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Evidence for Involvement of Phytochrome in Tumor Development on Plants[1]

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ABSTRACT

The regulation of nonpathogenic tumorous growths on tomato plants by red and far-red radiation was studied using leaf discs floated on water and irradiated from beneath. It was found that red light (600–700 nanometers) was required for the induction of tumors on tomato (*Lycopersicon hirsutum* Humb. & Bonpl. Plant Introduction LA 1625), while both blue (400–500 nanometers) and green (500–600 nanometers) light had little effect on tumor development. Detailed studies with red light demonstrated that tumor development increased with increasing photon flux and duration, though duration was the more significant factor. It was observed that tumor development could be prevented by the addition of far-red irradiance to red irradiance or by providing far-red irradiance immediately following red irradiance. The effectiveness of red and far-red irradiance in the regulation of tumor development indicates phytochrome involvement in this response. These findings should provide additional insight into the multiplicity of physiological factors regulating the development of nonpathogenic tumorous growths in plants.

Abnormal plant cell enlargement and division, resulting in growths commonly referred to as galls, tumors, or neoplasms, can be triggered by a number of factors. Many of these growths are induced by the action of pests such as insects, mites, and nematodes, or by pathogenic organisms, such as bacteria, fungi, or viruses (13). Others arise in the absence of inducing organisms. These latter are generically referred to as nonpathogenic or spontaneous tumors. Nonpathogenic tumors occur on a number of species, and exhibit a large degree of cultivar sensitivity. The best known examples of nonpathogenic tumorous growths are the 'genetic' tumors of *Nicotiana* (12), 'neoplastic pods' of peas (2), and edema (intumescence injury) which is prevalent in many Solanaceous species (10). The relationship between these various tumor types is not well understood, but it is suspected that most result from a similar underlying mechanism. Studies suggest that the potential for tumor development is inherited as a dominant genetic trait and results from aberrant regulation of plant growth substances, likely auxins and cytokinins (2). Nonpathogenic tumor development on the leaves of a tomato plant is shown in Figure 1.

It has been found that nonpathogenic tumorous growths are controlled by such external environmental factors as humidity and irradiance (11). These growths are often referred to as 'edema' since their occurrence has traditionally been attributed to water congestion under conditions of high humidity and reduced transpiration. However, previous work done in this laboratory (11) indicates that water congestion does not induce neoplastic growth, but makes tumor development only more pronounced. At present, the spectral quality of irradiation appears to be a primary factor in the regulation of nonpathogenic tumorous growths. Radiation in the near UV region of the electromagnetic spectrum has been demonstrated to inhibit or prevent the occurrence of these growths (11, 17). Because many glass and plastic greenhouse coverings and plastic lamp barriers in growth chambers absorb UV-B wavelengths (18), these environments are particularly conducive to tumor development on susceptible species. Visible wavebands also have been implicated in the regulation of tumor development. During some early work on *Hibiscus* (3), it was observed that intumescences would develop on plants grown under red 'glasses,' but not under blue, yellow or green 'glasses.' Other investigators have also alluded to this response (4), but careful studies of visible radiation effects on tumor development have not been reported. The purpose of this investigation was to define the wavelengths of visible irradiance responsible for regulating tumor development and, if possible, elucidate the mechanisms involved.

MATERIALS AND METHODS

Plant Material. The plant material used in these investigations was a species of wild tomato (*Lycopersicon hirsutum* Humb. & Bonpl. Plant Introduction LA 1625) known to be susceptible to tumor development (Fig. 1). Plantlets, from stem cuttings obtained from sterile shoot culture, were transplanted into a peat vermiculite medium and maintained in a growth chamber for experimental use. Irradiation was provided by CWF[2] lamps at a photosynthetic photon flux of approximately 300 μmol $m^{-2}s^{-1}$ for a 16 h photoperiod. The chamber did not have a lamp barrier so that plants received sufficient UV-B radiation from the CWF lamps to prevent tumor development. Day and night temperatures were maintained at 22±2°C, relative humidity was maintained at 60±10%, and plants were watered to excess with nutrient solution (6) four times daily using an automatic watering system.

