Light Quality Effects on Carbon Metabolism and Allocation in *Gracilaria verrucosa**

K. T. Bird**, C. J. Dawes and J. T. Romeo

Department of Biology, University of South Florida; Tampa, Florida 33620, USA

Abstract

Under conditions of new nitrogen availability and low light intensities, light quality (blue, green, or red light) was not found to affect carbon fixation patterns into major metabolic fractions (total ethanol soluble, carbohydrate, and protein) in preconditioned nitrogen enriched or limited apical tips of *Gracilaria verrucosa* Papenfuss. Within the ethanol soluble fractions of both nitrogen enriched and limited tips, blue light treatment led to a greater percentage of ^{14}carbon in amino acids as compared to non-ninhydrin reactive metabolites. A lesser effect was observed with red light, and green light did not appear to stimulate amino acid synthesis appreciably. The low intensity blue light effect in *G. verrucosa* appears to be an enhancement of non-photosynthetic carbon incorporation into amino acids, possibly through some form of the urea-ornithine cycle.

Introduction

The proportional allocation of carbon into different metabolic fractions such as proteins, carbohydrates, and amino acids is known to be influenced by both light intensity (Horváth and Szász, 1965; Morris *et al.*, 1974) and light quality (Ogasawara and Miyachi, 1970; Voskresenskaya, 1972; Miyachi *et al.*, 1978). Light quality effects on carbon allocation in algae are particularly noteworthy, as increased water depth causes selective filtering of certain portions in the incident light quality spectrum. In clear waters, low intensity blue light predominates at greater depths, while in turbid waters, the spectrum is shifted to accentuate the proportion of low intensity green light (Jerlov, 1970). The research on light quality effects, mostly comparing blue to red and/or white light, has generally pointed to an enhancement by blue light of carbon flow into nitrogenous metabolites such as amino acids, RNA, and proteins at the expense of carbohydrates (Kowallik, 1962; Steup and Ssmark, 1978; Miyachi *et al.*, 1978). It should be noted, however, that these workers have used algal cultures grown in a nitrogen enriched media, while in field conditions, algae may be nitrogen deficient (Ryther and Dunstan, 1970; Hanisak, 1979). Most of this light quality research has not included a comparison of green light, presumably due to the high use of chlorophycean cultures.

The lack of information on light quality effects in other algal groups led us to investigate the effects of low intensity blue, green, and red light on carbon allocation patterns in a marine rhodophyte *Gracilaria verrucosa* Papenfuss. Miyachi *et al.* (1978) have described phosphoenolpyruvate (PEP) carboxylase as important in the blue light effect on *Chlorella ellipsoidea*, so the use of a rhodophyte is particularly interesting, since no PEP carboxylase activity has been noted in a variety of red algae (Kremer, 1978, 1979). Effects of nitrogen preconditioning on algal responses to light quality were examined in experiments using preconditioned nitrogen enriched and limited thalli in experimental media with sufficient inorganic nitrogen for metabolic syntheses.

Materials and Methods

Nitrogen preconditioning of experimental thalli was carried out in laboratory cultures (22 °C). Thalli were acclimated to low light intensities under white fluorescent lighting at 50 $\mu E \cdot m^{-2} \cdot s^{-1}$, ca 40 $\mu E \cdot m^{-2} \cdot s^{-1}$ above the photosynthetic compensation point of fresh field material (K. T. Bird, unpublished data). Varying the concentrations of NH_4NO_3 added to the cultures resulted in nitrogen enriched or limited thalli. Cultures

* Harbor Branch Foundation Contribution No. 237

** Present address: Harbor Branch Foundation; Box 196, Fort Pierce, Florida 33450, USA

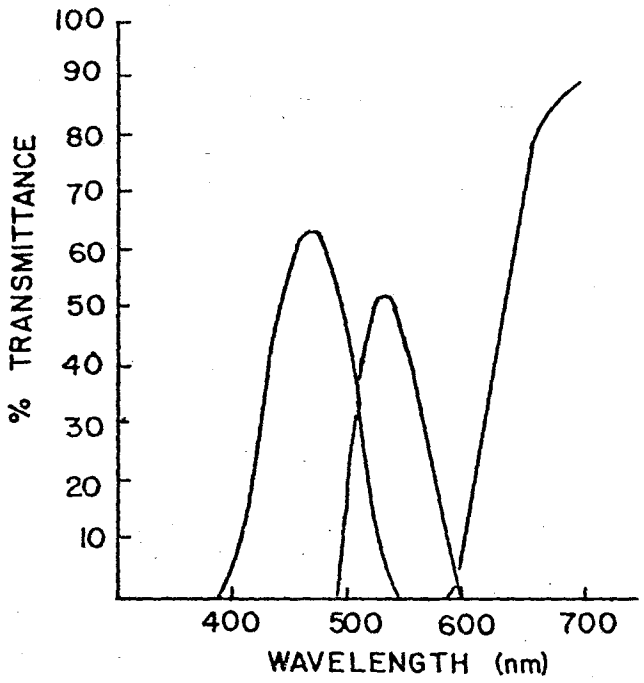

Fig. 1. Transmittance spectra of the Plexiglas filters: blue light, 380–540 nm, maximum at 475; green light, 480–600 nm, maximum at 530 nm; red light, 580–700

