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Two Light Sources Differentially Affected Ferric Iron Reduction and Growth of Cotton

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ABSTRACT

In growth chambers, low pressure sodium (LPS) plus incandescent (Inc) lamps and fluorescent cool-white (FCW) plus Inc lamps were used to determine their effects on growth of cotton (*Gossypium hirsutum* L.) and on the reduction of Fe^{3+} to Fe^{2+}. Cotton plants grown under LPS + Inc light developed chlorosis and grew poorly, whereas plants grown under FCW + Inc lights were green. The chlorophyll concentration and top and root weights of cotton grown under LPS + Inc were lower than those under FCW + Inc. In solution, FCW + Inc lamps reduced about eight times more Fe^{3+} to Fe^{2+} than did LPS + Inc lamps. Fe^{3+} is transported to plant tops as Fe^{3+} citrate and if we assume that FCW + Inc light reduces Fe^{3+} to Fe^{2+} in plant foliage as it did in the solutions, then reduction of Fe^{3+} by the light environment will make Fe^{2+} in the tops more available for biochemical reactions.

Intensity of irradiation, its spectral wavelengths, and duration of exposure are major factors affecting plant growth (5). FCW[1] lamps, supplemented with Inc lamps, have been used satisfactorily as a source of radiant energy in growth chambers (4). Cathey and Campbell (5) compared growth of several plant species with either FCW or LPS lamps with and without supplemental incandescent light. In their tests, *Lactuca sativa* L. cv. "Grand Rapids" developed chlorosis (symptoms similar to Fe-deficiency chlorosis) when grown under LPS lamps but the lettuce was green under FCW lamps. When Inc lamps were added to the LPS lamps, yields increased and the foliage was more green.

Green plants require a continuous supply of Fe as they grow, and their ability to absorb and translocate Fe is a regulated adaptive process in the roots that responds to Fe-deficiency stress (2). This mobilizing mechanism for Fe involves reduction of Fe^{3+} to Fe^{2+} at the root. For example, it was necessary for Fe^{3+} chelates to be reduced to Fe^{2+} chelates before Fe was absorbed and transported in soybean roots (6). Iron remained as Fe^{2+} in the protoxylem up to the juncture of the metaxylem (2). Here, Fe^{2+} was oxidized to Fe^{3+}, chelated by citrate, and was then transported in the xylem to the plant top (3, 7, 13, 14, 15). On the same basis, if light could reduce Fe^{3+} citrate to Fe^{2+} citrate in plant tops, the Fe^{2+} would probably be more available for plant use than Fe^{3+} because the equilibrium constant (K) is 2.5×10^{11} for Fe^{3+} citrate and only 2.4×10^{4} for Fe^{2+} citrate (15). This should make Fe more available to biochemical systems that require it for Chl synthesis.

[1] Abbreviations: FCW: fluorescent cool-white lamps; LPS: low pressure sodium lamps; Inc: incandescent lamps; FeHEDTA: Fe-hydroxyethylethylenediaminetriacetic acid; Ferrozine: disodium salt of 3-(2-pyridyl)-5,6-bis(4-phenylsulfonic acid)-1,2,4-triazine.

Our objectives were: (*a*) to determine the effect of LPS and FCW light on reduction of Fe^{3+} to Fe^{2+} in solution; (*b*) to measure plant growth under these two light sources; and (*c*) to determine if the results of Fe^{3+} reduction in solution were related to Chl synthesis and plant growth response.

MATERIALS AND METHODS

Growing the Plants. Growth of cotton, *Gossypium hirsutum* L. (genetic selection "M8"), was compared in two growth chambers. Chamber 1 contained low pressure sodium SOX-180 w and 60-w incandescent bulbs (LPS + Inc) and chamber 2 contained fluorescent, slimline, standard cool-white T-8 lamps and 60-w incandescent bulbs (FCW + Inc) as the light sources. The temperature in the two chambers was 25 ± 2 C with 16-h photoperiods at about 300 $\mu Em^{-2}\ s^{-1}$. The irradiance in the two chambers (Table I) was obtained as previously reported (4).