Experiments were carried out using a procedure to induce tumors on leaf discs (16). This procedure involved floating the discs adaxial surface down in small vessels for 3 d. Irradiation was provided from beneath the vessels and UV absorbing Plexiglas was placed between the vessels and the irradiance source to allow injury development. Injury levels on leaf discs were scored at the end of each experiment using a scale from 0 to 100%, with the rating corresponding to the proportion of leaf area covered by tumorous growths.

Irradiation. *Spectra.* A number of different radiation spectra were used in this study, each obtained with different lamp and filter combinations (Fig. 2). Filters were placed under each

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[2] Abbreviations: CWF, cool white fluorescent; PPE, phytochrome photoequilibrium.

exposure vessel since the vessels were irradiated from beneath. Two different red spectra were developed for this study, one (RED-A) was provided with CWF lamps (F20T12/CW) and the other (RED-B) with red fluorescent (RF) lamps (Sylvania No. 236). Both red spectra were generated by filtering the lamps with a No. 15 Roscolux filter (Musson Theatrical Inc., Santa Clara, CA) and a 15 mil red acetate sheet (Trancil Wrap, North Lake, IL) to exclude most wavelengths below 600 nm. As seen in Figure 2, the RF lamps (RED-B) provided a bandwidth more confined to the red region than that obtained with the CWF (RED-A) source. The far-red spectrum (FAR-RED) was generated by using far-red fluorescent (FRF) lamps (Sylvania No. 232). Because some blue and green wavebands were present in the FRF lamps, the same combination of filter materials used with both red sources was utilized. The blue spectrum was generated with CWF lamps filtered with a combination of a No. 85 Roscolux filter and a 15 mil blue acetate sheet (BLUE) (Trancil Wrap, North Lake, IL). The green spectrum (GREEN) used was provided by CWF lamps filtered with No. 15 and No. 86A Roscolux filters, and a 15 mil blue acetate filter.

FIG. 1. Nonpathogenic tumor development on leaves of a tomato (*Lycopersicon hirsutum*) plant.

Measurement. Measurement of the radiation spectra shown in Figure 2 were carried out using a LiCor LI-1800 spectroradiometer. All radiation measurements were made at the disc surface and expressed as μmol $m^{-2}s^{-1}nm^{-1}$. Phytochrome photoequilibria were calculated for some of the irradiance treatments used in this investigation to obtain an estimate of the photostationary equilibrium of phytochrome (as Pfr/Ptot) under a particular irradiance spectrum. These values were determined using the procedure and relative quantum efficiences described by Gardner and Graceffo (5).

Treatments. *Comparison of Red, Blue, and Green Irradiation.* Three different broad wavebands were compared in this study to determine their effectiveness in the induction of tumor development. Treatments consisted of the BLUE, GREEN, and RED-A spectra, and unfiltered CWF lamps (control). PPF in each treatment was maintained at 25 ± 2 μmol $m^{-2}s^{-1}$. Though tumor development did occur if leaf discs were irradiated for approximately 20 h and then placed in the dark for an additional 52 h, irradiance was maintained continuously for the entire 72-h period to obtain more consistent injury levels. The experiment consisted of three replicates of each treatment in separate dishes,

