caldwell (22). All of the dishes received approximately the same intensity of visible irradition (600–650 $\mu E \cdot m^{-2} \cdot sec^{-1}$ at 400–700 nm) but were exposed to three different UV-B irradiation levels (high, moderate, and no UV-B) by being placed in wire frames covered with various plastic filters. A high level of UV-B radiation (approximately 2,450 $J \cdot m^{-2}$ of biologically effective radiation, $UV\text{-}B_{BE}$, over 7 hr) was achieved by placing the Petri dishes in a frame with no filter. (The weighting for biological effectiveness is taken from a relationship recognized by Caldwell [3] and roughly follows the UV-B action spectra of effects mediated by nucleic acids and proteins. $UV\text{-}B_{BE}$ is calculated by multiplying the spectral irradiance by the relative biological effectiveness at each wavelength and integrating over the UV-B waveband.) The moderate UV-B treatment was a frame covered with two layers of Kodacel which transmitted about 975 $J \cdot m^{-2}$ of $UV\text{-}B_{BE}$ radiation over 7 hr, and the no UV-B treatment was a frame with one layer of 5-mil Mylar which transmitted virtually no $UV\text{-}B_{BE}$ radiation. The spectral irradiance of these regimes is illustrated in Figure 1.

At the end of the 7-hr irradiation period, the Petri dishes were covered and in some cases exposed to short periods of FR and/or R and then placed in the dark at 25 C. For time course experiments the diameters of the discs were measured at 32, 48, or 64 hr from the beginning of UV-B irradiation. In all other experiments the discs were measured at 49 to 51 hr after the treatment was first begun.

R and FR Light Sources. R light was obtained by filtering the radiation from four pink Sylvania F20T12 · PK fluorescent bulbs through one 3.2-mm-thick sheet of red Plexiglas (Rohm and Haas No. 2423) and 2 cm of 0.06 M $CuSO_4$ solution. FR light was obtained by filtering light from four 150-w Sylvania flood lamps through one 3.2-mm sheet of blue Plexiglas (Rohm and Haas No. 2424) plus one 3.2-mm sheet of amber Plexiglas (Rohm and Haas No. 2422) plus 6 to 8 cm of water. The spectral irradiance from these two light sources at the level of the irradiated leaf material is shown in Figure 2. Preliminary tests demonstrated that the R and FR exposure times used in the experiments were sufficient to affect leaf disc expansion, and that growth of discs exposed to R/FR or FR/R exhibited typical phytochrome-mediated reversibility.

Measurements of spectral irradiance of all radiation sources were conducted with a Gamma Scientific Co. spectroradiometer.

Anthocyanin Measurements. Following the diameter measurements, the discs were frozen, lyophilized, and the dry weight taken. Then the discs were extracted for anthocyanins following the methods of Mancinelli *et al.* (15). The dried discs were placed in a vial with 10 ml of methanol-water-concentrated HCl (80:20:1) and put on a shaker in the dark at 2 C. At the end of 48 hr the extract was filtered through Whatman No. 1 filter paper, taken to 12 ml, and the A was read at 530 and 657 nm. Anthocyanin concentration was determined by the formula [A] = A 530 − 1/3 A 657 and was given as A/g dry weight of leaf tissue. Using the methods of Harborne (5, 6) the major anthocyaninidin in the extracts was determined to be cyanidin.

Experiments were repeated at least three times.

