5. LAUSBERG, T. 1935. Quantitative Untersuchungen über die kutikuläre Exkretion der Laubblätter. Jahrb. Wiss. Botan. 81: 769–806.
6. SCHOCH, K. 1955. Erfassung der kutikularen Rekretion von K and Ca. Ber. Schweiz. Botan. Ges. 65: 205–50.
7. TUKEY, H. B., JR. AND J. V. MORGAN. 1964. The occurrence of leaching from above-ground plant parts and the nature of the materials leached. Proc. XVI Intern. Hort. Cong. 4: 146–53.
8. TUKEY, H. B., JR. AND H. B. TUKEY. 1962. The loss of organic and inorganic materials by leaching from leaves and other above-ground plant parts. In: Radioisotopes in Soil-Plant Nutrition Studies. Intern. At. Energy Agency, Vienna. p 289–302.
9. TUKEY, H. B., JR., H. B. TUKEY, AND S. H. WITTWER. 1958. Loss of nutrients by foliar leaching as determined by radioisotopes. Proc. Am. Soc. Hort. Sci. 71: 496–505.

Repression of Tissue Culture Growth by Visible and Near Visible Radiation [1, 2]

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That specific wavelengths of visible radiations are causal factors in plant morphogenesis needs no documentation other than to note that it was known in 1682 to Stephan Hales. Much of the research on photomorphogenesis is being done with intact plants or organ systems in which tissue interactions play a role in the observed responses, and it is at this level that plant tissue cultures can be profitably used. As a technical problem, too, the influence of visible radiations on the growth of tissue cultures is important, since virtually every laboratory known to the author uses a different light regime, ranging from total darkness to constant illumination.

Materials and Methods

Growth Procedures. Tissue cultures of *Parthenocissus tricuspidatus* Planch, and *Helianthus annuus* L. were maintained in 125 ml flasks containing 50 ml of an agar medium (12) at 25° ± 0.5 in darkness. One week prior to the start of an experiment, explants weighing 15 to 25 mg (fresh wt) were excised from the tissue mass and transferred to agar slants to allow the completion of the lag phase of growth prior to the experiment (10). The small pieces were weighed to the nearest milligram on a Roller-Smith balance, transferred, one piece to each 125 × 15 mm test tube containing 6 ml of medium, and incubated for 28 days at 25° ± 1 under appropriate illumination schedules. A minimum of 10 tubes were used per variable and some (but not all) experiments were repeated several times. At the end of an experiment, fresh weight values were determined, averages obtained, and growth expressed as a Growth Increment:

$$\frac{\text{Final wt} - \text{Initial wt} \times 100}{\text{Initial wt}}$$

To permit comparisons, data are presented as a percentage of the dark-growth control in each experiment. Differences of 10 to 15 % from the appropriate control are significant at the 5 % level of confidence.

Radiation Procedures. White light was supplied by banks of cool white fluorescent tubes and incandescent lamps in the ratio of one rated watt of incandescent light to 10 watts of fluorescent light. Illuminance was measured with a Weston Illumination Meter, Model 756, with a cosine-corrected photocell.

Monochromatic radiation was obtained from incandescent lamps (500 or 750 w heat-resistant, reflector-flood or General Electric 500 w Quartzline lamps) by filtering the radiation successively through liquid filters (22) and either cast gelatin filters (21) or combinations of Cinemoid plastic filters (11). The transmission curves were checked for windows in a Cary recording spectrophotometer. Radiant flux was controlled by variable transformers and was measured with a Photovolt photoelectric photometer whose photocell was calibrated against a certified thermocouple. Near ultraviolet (near-UV) radiation was supplied by General Electric BLB lamps; wavelengths shorter than 385 mμ or 375 mμ can be filtered

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out with either 2 thicknesses of a clear plastic filter (New England Plastic Shade Co., Boston) or 2 sheets of cellulose acetate coated with Uvinul D-49 (Antara Chemicals Division of General Aniline & Film Corp., New York), respectively.