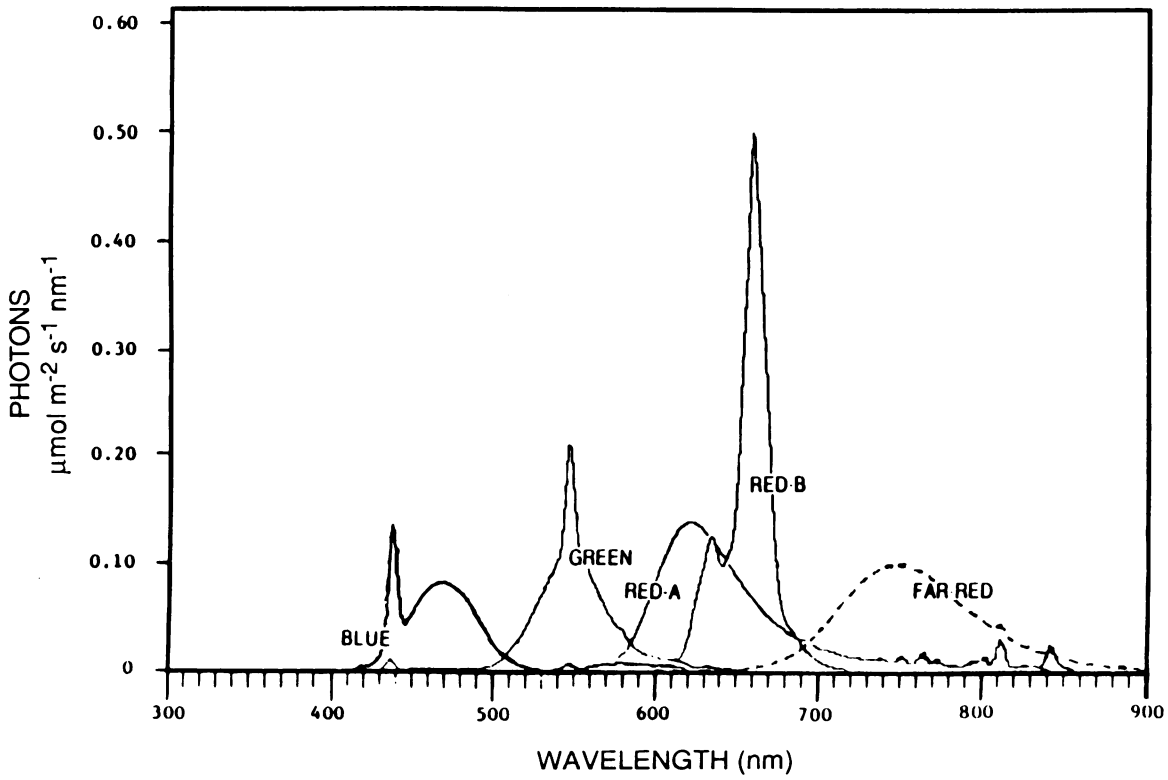

FIG. 2. Irradiance spectra developed for these investigations. The filters and lamps used for each spectrum are detailed in the text.

with four discs per replicate. Treatments and replicates were arranged in a randomized block design.

Quantification of Red Irradiance Effect. To determine the quantitative effect of red radiation on tumor development, leaf discs were irradiated continuously for 72 h using the RED-B spectrum at a photosynthetic photon flux of 2.5, 5.0, 10.0, 20.0, 40.0, or 80.0 μmol $m^{-2}s^{-1}$. Each treatment consisted of eight discs per vessel and was replicated three times.

To determine if tumor induction by red radiation exhibited reciprocity, a second experiment was undertaken with irradiance (RED-B) given for only a portion of each hour over the treatment period of 60 h. Discs were exposed to PPFs of either 10, 30, or 90 μmol $m^{-2}s^{-1}$ for durations of 5, 15, or 45 min out of each hour. Each treatment consisted of eight discs in one vessel, and treatments were replicated by repeating the experiment three times.

Interaction of Red and Far-red Irradiance. When red radiation was found to induce tumor development while blue did not, it was suspected that the red photoreceptor, phytochrome, was involved. Therefore, studies were undertaken to investigate the effects of red and far-red radiation interactions on tumor development. One study involved the addition of increasing far-red irradiance to a background red exposure (RED-A) of 15 ± 3 μmol $m^{-2}s^{-1}$. Far-red levels were controlled by shading the far-red lamps to varying degrees with a neutral density screen of cotton muslin. A series of six separate experiments were undertaken, each consisting of four different treatments. Experiments were run for 72 h. In each experiment, three of the treatments consisted of various elevated levels of far-red radiation, and the other treatment (designated as the control) was common to each experiment and consisted of RED-A irradiance provided by CWF lamps. The RED-A spectrum contained a low level (approximately 3 μmol $m^{-2}s^{-1}$) of far-red (700–800 nm) irradiance in addition to the 15 μmol $m^{-2}s^{-1}$ of red irradiance. Because there was some overlap between the red and far-red spectra used in this study (Fig. 2), it was found desirable to express each irradiation treatment as a phytochrome photoequilibrium value. The injury values obtained in each experiment were standardized based on the deviation between the control value for each treatment and the average control value for all experiments.

