Plant tops of treated plants contained a lesser percentage of potassium, sodium, and phosphorus and a greater percentage of boron and iron than did the untreated plants, table I. Roots of treated plants accumulated more calcium and less copper than roots of untreated plants. There was little or no difference in accumulation of calcium, copper, magnesium and zinc in tops of the plants nor in accumulation of boron, potassium, magnesium, manganese, and phosphorus in roots of the plants as between treated and untreated plants, table I.

Plant roots contained greater percentages of boron, copper, iron, magnesium, manganese, sodium, and phosphorus than plant tops in both treated and untreated plants. Calcium accumulated to a greater amount in roots than in tops of treated plants, altho no differences were noted in untreated plants. Potassium accumulated to a greater extent in tops than in roots of untreated plants, while no differences were noted in treated plants.

The content of phosphorus in the tops of the treated plants is much less than that of untreated plants. Grizzard et al (3) has demonstrated that rate of growth of tobacco parallels the rate of absorption of phosphorus.

The content of potassium in the tops of treated plants was 2/3 that of untreated plants.

Summary

Information on the action of growth regulators on mineral uptake of plants is rather scarce and somewhat contradictory.

Fifty ml of a 0.1 % solution of sodium salt of 2,4-D was poured into the sand around young tobacco plants grown in sand in eight-inch pots. Two weeks later plants were harvested, dried and analysed for mineral elements.

Content of phosphorus and potassium in the tops of treated plants was much less than in the tops of untreated plants.

Calcium accumulated to greater amount in roots than in tops of treated plants, but there was no difference in accumulation of calcium in roots and tops of untreated plants.

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A DARKROOM SAFELIGHT FOR RESEARCH IN PLANT PHYSIOLOGY[1,2]

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The selection of a suitable safelight system for handling plant material in the darkroom always has been a difficult problem. This is especially true for studies of chlorophyll synthesis and the photocontrol reactions of phototropism and photomorphogenesis, where measurable plant responses are obtained at very low irradiances near the threshold of vision. Most investigators of phototropism and auxin physiology have used a red safelight which emits energy in the general region of from 600 mμ out into the infrared, since the phototropic action spectrum has its maximum in the blue and negligible response in the orange, red and infrared, and because the classical organ for such studies, the Avena coleoptile, requires a pre-exposure to red radiant energy for suppression of the mesocotyl.

This practice is open to serious question in view of the observations by Kent and Gortner (5) that, in the pea test, little curvature in response to alpha-naphthalene acetic acid was secured without a pre-exposure to red radiant energy. Klein, Withrow and

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Elstad (6) found that the concentration of externally applied auxin required to produce a specified angle of the hypocotyl hook of bean was dependent on the dose of red energy. Conversely, it was demonstrated that the radiant energy required to produce a given hook-opening response was related to the external supply of auxin, i.e., the higher the level of auxin, the higher the incident energy required. Plant materials to be used in studies of this type usually are exposed to a safelight for varied and unmeasured periods of time depending upon the speed with which the operator can carry out the manipulations. This introduces an unknown variable which may condition the response.

Within the past five or six years, quite a few investigators have used green safelights, including Koski et al (7) for chlorophyll synthesis and Curry et al (3) for phototropism. However, the spectral characteristics of the system have usually not been specified. Nitsch and Nitsch (8) employed a green safelight devised by C. Yocum. This consisted of a green fluorescent lamp wrapped in three layers of "amber" and "green" cellulose acetate. Dr. Yocum (private communication, February, 1957) has indicated that little phototropic curvature resulted when Avena coleoptiles were exposed two inches from the source for one hour. He states that some red is visible with this source and, therefore, it would not appear to be satisfactory for studies involving the red, far-red group of reactions.