Results

Effect of White Light. Growth of the tissue cultures examined was repressed by continuous white light (fig 1). The responses differed, with Parthenocissus callus tissues being more sensitive than their nontumorous counterparts. Carrot crown-gall tissues were more sensitive than Parthenocissus crown-gall, a 50 % repression of carrot being noted at about 3000 ft-c hours (125 ft-c × a 24-hr photoperiod). The response to white light follows the Bunsen-Roscoe Reciprocity Law in that the biological effect of the product of duration and intensity of the radiation was a constant within the biological variation of the test system.

The permanence of the light-induced suppression of growth was examined by exposing cultures to darkness for 7 days followed by 4000 ft-c hours of light (170 ft-c × a 24-hr photoperiod) for one week and, finally, darkness for a third week. Upon returning tissues to darkness, the growth rate returned to control levels (fig 2).

Incandescent light from a 200 w incandescent lamp adjusted in height to give 200 ft-c at the level of the culture tubes was more repressive (35 % of the dark control) to Parthenocissus crown-gall tissue than was 200 ft-c of cool white fluorescent light (65 % of the dark control). The total radiant energy emitted in the 500 to 600 mμ range is almost 3 times greater from the incandescent than from the fluorescent lamps.

Effect of Monochromatic Radiation. The action spectrum for growth repression was determined using Parthenocissus crown-gall tissue culture. The radiations were each tested at a luminous flux of 0.43 Joules/day (50 μw/cm² × a 24-hr photoperiod).

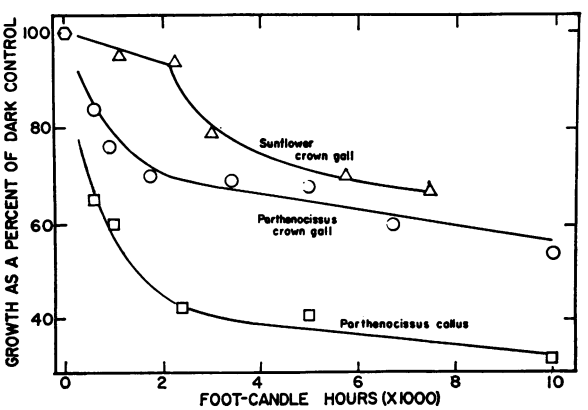

FIG. 1. Effect of white light on the growth capacity of several plant tissue cultures. Growth increment values for dark controls: sunflower crown-gall, 286; Parthenocissus crown-gall, 750; Parthenocissus callus, 367.

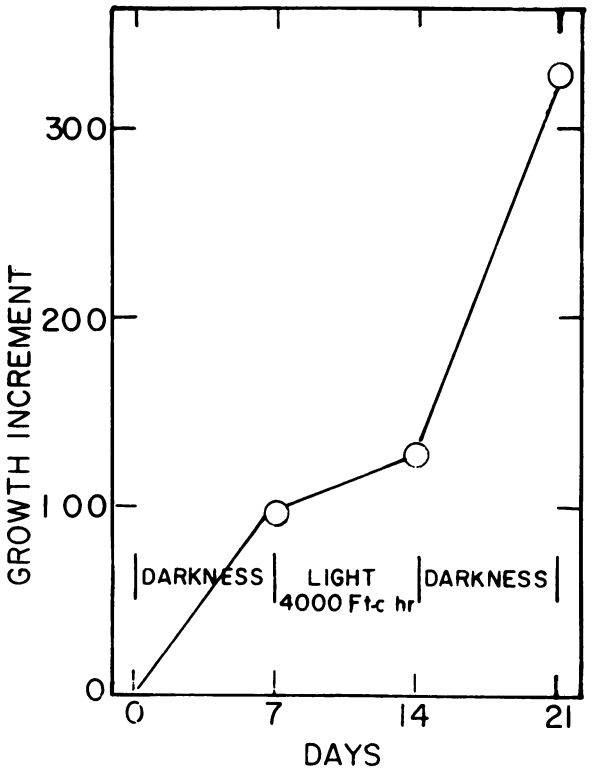

FIG. 2. Reversal of the light-induced suppression of the growth of Parthenocissus crown-gall tissue cultures by subsequent darkness.