A second study involved successive sequential exposures to RED-B and far-red irradiance during the 60 h treatment period. All treatments received 80 μmol $m^{-2}s^{-1}$ of red irradiance for 15 min each hour, and this was then followed by one of the following exposures; (a) 45 min of darkness, (b) 5 min of FR at 10 μmol $m^{-2}s^{-1}$ and 40 min of darkness, or (c) 5 min of FR at 10 μmol $m^{-2}s^{-1}$, 5 min of red at 10 μmol $m^{-2}s^{-1}$, and 35 min of darkness. The dosage used for the short term red and far red exposures (5 min at a photon flux of 10 μmol $m^{-2}s^{-1}$) was selected to ensure maximum effect and to simplify experimental setup. However, preliminary studies indicated that total reversal could be obtained at durations and PPFs significantly less than those used. Preliminary studies also indicated that with only 15 min of red irradiation provided during each hourly cycle, the initial PPF had to be near 80 μmol $m^{-2}s^{-1}$ to obtain moderate levels of injury. Each treatment consisted of three replicates in separate dishes, with 8 discs per replicate. Treatments and replicates were arranged in a randomized block design. The experiment was carried out twice and the data averaged.

RESULTS

Comparisons made between blue, green, and red wavebands demonstrated that red light promoted intumescence development while blue and green light appeared to have little or no effect (Table I). Discs receiving red light developed tumors over approximately 60% of their surface, compared to 0% for blue and 3% for green.

Table I. *Effects of Various Regions of the Visible Spectrum on Tumor Development*

All irradiance treatments were maintained at 25 ± 2 μmol m^{-2} s^{-1}.

Irradiance Spectrum[a]	Disc Area Covered by Tumors
	%
BLUE (400–500 nm)	0
GREEN (500–600 nm)	3 ± 4
RED-A (600–700 nm)	63 ± 16

[a] Lamp and filters detailed in text.

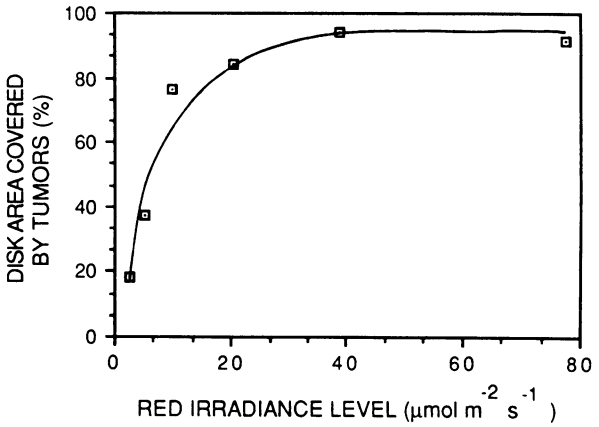

FIG. 3. Effect of increasing red (600–700 nm) light levels on tumor development. Experiment duration was 72 h.

Table II. *Effect of Interactions between Level and Duration of Red Irradiance (Red-B) on Tumor Development in Lycopersicon hirsutum Leaf Discs*

Underlined values are equivalent in terms of total irradiance (0.027 mol m^{-2}).

Exposure Duration (min h^{-1})	Disc Area Covered by Tumors (%) with Irradiance Level (μmol m^{-2} s^{-1}):		
	10	30	90
5	0.4	1.3	2.1
15	4.8	33.3	55.2
45	50.4	82.1	92.7

It was found that the development of tumorous growths increased with increasing levels of red light (Fig. 3). Maximum tumor development occurred at a PPF of approximately 30 μmol $m^{-2}s^{-1}$ (equal to a fluence of 7.8 mol m^{-2} over 72 h). At this level, tumors covered approximately 90% of the disc surface and the discs exhibited a slight downward curvature. At higher PPFs, no increase in tumor development could be discerned, but the discs exhibited increasingly severe downward curvature.

When red irradiance was provided for 5, 15, or 45 min out of each hour, it was found that tumor formation increased with both increasing irradiance duration and increasing PPF (Table II). However, irradiance duration was most significant since exposures of 5 min out of each hour produced very little tumor development, even at high PPFs, while exposures of 45 min out of each hour resulted in heavy tumor formation. At equivalent total irradiance (underlined values in Table II), the percent of the disc covered by tumors was only 2% for 5 min exposures at 90 μmol $m^{-2}s^{-1}$ as compared to 50% for the 45 min exposures at 10 μmol $m^{-2}s^{-1}$.