To be adaptable for a range of photobiological investigations, a safelight should have its maximum transmission near the region of maximal spectral sensitivity of the human eye and should exclude those wavelengths inducing phototropism, chlorophyll synthesis or photomorphogenesis. Weaver (10) and others have shown that the dark-adapted eye has maximum sensitivity at 510 mμ while the light-adapted eye, i.e., one which has been exposed to relatively high light intensities, has its maximum sensitivity at 555 mμ. When working under the lowest possible light intensities, the dark-adapted eye is color blind and makes use of rod vision only. Its spectral sensitivity is given in figure 1. It will be noted that the sensitivity in the green at 510 mμ is from 100 to 1000 times greater than in the orange-red. For the same visibility, it is possible to use a very much lower irradiance in the green than in the orange and red.

The processing of photographic material presents a problem similar to that of handling photosensitive plant materials. With orthochromatic emulsions, which have little orange and red sensitivity, an orange safelight is used. Panchromatic materials are sensitive throughout the entire visible spectrum and it is essential to use a safelight which yields maximum visibility per unit of transmitted energy. In this case, a green safelight is recommended. The green Wratten-type safelight filters designed for use in handling film, however, transmit in the far-red. The commercial safelight filters which have been tested in this laboratory begin to transmit just beyond 720 mμ. They are not, therefore, very suitable for handling plant material to be used in photomorphogenic studies, especially with incandescent sources, since photoinactivation of this process has its maximum activity in the general region of from 700 to 750 mμ (1, 12, 13).

The curves of figure 1 present the action spectra for the principal plant photochemical reactions, together with the spectral sensitivity of the dark-adapted eye taken from the data of Weaver (10). It will be noted that the phototropic action spectrum (4) falls to a minimum at about 520 mμ, while the maximum sensitivity of the dark-adapted eye is at 510 mμ. In the region of 500 to 550 mμ, there is a general low level of sensitivity for chlorophyll synthesis (7), photosynthesis (2), and photomorphogenesis (6, 12, 13).

The safelight we have devised for our work consists of two General Electric green fluorescent lamps filtered with dyed-gelatin films which we prepare in our laboratories according to the methods described previously (11). This safelight has been mentioned in a previous publication (6), but it has given us such excellent service for a variety of research problems that we feel it merits a more detailed description. The first filter (A, table I) we prepared was very satisfactory for red photomorphogenic studies but a weak bending response in Avena coleoptiles was excited. This filter was modified by adding more of the yellow dye and less of the green, so as to shift the band about 10 mμ to the longer wavelengths. These filters have been used for over six years in studies of photomorphogenesis and about one for phototropism. Transmission is from 500 to 550 mμ for filter A, and 510 to 580 mμ for filter B. The shaded curve of figure 1 gives the calculated relative spectral distribution of the combination of a green fluorescent lamp and two films of filter A. From figure 2 it can be seen that the green fluorescent lamp is a much more suitable safelight source than the incandescent lamp, especially for investigations involving the photomorphogenic red and far-red reactions. The incandescent lamp has its maximum energy in the infrared; this would be of little consequence except that organic dyes transmit freely in the near infrared and most of them in the far-red. With the green fluorescent lamp, the problem is minimized because there is little energy in the longer wavelengths except for that from the incandescent cathodes.

The transmittances of two safelight filters are given in table I, together with the formulas for their preparation. The filters do not begin to transmit in the infrared until about 800 mμ. This has been achieved by adding high concentrations of naphthol green B. Since the green transmission band of naphthol green B is broad, tartrazine and pontacyl green BL were added to narrow the band to the desired limits. The combination of the three dyes is dissolved in a solution of gelatin, sorbitol and sodium benzoate at the rate of 0.3 mg of each dye per cm^2 of gelatin-film surface. One film of filter A of 1000 cm^2 area