There were 2 wave bands which were effective in repressing growth, one in the green (filter system transmitting from 510 to 585 mμ; peak at 550 mμ; half band width 30 mμ), the other in the near ultraviolet (lamps transmitting from 300 to 420 mμ, peak at 360 mμ, half band width, 40 mμ) (fig. 3). Filtration of wavelengths below either 375 mμ or 385 mμ from the BLB lamps effectively prevented growth response, indicating that the effective radiation band was between 300 mμ and 375 mμ and is likely to be the 360 mμ mercury peak. Blue radiation (filter system transmitting from 385 to 490 mμ; peak at 420 mμ: half band width, 40 mμ), orange (filter system transmit-

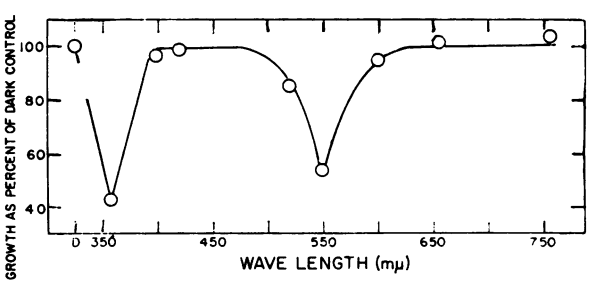

FIG. 3. Action spectrum for the suppression of the growth of Parthenocissus crown-gall cultures irradiated with continuously supplied radiation at 50 μw/cm². Growth increment for dark controls is 364.

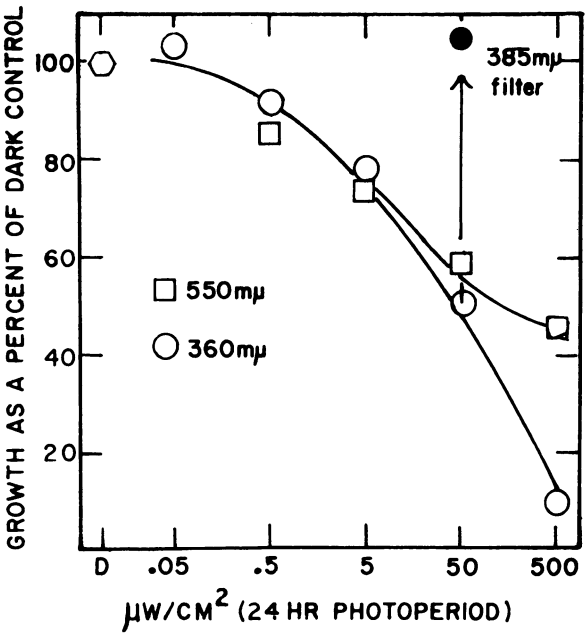

FIG. 4. Response of Parthenocissus crown-gall tissue cultures to green or near-UV radiation at different illuminances. Growth increment for dark control is 314.

ting from 555 to 645 mμ: peak at 600 mμ: half band width, 30 mμ), red (filter system transmitting from 620 to 685 mμ; peak at 660 mμ: half band width, 25 mμ) and far red radiation (filter system transmitting from 695 to 790 mμ; peak at 760 mμ; half band width, 30 mμ), did not alter either the growth capacity or the gross appearance of the tissues. A similar action spectrum was found for carrot crown-gall tissues.

Dose-response curves were obtained, again using Parthenocissus crown-gall cultures (fig 4). No differences were noted between the effectiveness of the 550 mμ and the 360 mμ radiations at irradiances up to 50 μw/cm² (24-hr photoperiod) but above that level the near-UV was more suppressive than was the green radiation. Tissues continuously irradiated with 500 μw/cm² of 360 mμ radiation showed necrotic areas within 14 days, while those under the green radiation appeared to be healthy. Simultaneous irradiation of Parthenocissus crown-gall tissue cultures with both 550 and 360 mμ radiations, each supplied at 0.43 Joules/day was only as effective as irradiation with either of the wavelengths alone.