also contained a nutrient concentrate which added the following constituents to the ambient seawater ($mg \cdot l^{-1}$): KH_2PO_4, 20.4; $FeSO_4$, 1.4: Na EDTA, 1.5; $MnCl_2$, 0.01, and $NaHCO_3$, 5.0. The degree of nitrogen preconditioning was determined using a procedure similar to that of Hanisak (1979) in which apical tips (< 25 mm length) were cultured in 6 concentrations of NO_3^-, 8, 12, 20, 79, 104, and 204 μg-at $N \cdot l^{-1}$, in triplicate samples. The media was changed every 3 d for a period of 3 wk, after which growth was determined by increases in weight and the tips analyzed for percent protein (Lowry *et al.*, 1951) and carbohydrate (DuBois *et al.*, 1954). The whole procedure was again repeated. Only NO_3^- was used in these experiments to avoid NH_4^+ toxicity at higher concentrations. Ambient NH_4^+ concentration was 0.4 μg-at $N \cdot l^{-1}$. When the data was examined by a procedure similar to that of Hanisak (1979), it was found that those apical tips with a protein:carbohydrate ratio of 0.4 or greater were nitrogen enriched with respect to growth. The protein:carbohydrate ratios of the experimental nitrogen enriched tips were 0.50 ± 0.08, and for the nitrogen limited tips, 0.13 ± 0.04.

The experimental design consisted of using preconditioned nitrogen enriched and limited apical tips under blue, green, and red light treatments. The spectra of the filters used to produce these light quality regimens are depicted in Fig. 1. Photosynthetic rates were set equal under the different light quality treatments as light intensity, hence photosynthetic rates, can also affect carbon allocation patterns (Morris *et al.*, 1974; Miyachi *et al.*, 1978). The experimental media contained a high concentration of NH_4^+ and NO_3^- (20 μg-at $N \cdot l^{-1}$ of each). Ammonium nitrate was used to avoid pH problems sometimes encountered with use of a single nitrogen species (Haynes and Gon, 1978). Photosynthetic rates were monitored in a Gibson respirometer. Pretreatment consisted of holding the apical tips for 1.5 h in the dark, after which tips were exposed to different light treatments and photosynthetic rates set equal by varying light intensity. Experiments were run using triplicate samples, and then repeated. After a 15 min adjustment period under a light quality treatment, ^{14}C-bicarbonate was injected to produce a final concentration of 62 $\mu Ci \cdot m$ mol bicarbonate. After 30 min under the light quality treatment, the apical tips were removed, frozen in liquid N_2, and subsequently extracted to yield total ethanol soluble, TCA (soluble carbohydrate), NaOH (protein), and pellet fractions. Fractions were counted and corrected for quenching. The ethanol fractions were chromatographed to separated amino acids and a non-ninhydrin reactive fraction (Bird *et al.*, 1980). Carbohydrate and protein determinations were performed in the respective fractions. Controls using frozen-rethawed tips, as well as controls for excretion, were also performed under the 3 light treatments.

Results and Discussion

There were no apparent light quality effects on carbon allocation into major metabolic fractions (total ethanol, carbohydrate, and protein) in either nitrogen enriched or limited apical tips (Table 1). A Student's *t*-test ($P < 0.05$) indicated no significant differences between net ^{14}C fixation rates under the 3 different light treatments for either nitrogen enriched or limited tips. Slightly higher quantum irradiances were used for nitrogen limited tips as a high degree of unpredictability in photosynthetic rates had been found at lower light intensities (Table 1). The absence of light quality effects on carbon allocation into major metabolic fractions contrasts with the results of Miyachi *et al.* (1978). However, these workers used saturating light intensities for cultures of *Chlorella ellipsoidea*. The low light intensities employed here may not have increased metabolic rates sufficiently to affect carbon distribution in major fractions. Horváth and Szász (1965) noted similar responses by *Phaseolus vulgaris* at low light intensities. The controls showed that no significant excretion of metabolites occurred under the different light quality treatments, nor was there noticeable uptake of carbon by frozen-rethawed apical tips.

