Temperature and Plant Adaptation.

I. Interaction of Temperature and Light in the Synthesis of Chlorophyll in Corn

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Summary. The effect of temperature and light intensity have been studied in relation to the greening of etiolated corn (Zea mays cv. Pioneer 309-B) seedlings. Chlorophyll accumulation is rapid at high temperature (28°) under all conditions of light intensity. At low temperature (16°), and particularly in combination with high light intensity (3000-4500 ft-c), the accumulation of both chlorophyll and carotene is inhibited.

Low pigment content at 16° is not directly due to a block in the pigment synthesizing mechanism, but rather to the photodestruction of chlorophyll prior to its stabilization in the membrane structure of the chloroplast lamellae. The parallel reduction in carotene content at high light intensity is probably a contributing factor, because of its role in protecting chlorophyll from photodestruction. The greater severity of photo-oxidation of chlorophyll at low temperature in corn when compared with wheat, appears to be due to a slower rate of protochlorophyllide synthesis and subsequent esterification. Thus in corn at 16° there is a prolongation of the photosensitive stage during chlorophyll synthesis. Photo-oxidation at 16° has also been shown to be a function of the incident light energy, with the photosynthetic pigments acting as receptors for their own destruction.

In comparison with the behavior of corn, wheat seedlings green rapidly at high light intensity at both 16° and 28°. This contrasting temperature response with respect to chlorophyll synthesis may underlie a fundamental difference in adaptation of these 2 species to growth in the temperate zones of the world.

Many tropical plants are unable to grow at temperatures well above freezing (25) and often show severe symptoms of chlorosis and even death, when germinated and grown at temperatures between 10 to 15° (17). This behavior is in sharp contrast with the active growth of temperate zone species when exposed to the same temperature range. Alleviation of low-temperature biochemical deficiencies by nutritional supplements has been reported for a few vascular plants (13, 18), but in general, this experimental approach has not been used as extensively as with bacteria (16). Biochemical lesions occurring in tropical plants may represent a breakdown in the genetic control mechanism for enzyme production, or a disturbance of metabolic reaction rates, or cold-induced overbonding of 1 or more particularly susceptible enzymes (17). Through any or all of these means, lowered temperatures may result in a reduction in the rate of synthesis of some essential substance, and growth will be limited.

Chlorosis, which is apparent in many thermophilic plants subjected to low temperature, may occur because the synthesis of chlorophyll precursors is depressed. Another possibility is that the rate of photodestruction of chlorophyll and associated pigments occurs more rapidly than synthesis. Evidence for rapid photodestruction has been found in wheat and barley (3, 22, 23, 24).

In the present experiments, the effects of both temperature and light intensity have been studied in relation to the greening of etiolated corn seedlings. An attempt has been made to establish the degree of interaction of temperature and light; and, in particular, an assessment has been made of the importance of photobleaching under conditions that limit the rate of chlorophyll synthesis.

Materials and Methods

Seeds of corn (Zea mays cv. Pioneer 309-B) were germinated in moist vermiculite in the dark...
at 16° and 28° and grown until the first leaf ruptured the tip of the coleoptile sheath. In certain experiments, seedlings of winter wheat (Triticum aestivum cv. Knox) were used for comparative purposes. The wheat and corn seedlings were transferred from the dark directly into artificially lit growth cabinets maintained at either 16° or 28° constant temperature, and grown for 4 and 6 days respectively. The light was supplied by a bank of 125 w cool/white fluorescent tubes supplemented by 40 w incandescent sources. Light intensities in the cabinets were measured in ft-c using a cosine-corrected meter and were varied from 300 to 4500 ft-c by means of neutral density filters. Also, mylar coated with a UV absorbing film was used to exclude light transmission below 400 μm. Intensities were measured in μw·cm⁻² using a spectroradiometer (ISCO, Lincoln, Nebraska) when comparing transmission through interference filters.

Chlorophylls were extracted from leaf samples with 80 % (v/v) acetone and their concentration (expressed as μg/g fr wt) determined spectrophotometrically using Arnon's procedures (2). In severely bleached samples where little pigment remained, the chlorophyll concentration was obtained from fluorescence spectra resulting from excitation at 430 μm (7).