Zinc Deficiency and Light Effects. Skoog (18) reported that zinc-deficient tomato plants appeared to overcome the growth reductions caused by limiting zinc when the plants were grown under red light; blue light appeared to intensify the severity of the growth responses. Ozanne (16) reported that zinc deficiency symptoms in sweet clover increased with increases in light intensity. Zinc-deficient cultures of Parthenocissus crown-gall tissues were obtained by successive transfers on zinc free medium (13) purified by the methods of Price and Vallee (17). When such cultures were handled as in other radiation studies reported here, we were unable (table I) to observe any radiation effect, nor were we able to either replace the zinc requirement with gibberellic acid as reported by Dycus (5) for bean seedlings, or observe Zn: GA synergism (3). Whether the differences in results are due to the very different test systems used or reflect modifications in radiation and temperature control is currently unknown.

Discussion

There are very few reports on the response of plant tissue cultures to light. Steinhart et al. (19) noted that the growth of spruce tissue cultures was more vigorous in darkness than in low light. Goris (8), Naef (14) and Gautheret (7) reported that illumination promoted several metabolic processes and differentiation in various tissue cultures. de Capite (4), studying the interaction of light and temperature, found that growth was promoted by light but indicated that this was due, at least in part, to changes in temperature. There is little doubt that there are species or even clonal specificities in the responses of tissue cultures to visible radiation. The growth of some tissue cultures such as those from Rumex, may be stimulated by light (15; Gentile, unpublished). Even here, the role of photosynthesis should not be ignored. The present work serves as a notice that illumination of plant tissue cultures may be contra-indicated.

Table I. *Growth of Zinc-Deficient and Zinc-Sufficient Parthenocissus Tissue Cultures on Different Media and Exposed to Either Visible Radiation or to Gibberellic Acid.*

Growth increment of dark + Zn control is 300.

Tissue	Medium	Dark control	Growth as a % of dark control			
			Irradiated*		Gibberellic acid	
			blue	red	1 mg/liter	10 mg/liter
+ Zn	+ Zn	100	104	99	103	101
− Zn	+ Zn	88	85	85	87	91
+ Zn	− Zn	102	99	93	93	97
− Zn	− Zn	63	63	66	63	66

* Blue radiation: 385 to 490 mμ, peak at 420 mμ, 600 μw/cm² × 24 hr.
Red radiation: 625 to 705 mμ, peak at 650 mμ; 560 μw/cm² × 24 hr.

More important is the cause of the observed growth repressions. It is not phytochrome, carotene, or flavin, the 3 photoreceptors most frequently implicated in photomorphogenesis. The action spectrum does coincide with the absorption spectrum of vitamin B_{12} and, equally interesting, with the action spectrum for the photooxidation of the adenyl cobamide coenzyme (1, 20). Under the influence of visible radiation, the B_{12} coenzyme is oxidized into 2 components, neither of which retain biological activity (9). In spite of earlier reports to the contrary, vitamin B_{12} is found in higher plants (6). The known role of B_{12} in nucleic acid biosynthesis (2) is not at variance with the data and the hypothesis presented here. Work in progress is designed to test the hypothesis that green and near-UV radiations repress plant growth because they inactivate the B_{12} coenzyme function in nucleic acid biosynthesis.

Summary

Green and near ultraviolet radiation were found to repress the growth of Parthenocissus plant tissue cultures. The effects were reversible by subsequent darkness. There was no interaction between the effective radiations.

Neither visible radiations nor gibberellic acid permitted growth of zinc-deficient tissue cultures.