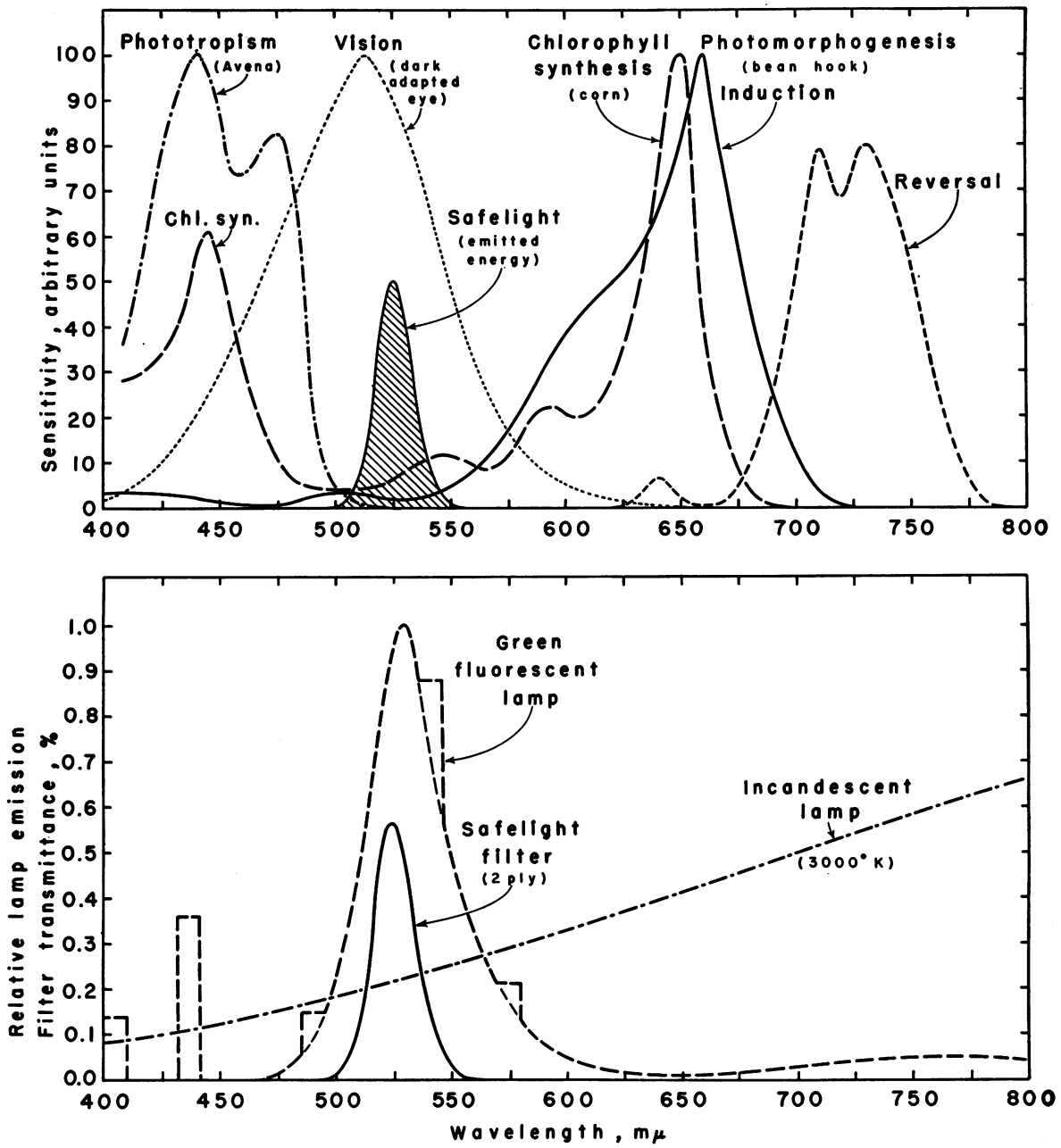

FIG. 1 (*above*). Action spectra of various biological photochemical reactions and spectral energy distribution of the safelight.

FIG. 2 (*below*). Spectral transmittance of safelight filter and relative emission of green fluorescent and incandescent lamps.

may be prepared as follows: dissolve 300 mg each of the three dyes, 8 gm sorbitol, 15 mg benzoate and 1 ml ammonium hydroxide in sufficient hot water to make a total volume of 200 ml. Cool to room temperature and add 23 gm food grade gelatin. The mixture is then heated on a water bath to about 60° C, poured onto a level glass plate and allowed to dry. We use two films in each safelight filter to eliminate possible leakage through pinholes or thin spots due to air bubbles formed during the casting.