RESULTS

Characteristics of Young Leaf Disc Growth and Anthocyanin Production. The growth of leaf discs in darkness following UV-B irradiation at three levels is shown in Figure 3. Significant differences ($P < 0.05$) in expansion between the high and no UV-B treatments were apparent 32 hr after the experiment had begun, and these differences persisted to the conclusion of the experiments at 64 hr. The growth of the discs exposed to high or moderate

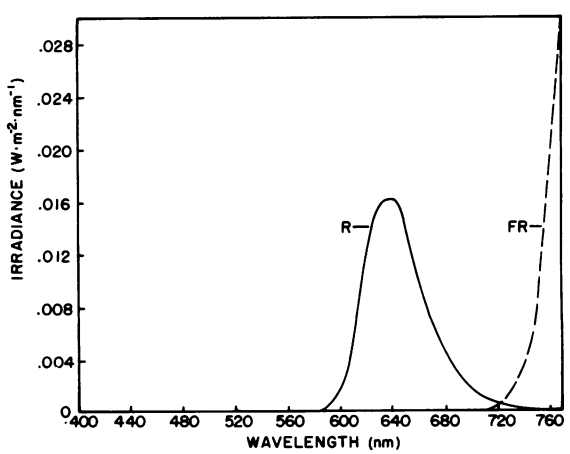

FIG. 2. Spectral irradiance from the R and FR light sources. The R light source consisted of radiation from four pink fluorescent bulbs filtered through one 3.2-mm-thick sheet of red Plexiglas and 2 cm of 0.06 M $CuSO_4$ solution. The FR light source consisted of radiation from four 150-w flood lamps filtered through one 3.2-mm-thick sheet of blue Plexiglas, one 3.2-mm-thick sheet of amber Plexiglas and 6 cm of water.

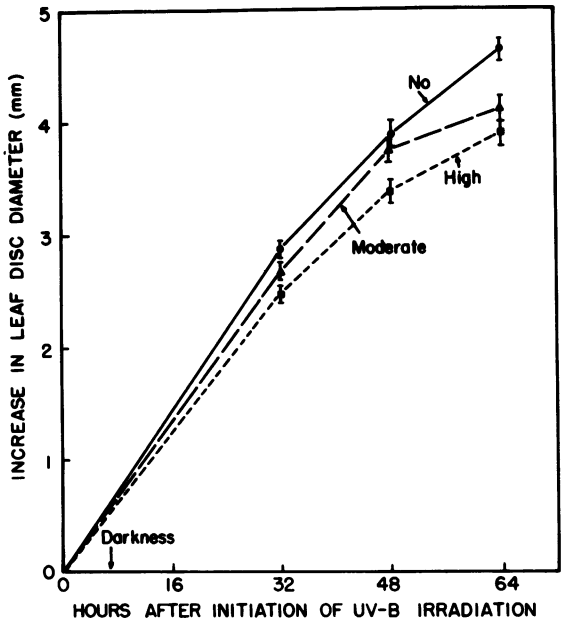

FIG. 3. Time course of increase in leaf disc diameter in darkness at 25 C following 7 hr visible plus no, moderate (975 J · m^{-2}UV-B_{BE}), or high (2,450 J · m^{-2}UV-B_{BE}) UV-B irradiation treatments. Vertical bars represent ± 1 SE of the mean.

Table I. Anthocyanin concentrations in leaf discs 32, 48, and 64 hr after initiation of experiment.

Average concentrations ± standard deviations are given as A/g dry weight. Discs were treated with 7 hr visible plus one of the three levels of biologically effective UV-B radiation and then placed in darkness at 25 C. Means judged significantly different (Student's t test, $P < 0.05$) are given different letters within each time category.

UV-B_{BE} Dose ($J \cdot m^{-2}$)	Time (hr after experiment initiated) 32	48	64
0	1.33 ± 0.29^a	1.18 ± 0.25^a	1.21 ± 0.34^a
975	2.09 ± 0.36^b	1.69 ± 0.24^b	1.60 ± 0.36^a
2450	3.14 ± 0.65^c	2.57 ± 0.51^c	2.51 ± 0.45^b

UV-B radiation appeared always to remain at a lesser rate than the expansion rate of discs exposed to no UV-B radiation.

Anthocyanin concentration in the leaf tissue was also responsive to UV-B radiation (Table I). From 32 to 64 hr after beginning of exposure, anthocyanin concentration was significantly greater ($P < 0.05$) in discs exposed to high UV-B than in discs exposed to moderate UV-B, and anthocyanin concentration in discs exposed to moderate UV-B was significantly greater than in discs exposed to no UV-B at 32 and 48 hr. The highest concentration of anthocyanin in the leaf tissue following UV-B exposure was reached at or before 32 hr.