Literature Cited

1. Barker, H. A., R. D. Smyth, H. Weissbach, A. Munch-Peterson, J. I. Toohey, J. N. Ladd, B. E. Volcani, and R. M. Wilson. 1960. Assay purification and properties of the adenyl cobamide coenzyme. J. Biol. Chem. 236: 181–90.
2. Beck, W. S., S. Hook, and B. H. Barnett. 1962. Participation of vitamin B_{12} in the biosynthesis of DNA and its water-soluble precursors. Biochim. Biophys. Acta 55: 470–78.
3. Dancer, J. 1959. Synergistic effect of zinc and gibberellin. Nature 183: 901–02.
4. de Capite, L. 1955. Action of light and temperature on growth of plant tissue cultures in vitro. Am. J. Botany 42: 869–73.
5. Dycus, A. M. 1961. The action and interaction of IAA and gibberellic acid on zinc-deficient plants. Plant Physiol. 36: xii.
6. Fries, L. 1962. Vitamin B_{12} in *Pisum sativum* L. Physiol. Plantarum 15: 566–71.
7. Gautheret, R. J. 1961. Action de la lumiére et de la temperature sur la neoformation de racines par des tissues de topinambour cultivés in vitro. C. R. Acad. Sci. (Paris) 252: 2791–96.
8. Goris, A. 1952. Influence de l'eclairment sur la teneur en fructose de diverses souches de tissus de carotte et de crown-gall de vigne cultivés in vitro. Bull. Soc. Chim. Biol. 34: 527–31.
9. Hogenkamp, H. P. C. and H. A. Barker. 1962. Nucleoside photolysis products of coenzyme B_{12}. Federation Proc. 21: 470.
10. Klein, R. M. 1957. Growth and differentiation of plant tissue cultures. In: Rhythmic and Synthetic Processes in Growth. D. Rudnick, ed. Princeton Univ. Press, New Jersey. p 31–58.
11. Klein, R. M. 1963. Apparatus for photomorphogenic studies on plants. Am. Biol. Teacher. 25: 96–100.
12. Klein, R. M. and G. E. Manos. 1960. Use of metal chelates for plant tissue cultures. Ann. N.Y. Acad. Sci. 88: 416–25.
13. Klein, R. M., E. M. Caputo, and B. A. Witterholt. 1962. The role of zinc in the growth of plant tissue cultures. Am. J. Botany 49: 323–27.
14. Naef, J. 1959. Action de la lumiére sur l'utilization du glucose par les tissus végétaux cultivés in vitro. C. R. Acad. Sci. (Paris) 259: 1706–08.
15. Nickell, L. G. and P. R. Burkholder. 1950. Atypical growth of plants. II. Growth in vitro of virus tumors of Rumex in relation to temperature, pH and various sources of nitrogen, carbon, and sulfur. Am. J. Botany 37: 538–47.
16. Ozanne, P. G. 1955. The effect of light on zinc deficiency in subterraneum clover (*Trifolium subterraneum* L.). Australian J. Biol. Sci. 8: 344–53.
17. Price, C. and B. Vallee. 1962. *Euglena gracilis*, a test organism for study of zinc. Plant Physiol. 37: 428–33.
18. Skoog, F. 1940. Relationships between zinc and auxin in the growth of higher plants. Am J. Botany 27: 939–51.
19. Steinhart, C., L. Anderson, and F. Skoog. 1962. Growth promoting effects of cyclitols on spruce tissue cultures. Plant Physiol. 37: 60–66.
20. Weissbach, H., J. N. Ladd, B. E. Volcani, R. D. Smyth, and H. A. Barker. 1960. Structure of the adenyl cobamide coenzyme: degredation by cyanide, acid, and light. J. Biol. Chem. 235: 1462–73.
21. Withrow, R. B. and L. Price. 1953. Filters for the isolation of narrow regions in the visible and near visible spectrum. Plant Physiol. 28: 105–14.
22. Withrow, R. B. and V. Elstad. 1953. Water-cooled lamp systems with refluxing aqueous filters. Plant Physiol. 28: 334–38.