The tissues were extracted in very dim green light that was ineffective in transforming protochlorophyll. Equivalent solvent dilutions were used in all determinations. The relative concentrations of protochlorophyllide formed in the dark at 16° and 28° following a 5 minute exposure to red light (1000 μw·cm⁻²) were obtained from fluorescence spectra following excitation at 430 μm (7). For carotenoid determinations the leaf samples were extracted in a mixture of acetone and hexane, and after removal of chlorophylls and xanthophylls on a column, absorbance was read at 436 μm in a spectrophotometer (12).

For electron microscopy, small pieces of leaf tissue were fixed in glutaraldehyde-potassium permanganate, dehydrated in alcohol and embedded in epon. Sections were cut on an ultramicrotome. They were then examined and photographed with a Phillips EM 200.

**Results and Discussion**

The effect of increasing light intensity on the chlorophyll content of corn after 6 days and wheat seedlings after 4 days at 16° and 28° is shown in figure 1. At 28°, both species greened rapidly at all light intensities and chlorophyll was visible within a few hours’ exposure. Corn accumulated slightly more chlorophyll at low light intensity than at high and wheat the reverse, but it is not known for how long these trends persist. At 16° there was a marked species difference. Wheat produced approximately the same amount of chlorophyll at all light intensities but chlorophyll accumulation in corn was severely restricted at the higher light intensities. At 4500 ft-c the seedlings appeared bleached and contained only a small amount of chlorophyll a and no chlorophyll b. Protection from light transmission below 400 μm resulted in only a small improvement in chlorophyll accumulation, in proportion to the reduced light intensity. At high light intensities, all corn seedlings died as soon as seed reserves were exhausted.

The concentration of carotene was also determined in corn seedlings at 16° over a range of light intensities. At 300 ft-c there was an accumulation of carotene over and above the level in the dark grown seedlings, but at higher intensities the level declined, and at 4500 ft-c only 25 % of that present on exposure of the seedlings to light was found, (fig 1). The parallel reduction of both chlorophyll and carotene at high light intensity is undoubtedly related. Carotenoids are known to participate in the capture and transfer of light energy. They also function as an important light screening pigment, and play a role in protecting chlorophyll and other porphyrins such as catalase and cytochrome from photodestruction (1,11,19).

The contribution of photosynthate to the green-
The correlation between photobleaching and the total amount of white light received can be interpreted to mean that the metabolic pathway for the formation of protochlorophyllide is blocked or inactivated, or that the unphytylated chlorophyll itself is undergoing photodestruction as suggested by Augustinussen and Madsen (3). The first alternative was investigated by feeding [(4C)]leuvulinic acid to the leaves of corn seedlings during greening at 16°C. Activity was recovered in acetone-ether extracts of seedlings treated at 300, 2000 and 4500 ft-c. Apparently, porphyrin and possibly chlorophyll synthesis occurred even at the highest light intensity. Without definite identification of intermediates, however, it is not profitable to say more about them.

Electron micrographs of chloroplasts from corn leaves bleached at 4500 ft-c (16°C) show a higher degree of organization than found in dark grown plastids. A primary lamellar structure is well developed and in some sections the remains of the prolamellar body can be seen. In no instance, however, was there evidence of grana formation. This may be related to the absence of chlorophyll b, or to the low concentration of chlorophyll a as energy absorbers in the plastids. Similar deviations from normal plastid development have been described in cases where etiolated leaves were exposed to low light (6) or low temperature (14). In both of these reported instances only a very limited accumulation of chlorophyll occurred.

The results thus far described, plus the observation that light-bleached corn seedlings green rapidly at high light intensity when the temperature is raised to 26°C indicates that light when used in conjunction with low temperature does not damage the mechanism leading to chlorophyll production.

Bleaching appears to result not only from photo-oxidation of chlorophyll but of chlorophyllide as well (4). During the lag phase in greening, following exposure of etiolated seedlings to light, a change was observed in the absorption maximum of chlorophyllide from 685 to 673 mµ. This absorption shift possibly results from disaggregation following disruption of proplastid structure (5), or the separation of chlorophyllide from the holochrome, and a free condition prior to phytlylation and incorporation into the membrane structure of the lamellae (10). Both forms of chlorophyllide have been shown to be photolabile. Stability is achieved only after phytlylation and an ordered association with carotenoids and other components of the photosynthetic apparatus is achieved in the lamellae (9).