Response to R and FR Treatments Following UV-B Exposure. To test the hypothesis that phytochrome-mediated signals may modify UV-B inhibition of leaf disc expansion or UV-B stimulation of anthocyanin production, leaf discs were given R or FR following exposure to different levels of UV-B irradiation. Ten to 15 min of R following exposure of discs to any of the three UV-B treatments did not significantly alter the pattern of growth from discs which received only darkness following exposure to UV-B (dark controls) (Fig. 4). Forty min of FR, however, significantly ($P < 0.05$) depressed expansion in leaf discs treated first with 7 hr moderate or no UV-B compared to moderate or no UV-B dark controls, but leaf discs given high UV-B and then FR were not significantly different from high UV-B/dark controls. In fact, discs from all three UV-B treatments exhibited about the same growth following irradiation with FR, and this growth was about the same as growth of discs given high UV-B followed by darkness or R.

Anthocyanin concentrations were also responsive to FR treatment. The action of FR in this process was to suppress the effects of the high and moderate UV-B treatments (Fig. 5). Anthocyanin levels of discs treated with UV-B/R were not significantly different from levels of UV/dark controls at all three UV-B levels.

The results of the R and FR treatments on leaf disc expansion suggested that FR might either be duplicating the effects of the highest level of UV-B radiation or that it might be acting inde-

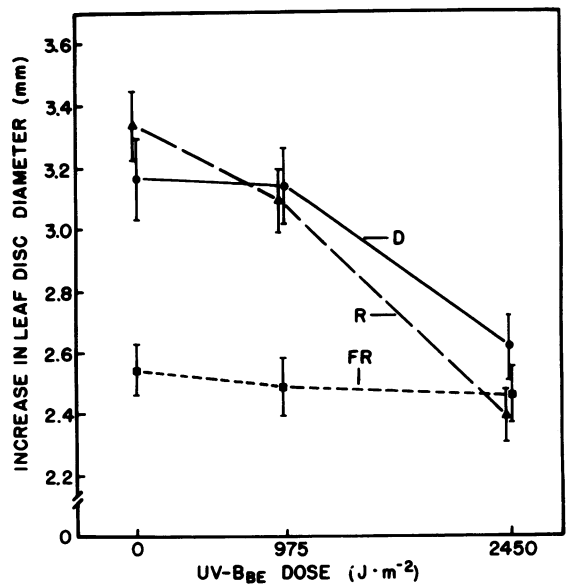

FIG. 4. Increase in leaf disc diameter of discs treated with R, FR, or darkness (D) following 7 hr visible plus 0, 975, or 2,450 J · m^{-2}UV-B_{BE} radiation. The UV-B-irradiated discs were either immediately placed in darkness at 25 C for 42 to 44 hr or were given 10 to 15 min R or 40 min FR and then placed in darkness. Vertical bars represent ± 1 SE of the mean.