The cotton seeds were aerated in deionized H_2O for 24 h and then germinated between layers of moist cheesecloth on stainless steel screens. When the roots were about 2 cm long, the seedlings were transferred to holes in a plastic ring supported on a 10-liter Pyrex jar containing a 1/5 Steinberg nutrient solution (8, 11). After the seedlings had elongated for 3 days, they were transferred to 8 liters of 1/5 Steinberg nutrient solution, containing 3 mg P/l and 1 mg Fe/l as FeHEDTA. The jars of nutrient solution were in boxes and the plants were supported by corks placed in lids that covered each box. The pH of the nutrient solution was adjusted to 6.6. Each jar treatment contained four plants and each treatment was replicated four times. The plants were 18 days old when harvested.

Harvest and Analysis of Plants. From one cotton plant in each jar, the second true leaf was harvested, weighed, its area measured, and its Chl content determined (1). The remaining plant material (including three other plants in each jar) was dried at 70 C in a forced-air oven and ground in a stainless steel mill to pass a 40-mesh stainless steel screen. The P, K, Ca, Mg, Mn, Fe, B, Cu, Zn, Mo, and Na concentrations were determined by emission spectrography. Iron and P were also determined in the plant material by the *o*-phenanthroline (10) and vanadomolybdophosphoric yellow (9) colorimetric methods, respectively.

Measuring Reduction of Fe^{3+} in Solution. Ferrozine forms a stable magenta complex species with Fe^{2+} that is very water-soluble (12). This compound was placed in solution and exposed to LPS + Inc and FCW + Inc light. The color change of this complex was used as a measure of Fe^{3+} reduced to Fe^{2+} by light (12). The solutions were made up with demineralized water as follows:

1. Ferrozine (90 μM).
2. Ferrozine (90 μM) + Fe^{3+} as $FeCl_3$ (45 μM).
3. Ferrozine (90 μM) + Fe^{3+} as $FeCl_3$ (45 μM) + HEDTA (2 μM).

4. Fe^{3+} as $FeCl_3$ (45 μM).
5. Fe^{3+} as $FeCl_3$ (45 μM) + HEDTA (2 μM).

The pH of each solution was adjusted to pH 4.0 with either 0.1 N HCl or 0.1 N NaOH.

Experiment 1. From the above solutions, solution No. 3 (500 ml, replicated five times) contained in 600-ml Pyrex beakers was placed near the cotton in the growth chambers under LPS + Inc or FCW + Inc lamps. At intervals of 0.5, 1.5, 2.5, and 3.5 h the Fe^{3+} reduced to Fe^{2+} was determined as Fe^{2+}Ferrozine in a spectrophotometer at 562 nm.

Experiment 2. Using 600-ml Pyrex beakers, 500 ml of each of the above solutions (either covered or open, replicated three times) was placed under the FCW + Inc lamps. To exclude light, some of the test samples were covered with a plastic film and the beakers were wrapped with aluminum foil. All of the test samples (covered and open) were exposed to the light for 3 h. Some covered and some open samples were then mixed to determine if FCW + Inc light had reduced Fe^{3+} to Fe^{2+}. The absorption spectra (200–700 nm) were determined for each solution in a spectrophotometer.

RESULTS

Plant Growth. Cotton plants grown under LPS + Inc light developed some chlorosis, whereas plants under FCW + Inc were green. Chl concentration under LPS + Inc was only 55% of that of cotton grown under FCW + Inc (Table II). Top and root weights were significantly higher for FCW + Inc grown cotton than for LPS + Inc grown cotton and Fe concentrations were larger but not significantly so (Table II). Other nutrient elements were comparable in the plant material and these data are not given.

Reduction of Fe^{3+} in Solution Experiment 1. FCW + Inc light reduced approximately eight times more Fe^{3+} to Fe^{2+} than LPS + Inc light in exposed solutions (Fig. 1).

Experiment 2. When either $FeCl_3$ or $FeCl_3$ + HEDTA solutions were exposed to FCW + Inc light, the exposed solutions transmitted more light below 450 nm than the unexposed solutions (Fig. 2). This shift was due to reduction of Fe^{3+} to Fe^{2+}. When these solutions were mixed with Ferrozine, Fe^{2+}Ferrozine was formed (Fig. 3) for exposed but not for unexposed solutions. Formation of Fe^{2+}Ferrozine was greater when the iron solution and Ferrozine were mixed and then exposed to FCW + Inc light (Fig. 3) than when either solution was unexposed and then mixed.