Far-red radiation was also found to regulate tumor development. The addition of far-red radiation to a background red

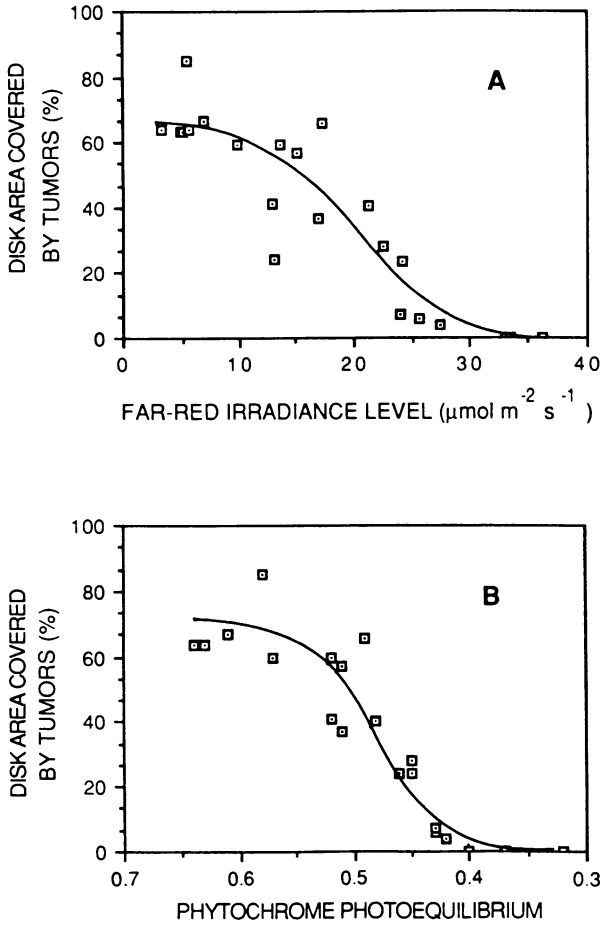

FIG. 4. Inhibition of tumor development by the addition of varying levels of far-red radiation (700–800 nm) to a constant background level of red radiation (600–700 nm) at 15 μmol $m^{-2}s^{-1}$ for a 72-h period. Data are presented as (A) far-red photon flux and (b) phytochrome photoequilibrium values.

radiation exposure of 15 ± 3 μmol $m^{-2}s^{-1}$ was observed to inhibit the promotive effect of the red radiation. Increasing levels of far-red radiation resulted in decreasing tumor development, and if sufficient far-red radiation was added, tumor development could essentially be prevented. The level of far-red radiation has been expressed both as photon flux and as PPE values (Fig. 4). Tumor development was inhibited to a maximum extent at far-red levels greater than apx. 30 μmol $m^{-2}s^{-1}$ (equal to a fluence of 7.8 mol m^{-2} over 72 h) and at PPE values less than 0.40. For reference, sunlight has an approximate PPE value of 0.59 and does induce tumor development on greenhouse grown plants when UV-B wavelengths are absorbed by glass or plastic greenhouse coverings.

Far-red radiation was also effective in preventing injury development when given immediately following inductive red radiation exposures of 15 min at 80 μmol $m^{-2}s^{-1}$ out of each hour (Fig. 5). Approximately 66% of each leaf disc surface was covered with tumors in treatments receiving red radiation alone. The lower injury level observed in Table II under a similar exposure regime is probably due to slight differences in plant material between the two experiments. Following each hourly red radiation exposure with far-red irradiance at 10 μmol $m^{-2}s^{-1}$ reduced this injury to only 1%. However, following each far-red exposure with a subsequent red exposure for 5 min at a photon flux of 10 μmol $m^{-2}s^{-1}$ nullified this effect, resulting in tumor development essentially equal to that observed when red exposures were given alone.