One simple method of mounting the films is to wrap two layers around a small fluorescent lamp and exclude any emission from the ends of the tube by black tape. Films do not stand up for extended periods under these conditions, but the method is simple and they can be used for short periods in

TABLE I

PERCENT TRANSMITTANCE (T) AND COMPOSITION OF SINGLE AND DOUBLE FILMS OF THE SAFELIGHT FILTERS

WAVELENGTH, mμ	FILTER A		FILTER B	
	1 PLY	2 PLY	1 PLY	2 PLY
320	0	0	0	0
480	0	0	0	0
490	0.1	0	0	0
500	0.8	0.006	0	0
510	3.9	0.15	0.2	0
520	7.3	0.53	4.7	0.22
525	7.5	0.56	8.8	0.78
530	6.8	0.46	13.0	1.7
540	3.8	0.14	16.0	2.6
550	1.5	0.023	14.0	2.0
560	0.2	0.004	7.9	0.62
570	0	0	3.7	0.14
580	0	0	1.0	0.01
590	0	0	0.2	0
600	0	0	0	0
760	0	0	0	0
780	0.1	0	0.1	0
800	0.2	0	0.2	0
820	0.6	0.004	0.6	0.004
840	1.5	0.02	1.5	0.02
860	3.5	0.12	3.5	0.12
880	8.5	0.72	8.5	0.72
900	19.0	3.6	19.0	3.6

Composition of one ply of a two-ply filter:

	A	B	
Dyes, duPont fabric			
Naphthol green B (C.I.* 5)	0.3	0.3	mg/cm²
Pontacyl green BL, 200% (C.I. 666)	0.3	0.1	mg/cm²
Tartrazine (C.I. 640)	0.3	1.0	mg/cm²
Gelatin solution			
Gelatin, food grade	150		gm
Sorbitol	50		gm
Sodium benzoate	1		gm
Water	1000		ml
Rate of pouring	0.2		ml/cm²

* C.I. = Colour Index (9).

rooms at moderate humidity. Coating the film with a clear plastic, such as photographic film lacquer, is of considerable help in preventing deterioration under high humidity conditions. A second method is to mount the two filter plys between two glass panes, together with one or more layers of diffusing material, such as tracing or filter paper. The paper diffuses the light and eliminates bright areas in the background. The assembly can be taped together with black plastic electrical or photographic tape and mounted on the front of a box containing two 15-watt, 18-inch green fluorescent lamps. The box for the lamps can be made from a $3'' \times 10'' \times 23''$ standard electronic steel chassis. A convenient fluorescent fixture is the lighting strip type in which the ballast is mounted in a small narrow metal case with a lamp on the outside. Most of these units are narrow enough to fit inside a 3-inch deep electronic chassis. The open side (bottom) of the chassis is then used for mounting the filter assembly. A third method which we use is to mount the glass-enclosed safelight filter in a table top; this gives an excellent working surface for the handling of plant material. The use of two fluorescent lamps reduces the possibility of total lamp failure in the middle of an experiment.

The light intensity required for just barely seeing such materials as Avena coleoptiles and bean hooks ranges from 0.01 to 0.1 ft-c. At 0.1 ft-c with filter A, bean hooks can be exposed for about one hour without any significant hook opening response (6). An exposure to 0.1 ft-c for two hours from the B filter induced no phototropic curvature in Avena. One can work with moderate ease on a clean white surface with only 0.01 ft-c if red goggles have been worn for a half hour or more prior to working in the darkroom. Red goggles for dark-adapting the eyes are available from hospital supply houses, since this is the technic used by radiologists who need to dark-adapt their eyes for seeing the weak images of an x-ray fluorescent screen.

SUMMARY

A safelight for darkroom handling of plant material is described. During extensive tests it has been found without effect on phototropic bending of the Avena coleoptile, on photomorphogenesis in bean and pea seedlings and in chlorophyll synthesis. It has its maximum transmission in the region of maximal sensitivity of the human eye.

The safelight consists of one or more green fluorescent tubes and a dyed-gelatin filter. The composition of the filter can be varied so as to shift the transmission region either toward the blue or red, depending on the problem under investigation.

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