Since protochlorophyllide synthesis is temperature sensitive (22), any reduction in the rate of its synthesis caused by exposure to low temperature would tend to accentuate apparent photodestruction (20,21,23). At 28°C the initial rate of synthesis was very rapid; and, after 10 to 15 minutes in the dark, synthesis was virtually complete. At 16°C, the initial rate was only about 17% of the rate at 28°C.

![Graph](image-url)

**Fig. 2.** Effect of high light intensity (4500 ft-c) on chlorophyll accumulation over a 4-day period in corn and wheat seedlings previously greened at low light intensity. Points on ordinate represent chlorophyll concentration at 1x. Temperature throughout 16°C, plants exposed to 4500 ft-c after preliminary greening; ------, controls retained at 300 ft-c.

ing of etiolated tissue has been investigated by Klein and Neuman (15). They showed that inhibition of photosynthesis by C.M.U. [(3-4 chlorophenyl)-1,1-dimethyl urea] causes a reduction in chlorophyll synthesis, but this effect is not specific, and was overcome by providing the tissue with sugar. In the present experiment, 0.2 m sucrose was supplied to plants continuously during high light exposure at 16°C but chlorophyll accumulation was not promoted.

Once chlorophyll has formed and, presumably, became complexed with the chloroplast lamellae, it appears to be protected from rapid photodestruction. Corn plants allowed to green at 16°C and 300 ft-c retained their chlorophyll when subsequently exposed to 4500 ft-c for 96 hours. As anticipated, a similar result was obtained with wheat (fig 2).

Photo-oxidation of chlorophyll and carotene in corn at 16°C, during the greening phase, appears to be a function of the incident light energy, with the photosynthetic pigments acting as the primary receptors. The rate of photo-oxidation of chlorophyll extracts was measured immediately following exposure to 3 narrow bands of the visible spectrum. Intact plants were not used because of the difficulty of obtaining light of the required wavelength at an intensity sufficient to cause photobleaching. Absorbance by acetone extracts of corn chlorophyll was measured in a spectrophotometer following exposure at 16°C to light at 3 different wavelengths, but of equal intensity and energy value (500 \( \mu \text{W cm}^{-2} \)). Although the spectral sample was limited, it appeared that the efficiency of photo-oxidation followed a typical absorption spectrum for chlorophyll, with the maximum rate of photo-oxidation in the red (650 mµ) and blue (450 mµ) and the lowest in the green (550 mµ).

The correlation between photobleaching and the
and this early difference in chlorophyll production was not made up even after 1 hour in the dark (fig 3). Thus with corn, low temperature causes a reduction in the rate of protochlorophyllide synthesis and hence in the rate of chlorophyllide accumulation. Furthermore, the work of Wolff and Price (26) indicates that the enzyme-catalyzed esterification of chlorophyllide to form chlorophyll a is a temperature, as well as light, sensitive step.

As a result, at low temperatures (around 16°) the final stage in chlorophyll synthesis in corn was prolonged, and exposure to high light intensity caused photodestruction of pigment at a rate faster than its formation. At the higher temperature (28°) there was a rapid accumulation of chlorophyll even at high light intensity. A favorable differential in the rate of chlorophyll synthesis versus destruction at 16° can account for the different greening response of wheat when exposed to high light intensity.

Both corn and wheat are crop plants descended from wild ancestral forms that originated in tropical and temperate zones, respectively. It seems likely that the genera to which these species belong have different temperature ranges for several enzyme systems. Temperate zone plants can function competitively over a temperature range from near 0° to 35°, whereas the corresponding range for tropical zone plants is considerably higher, from about 15° to 45° (17). In the non-overlapping regions, temperature induced metabolic lesions are likely to be found. Bleaching in corn at low temperature, as reported in this paper, is an example of a lesion occurring at a temperature corresponding with that of the coolest sections of the temperate zone. Bleaching at low temperature (16°) has also been found in 2 other tropical grasses *Pennisetum* and *Sorghum*. Possibly this is a general phenomenon among tropical species. An analogous high temperature effect has been found in a temperate species, Marquis wheat. When grown at 34°, it develops a severe chlorosis (8).

Temperature control of chlorophyll synthesis may well be of critical importance in adaptation of plants to the major climatic zones. Rapid formation of a functional photosynthetic system is crucial to survival.

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**Literature Cited**