DISCUSSION

These results indicate that red light is required for the development of nonpathogenic neoplastic growth in tomatoes. The promotion of injurious neoplastic growth by red light is surprising as this portion of the solar spectrum is most efficient in driving photosynthesis (14). For this reason it was suspected that neoplasm development was associated with photosynthetic activity. This suspicion was supported by the observation that neoplasms could not be induced in darkness. However, the inability of blue or green wavelengths to provide any significant stimulation of injury made this supposition appear unlikely. Inhibition of tumor development by UV and far-red wavelengths also argued against photosynthesis. Rather, the inhibition of neoplasms by far-red wavelengths indicates that phytochrome is a primary regulator of this response.

The level of red irradiance required to initiate neoplasm development is significantly greater than the level of red irradiance required to reverse far-red effects during successive red/far-red exposures. Thus there appears to be two separate irradiance responses involved in neoplasm development, a prolonged red response and a reversible red/far-red response. It appears that prolonged red irradiance controls the induction of neoplasm development, while the reversible red/far-red response regulates the expression of neoplasm development. The possibility that two responses are present and involved in regulating tumor development is also supported by evidence that tumor initiation and tumor expression in *Nicotiana* each involve separate genetic components (1). Observations made during this study suggest that these responses may involve both the high irradiance phytochrome response and the low fluence phytochrome response as reported for inhibition of hypocotyl elongation in *Cucumis* (7) and *Sinapis* (19), and the stimulation of anthocyanin formation in *Sinapis* (19). The requirement for prolonged exposures of red irradiation to induce tumors and the fact that this response

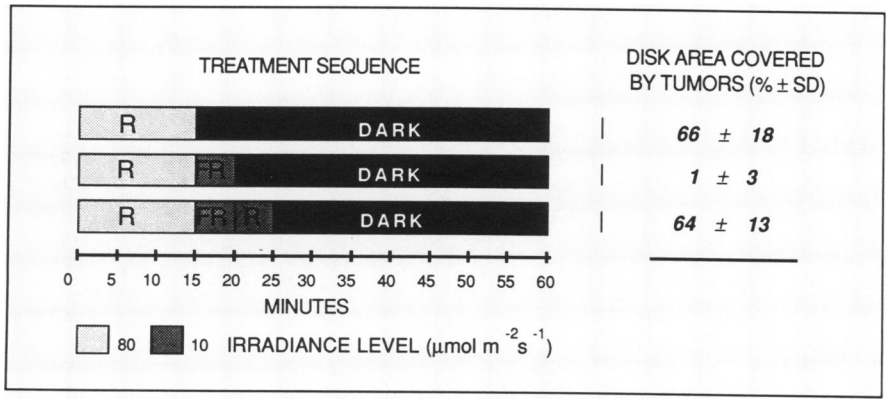

FIG. 5. Regulation of tumor development on leaf discs of tomato by successive hourly cycles of alternate red and far-red irradiation. Exposures were carried out for 60 h, after which time leaf discs were scored for neoplasm development. Photon flux was 80 μmol $m^{-2}s^{-1}$ for the initial 15 min exposures and 10 μmol $m^{-2}s^{-1}$ for the subsequent 5 min exposures (both red and far-red).

was photon flux (fluence rate) dependent and did not exhibit reciprocity, is characteristic of the high irradiance mode of phytochrome action. However, the apparent lack of response to blue light is not typical of high irradiance responses (8). Therefore, this response needs to be examined further. The fact that the duration of the prolonged exposure was more critical to the induction of neoplasm development than was actual photon flux may indicate that this response involves the production of some substance or the release of some substance from an inactive form in order for neoplasm development to proceed. The reversibility of neoplasm development, the ability to prevent this response by following red exposures with far-red irradiance and to cancel the far-red effect with a subsequent red exposure, is characteristic of low fluence responses (8). Though the kinetics of the red and far red reversible response were not determined, preliminary data (not shown) indicates that the photon fluence effective in this response falls within the 1 to 1000 μmol m^{-2} range characteristic of low photon flux responses (8). Work is currently in progress to determine escape times for this response.