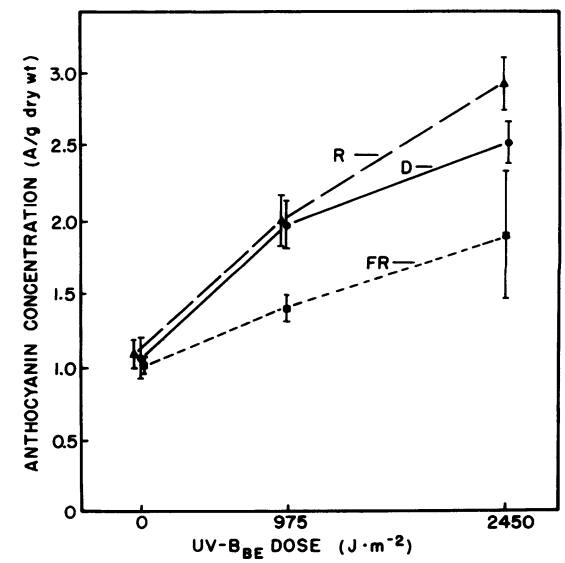

FIG. 5. Anthocyanin production in leaf discs treated with R, FR, or darkness (D) following 7 hr visible plus 0, 975, or 2,450 J · m^{-2}UV-B_{BE} radiation. The UV-B-irradiated discs were either immediately placed in darkness at 25 C for 42 to 44 hr or given 10 to 15 min R or 40 min FR and then placed in darkness. Vertical bars represent ± 1 SE of the mean.

pendently of U-B to depress expansion. Since growth was already depressed in the high UV-B radiation treatment, these discs might not show a change in expansion from discs exposed to high UV-B followed by darkness. If FR was acting on the phytochrome system independently of UV-B radiation, then FR followed by R should reestablish the different levels of expansion in discs exposed to different UV-B treatments. This was tested in experiments in which 20 to 30 min R, 40 min FR, or 40 min FR followed by 20 to 30 min R was given subsequent to exposure of the discs to the three UV-B treatments. The results are shown in Figure 6. Growth of discs given moderate or no UV-B radiation followed by R was significantly greater ($P < 0.05$) than growth of discs given the same UV-B treatment followed by FR but was not significantly different from the growth of discs given UV-B/FR/R. Growth of the UV-B/FR/R discs was restored to nearly the same level as the growth of the UV-B/R discs. R was also able to reverse the effect of FR on anthocyanin content (Fig. 7). There was no significant difference between anthocyanin concentrations in R-treated discs and FR/R-treated discs at all three UV-B irradiation levels.

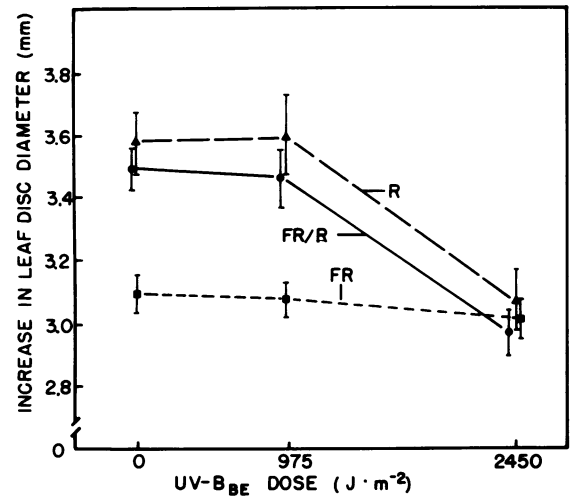

FIG. 6. Increase in leaf disc diameter of discs treated with R, FR, or FR/R following 7 hr visible plus 0, 975, or 2,450 J · m $^{-2}$UV-B$_{BE}$ radiation. The UV-B-irradiated discs were given 20 to 30 min R, 40 min FR, or 40 min FR followed by 20 to 30 min R (FR/R) radiation and then placed in darkness at 25 C for 42 to 44 hr. Vertical bars represent ± 1 SE of the mean.