DISCUSSION

The spectral radiant power curve for the LPS lamps is 560 and 610 nm, with no radiation emitted below 550 nm (4). In contrast, the FCW curve extends from below 350 to above 700 nm. The

Table I. Irradiance data for growth chambers 1 and 2 containing LPS + Inc and FCW + Inc lamps, respectively. The distance from the bottom of the lamps to table top was 118 cm. The boxes that contained the jars with nutrient solution and supported the plants were 32 cm high. Irradiance measurements were made 46 cm above the boxes as described previously (3).

Wavelength nm	Chamber 1 LPS	Inc	LPS + Inc	% Energy
		W/m^2		
320–400	0.06	0.05	0.11	0.1
400–580	1.00	1.00	2.00	3.6
580–700	39.00	3.00	42.00	74.9
700–850	6.00	6.00	12.00	21.4
320–850	46.06	10.05	56.11	100.0
	Chamber 2			
	FCW	**Inc**	**FCW + Inc**	**% Energy**
		W/m^2		
320–400	1.46	0.09	1.55	2.6
400–580	28.00	2.00	30.00	50.4
580–700	15.00	4.00	19.00	31.9
700–850	1.00	8.00	9.00	15.1
320–850	45.46	14.09	59.55	100.0

Table II. Dry-matter yield, iron, and chlorophyll concentrations of 'M8' cotton grown in nutrient solutions under FCW + Inc and LPS + Inc lamps, respectively. The nutrient solutions were in 10-l jars that were enclosed in boxes which supported the plants. See Table I for irradiance data for growth chambers.

Light source	Plant part	Yield, g, fresh wt.	Yield, g, dry wt.	Fe μg/g	Chlorophyll μg/g
FCW + Inc	Tops	16.0a*	1.69a	108a	1.43a
LPS + Inc		11.4 b	1.04 b	92a	0.79 b
FCW + Inc	Roots	-	0.53a*	515a	-
LPS + Inc		-	0.28 b	327a	-

* For each column, values followed by the same letter are not significantly different at the 1% level, according to Duncan's multiple range test. Values are means of 4 replicates.

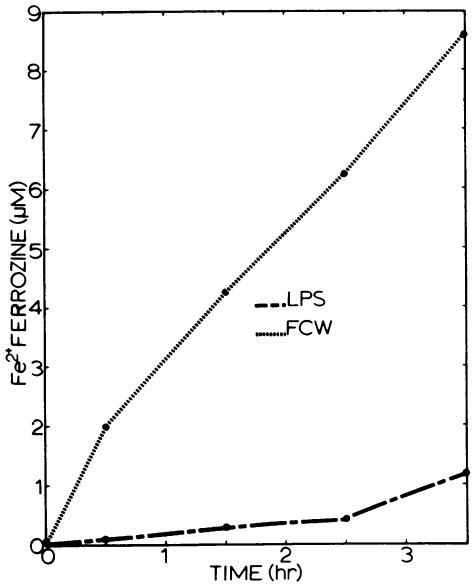

FIG. 1. FCW + Inc light reduced more Fe^{3+} to Fe^{2+} in a ferric chloride-Ferrozine solution over a period of 4 h than LSP + Inc light. The Fe^{3+} reduced to Fe^{2+} was determined as Fe^{2+}Ferrozine in a spectrophotometer at 562 nm (12). Ferrozine forms a stable magenta complex species with Fe^{2+} (Fe^{2+}Ferrozine) that is soluble in water.