The actual sequence of events which occur between red light stimulation and the subsequent abnormal cell enlargement observed during neoplasm development is not understood. Auxin is involved in normal cell enlargement (15), and work involving interspecific crosses of tobacco (2) suggests that neoplastic growth on leaves may be an aberration of normal tissue development due to the inheritance of duplicate sets of auxin producing genes. It is therefore possible that an auxin related mechanism is involved in neoplasm development in tomatoes. The inhibition of neoplastic growth by UV radiation might then involve the degradation of auxin or auxin precursors (9). It would be of interest to determine if UV radiation is exerting its effect directly through an interaction with phytochrome, or through a mechanism involving auxin. Nonpathogenic tumorous growths in some plant species are characterized by cell division as well as by cell enlargement, indicating the possible involvement of cytokinins in addition to auxin. However, it needs to be determined if the abnormal division of cells observed in these species responds to red and far-red irradiance in a manner similar to that observed for the abnormal enlargement of cells in tomatoes as studied herein.

This paper confirms earlier work implicating the involvement of red irradiance in neoplasm development and, as far as is known, provides the first substantial evidence for phytochrome regulation of neoplasm development.

LITERATURE CITED

1. AHUJA MR 1968 An hypothesis and evidence concerning the genetic components controlling tumor formation in *Nicotiana*. Mol Gen Genet 103: 176–184
2. BAYER MH 1982 Genetic tumors: physiological aspects of tumor formation in interspecies hybrids. *In* G Kahl, JS Schell, eds, Molecular Biology of Plant Tumors. Academic Press, New York, pp 33–67
3. DALE E 1901 Investigations on the abnormal outgrowths or intumescences on *Hibiscus vitifolius* Linn.—A study in experimental plant pathology. Phil Trans Roy Soc Lond B 194: 163–182
4. DODDS KS, P MATTHEWS 1966 Neoplastic pod in the pea. J Hered 57: 83–85
5. GARDNER G, MA GRACEFFO 1982 The use of a computerized spectroradiometer to predict phytochrome photoequilibria under polychromatic irradiation. Photochem Photobiol 36: 349–354
6. HAMMER PA, TW TIBBITTS, RW LANGHANS, JC MCFARLANE 1978 Base-line growth studies of 'Grand Rapids' lettuce in controlled environments. J Am Soc Hort Sci 103: 649–655
7. HILLMAN WS, WK PURVES 1966 Light responses, growth factors and phytochrome transformations of *Cucumis* seedling tissue. Planta 70: 275–284
8. KRONENBERG GHM, RE KENDRICK 1986 The physiology of action. *In* RE Kendrick, GHM Kronenberg, eds, Photomorphogenesis in Plants. Martinus Nijhoff Publishers, Boston, pp 99–134
9. KLEIN RM 1978 Plants and near-ultraviolet radiation. Bot Rev 44: 1–127
10. LANG SP, BE STRUCKMEYER, TW TIBBITTS 1983 Morphology and anatomy of intumescence development on tomato plants. J Am Soc Hort Sci 108: 266–271
11. LANG SP, TW TIBBITTS 1983 Factors controlling intumescence development on tomato plants. J Am Soc Hort Sci 108: 93–98
12. LEVINE M 1937 Tumors of tobacco hybrids. Am J Bot 24: 250–256
13. MANI MS, ed, 1964 Ecology of plant galls. *In* WW Weisbach, P Van Oye, eds, Monographiae Biologicae Vol 12. Dr. W. Junk, Publisher, The Hague
14. MCCREE KJ 1972 The action spectrum, absorption and quantum yield of photosynthesis in crop plants. Agric Meterol 9: 191–216
15. MOORE TC 1979 Biochemistry and physiology of plant hormones. Springer-Verlag, NY
16. MORROW RC, TW TIBBITTS 1987 Induction of intumescence injury on leaf discs. J Am Soc Hort Sci 112: 304–306
17. NILSEN KN, NR LERSTEN 1977 UVB-attenuated irradiance environments and the induction of neoplasms on leaves of tomatoes (*Lycopersicon esculentum* Mill.): morphological and anatomical aspects. HortScience 12: 45
18. NILSEN KN 1971 Plant responses to near-ultraviolet light. HortScience 6: 26–29
19. SCHAFER E, CJ BEGGS, L FUKSHANSKY, MG HOLMES, M JABBEN 1981 A comparative study of the responsivity of *Sinapis alba* L. seedlings to pulsed and continuous irradiation. Planta 153: 258–261