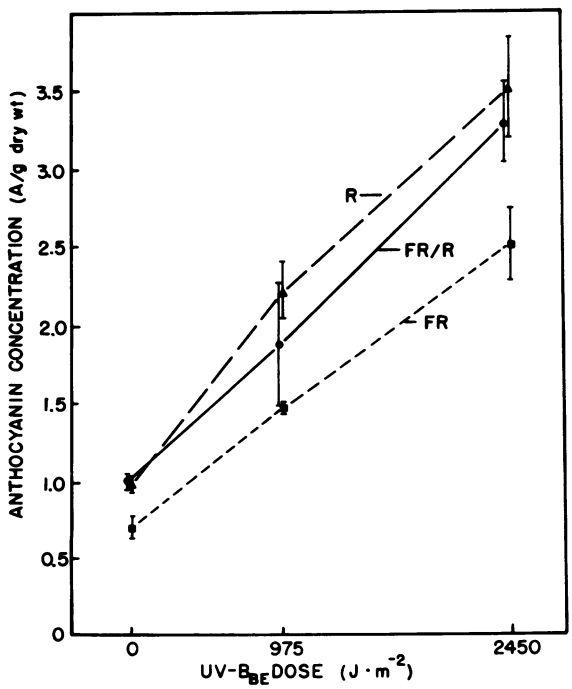

FIG. 7. Anthocyanin production in leaf discs treated with R, FR, or FR/R following 7 hr visible plus 0, 975, or 2,450 J · m^{-2}UV-B$_{BE}$ radiation. The UV-B-irradiated discs were given 20 to 30 min R, 40 min FR, or 40 min FR followed by 20 to 30 min R (FR/R) radiation and then placed in darkness at 25 C for 42 to 44 hr. Vertical bars represent ± 1 SE of the mean.

DISCUSSION

Both the low irradiance phytochrome system and enhanced levels of UV-B radiation have been shown to affect leaf disc expansion and anthocyanin concentration. It is possible to demonstrate that the two apparently operate independently through experiments in which R following FR caused reversal of the effects of FR but did not reverse the effects of high or moderate levels of UV-B radiation. The experiments performed here were concerned with an interaction between UV-B radiation and the low irradiance phytochrome system, and thus relatively short term, low irradiance doses of R and FR were used in an attempt to modify the UV-B treatments. More work is necessary to examine the possibility of UV-B irradiation acting on HIR.

Blue and UV radiations are often effective in HIR. The action spectrum for control of lettuce seedling hypocotyl lengthening has several peaks in the UV and blue as well as one near 720 nm (7). Hartmann suggests that phytochrome may be the photoreceptor for the UV-blue wavelengths as well as the FR, since short wavelength radiation will maintain photostationary states (7), and in three HIR-mediated processes the responses to UV-blue wavelengths can be overcome by simultaneous irradiation with black-red (760–800 nm) (8, 9). Several workers have presented evidence indicating that the blue photoreceptor is more likely to be a flavin or a carotenoid (1). Steiner (25) suggests that in a polarotropic response of liverwort, R acts through the phytochrome system and is able to modify the action of near UV radiation even though phytochrome may not be the photoreceptor for near UV. Regardless of what the UV-blue photoreceptor is for the HIR, studies using blue radiation to explore an effect of UV-B radiation treatments on the HIR would be complicated by the fact that blue radiation is effective in photoreactivation of UV radiation-induced DNA damage (12). Furthermore, long term, high energy blue radiation may affect repair and growth processes in plant tissues through increased photosynthesis.

Two other lines of research have suggested an effect of UV radiation on the phytochrome system, although neither duplicates the conditions being examined in this work. Chloroplast movement in the alga, *Mougeotia,* is controlled by the phytochrome system. In the low irradiance-controlled movement, the normal reversal of R by subsequent FR could be nearly completely prevented if near UV (370 nm) was given between the R and FR. If the sequence was UV/R/FR then the UV had no effect and FR was able to reverse the response to R (11). This phenomenon has been called UV stabilization and has been found in some higher plant processes controlled by low irradiance phytochrome system (10, 20). The UV-B treatments used in the present work (given simultaneously with visible radiation) appeared to have no effect on the ability of subsequent FR to inhibit leaf expansion and anthocyanin accumulation.

Wellman (26) has reported on an interaction between UV radiation and phytochrome in control of flavonoid synthesis. Flavone glycoside synthesis in parsley cell suspension cultures was stimulated by UV radiation (280–345 nm), and this was enhanced by subsequent irradiation with continuous FR. Further, the FR effect was reversed by R following FR, and neither R nor FR had an effect without previous UV irradiation. In the

experiments performed in the present study the effects of R or FR did not depend on pretreatment with UV-B.

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