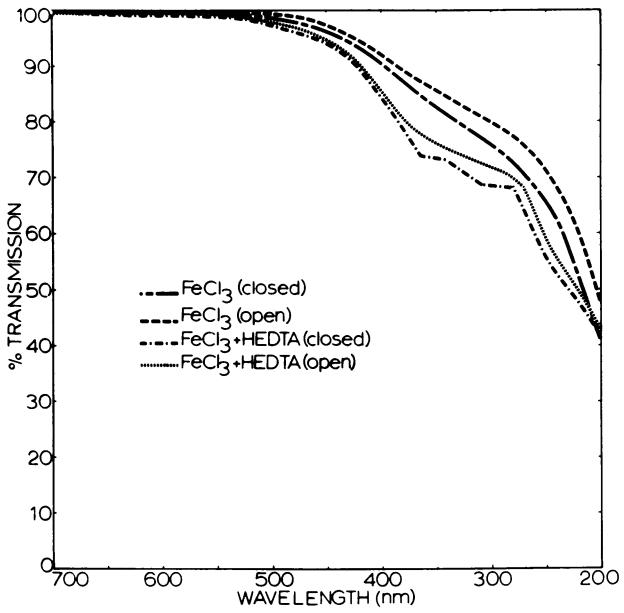

FIG. 2. FCW + Inc light reduced Fe^{3+} to Fe^{2+} in $FeCl_3$ solutions (with and without HEDTA), which caused more light to be transmitted below 450 nm of the spectral curves for exposed (open) than unexposed (closed) $FeCl_3$ solutions. The solutions were exposed to light for 3 h where applicable.

wavelengths at which Fe^{3+} was reduced to Fe^{2+} are below 500 nm, with maximum reduction below 400 nm.

The reduction of Fe^{3+} by wavelengths below 500 nm may explain why M8 cotton grew better and contained more Chl under FCW + Inc light than under LPS + Inc light. Iron chlorosis results when Fe (Fe^{2+}) is not made available for use by the plant (6, 7, 13, 14). The light sources did not affect nutrient element uptake or concentrations of these elements in plant tops.

Cathey and Campbell (5) found that incandescent light added to LPS lamps increased yields and partially corrected chlorosis in lettuce. They grew 20 species of floral and nursery crop plants

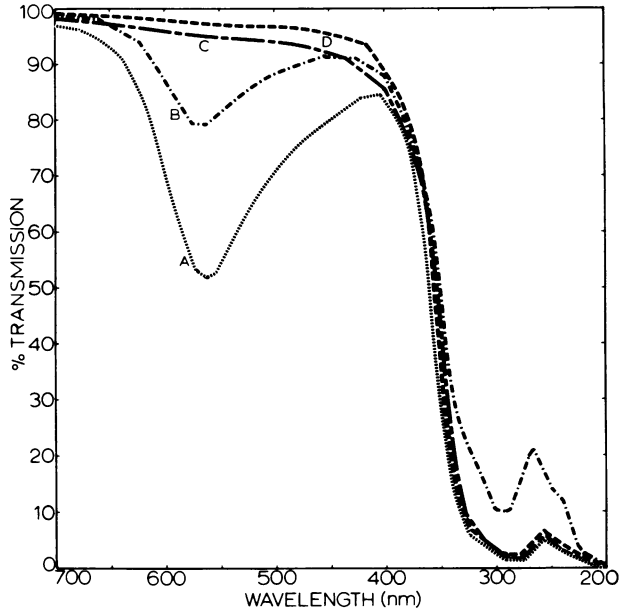

FIG. 3. Formation of Fe^{2+}Ferrozine (562 nm) was greater when $FeCl_3$ and Ferrozine were mixed and then exposed to FCW + Inc light than when either solution was unexposed and then mixed. These spectral curves are: (A) $FeCl_3$ + HEDTA, mixed with Ferrozine and then exposed to light 3 h; (B) $FeCl_3$ + HEDTA exposed to light 3 h and then mixed with Ferrozine; (C) $FeCl_3$ + HEDTA + Ferrozine and unexposed to light 3 h, and (D) $FeCl_3$ + HEDTA unexposed to light for 3 h and then mixed with Ferrozine. These spectral curves show that FCW + Inc light is reducing Fe^{3+} to Fe^{2+} and not affecting Ferrozine *per se*.

under LPS and FCW lamps, respectively. All plants except lettuce, African violet, and *Impatiens* grew well without supplemental incandescent light indicating that species differ in response to light regimes.

We know that Fe^{3+} is transported to plant tops as Fe^{3+} citrate (15), and that FCW + Inc light reduces Fe^{3+} to Fe^{2+} in solutions. If FCW + Inc light more rapidly reduces Fe^{3+} to Fe^{2+} in plant foliage than LPS + Inc light then FCW + Inc might increase Fe^{2+} availability for plant biochemical reactions. This implies that ionic species (Fe^{3+} or Fe^{2+}) may be as important to plant growth as Fe concentration in plant tops. The presence of a greater concentration of Chl and greater plant dry weight in cotton grown under FCW + Inc lamps than cotton grown under LPS + Inc lamps supports this hypothesis. In the construction of growth rooms, it appears that the spectral region of light below 450 nm should be included equivalent to that produced by FCW + Inc lamps.

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