Analyses of the ethanol fractions revealed that significantly greater percentages of ^{14}C were found in amino acids after blue light treatments of both nitrogen enriched and limited apical tips than after red or green light. Red light showed a greater effect than green (Table 2). These data agree with the results of other workers who have also noted that blue light

Table 1. *Gracilaria verrucosa.* Total carbon fixation rates and distribution of ^{14}C in nitrogen enriched and limited thalli under blue, green, and red light. Numbers in parentheses are percentages of total. ($\bar{X} \pm SD$) $n = 6$

	Light treatment		
	Blue	Red	Green
		Nitrogen enriched thalli	
Light quantum irradiances ($\mu E \cdot m^{-2} \cdot s^{-1}$)	24	13	14
nCi $^{14}C \cdot g^{-1} \cdot h^{-1}$ in:			
Total	1309.8 ± 152.8	1398.4 ± 286.6	1386.0 ± 68.9
Ethanol fraction	648.5 ± 70.8 (52)	715.6 ± 126.7 (51)	696.1 ± 78.6 (50)
TCA fraction	497.4 ± 75.0 (41)	555.4 ± 126.0 (40)	568.3 ± 15.3 (41)
NaOH fraction	120.6 ± 26.6 (8)	121.1 ± 26.7 (9)	117.1 ± 17.5 (8)
Pellet	7.2 ± 0.8 (< 1)	9.3 ± 2.9 (< 1)	4.6 ± 0.7 (< 1)
		Nitrogen limited thalli	
Light quantum irradiances ($\mu E \cdot m^{-2} \cdot s^{-1}$)	30	30	35
nCi $^{14}C \cdot g^{-1} \cdot h^{-1}$ in:			
Total	2086.7 ± 94.6	2112.3 ± 198.4	2145.1 ± 76.6
Ethanol fraction	923.6 ± 67.8 (44)	925.0 ± 139.4 (44)	961.8 ± 47.1 (45)
TCA fraction	1005.7 ± 92.7 (48)	1017.9 ± 47.2 (48)	1036.2 ± 27.8 (49)
NaOH fraction	136.5 ± 7.7 (7)	142.7 ± 29.9 (7)	131.8 ± 12.3 (6)
Pellet	21.6 ± 2.8 (1)	26.8 ± 6.9 (< 1)	15.3 ± 4.3 (< 1)

Table 2. *Gracilaria verrucosa.* Percentage distributions of metabolites in ethanol fractions of nitrogen enriched and limited thalli under blue, green, and red light. ($\bar{X} \pm SD$) $n = 6$

	Blue	Red	Green
		Nitrogen enriched thalli	
Aspartate	25.2 ± 2.2	34.9 ± 3.1	20.5 ± 1.0
Glutamate	9.1 ± 1.5	6.9 ± 1.6	4.5 ± 1.0
Glycine-serine	28.1 ± 3.7	16.9 ± 2.9	15.3 ± 1.8
Citrulline	18.9 ± 1.5	17.5 ± 1.3	33.2 ± 1.9
Arginine	2.4 ± 0.2	1.9 ± 0.6	0.8 ± 0.4
Unknown*	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.1
Total	83.7 ± 0.2 >**	78.2 ± 0.5 >**	74.9 ± 1.9
Other***	16.3 ± 0.2	21.8 ± 0.6	25.1 ± 1.9
		Nitrogen limited thalli	
Aspartate	7.2 ± 2.0	6.6 ± 1.7	6.9 ± 1.0
Glutamate	11.4 ± 1.2	4.7 ± 0.2	3.6 ± 0.4
Glycine-serine	14.7 ± 1.8	20.9 ± 1.9	20.9 ± 2.4
Citrulline	30.6 ± 4.7	28.7 ± 5.9	14.8 ± 3.1
Alanine	4.9 ± 1.2	3.8 ± 1.3	2.8 ± 0.4
Total	68.5 ± 3.1 >**	63.7 ± 3.1 >**	49.1 ± 2.6
Other***	31.5 ± 4.1	36.3 ± 4.0	50.9 ± 2.3

* unknown is a guanidine amino acid (Bird *et al.*, in press)
** Analysis of variance, Tukey's w-procedure ($P < 0.05$). Totals calculated by subtraction. Percentage data transformed by arcsin $\sqrt{X}$
*** Non-ninhydrin reactive compounds

enhances amino acid synthesis (Hauschild *et al.*, 1962; Ogasawara and Miyachi, 1970).

The distribution of ^{14}C within the amino acids pools (Table 2) revealed differences in carbon allocation patterns due to light treatments and nitrogen status of the apical tips. In preconditioned nitrogen enriched tips, there was a greater percentage of ^{14}C incorporated into acidic amino acids (glutamate and aspartate) under blue and red light than under green. After green light exposure, citrulline contained the highest percentage of ^{14}C. In nitrogen limited tips under blue and red light, however, the greatest percentages of ^{14}C were found in citrulline. These patterns of carbon allocation probably resulted from both the pathway of carbon fixation into amino acids and the physiological status of the apical tips. Bird *et al.* (1980) suggested that non-photosynthetic carbon fixation in *Gracilaria verrucosa* proceeds through some form

of the urea-ornithine cycle into citrulline. Ogasawara and Miyachi (1970) found that low intensity blue light stimulates the non-photosynthetic incorporation of bicarbonate into amino acids, and noted that under low intensities of blue and red light, citrulline was the first stable product of carbon fixation (< 5 min) followed by subsequent fixation into acidic amino acids. In the nitrogen enriched apical tips under blue and red light, a similar fixation of carbon into citrulline and then into acidic amino acids might have occurred, but would not be detected due to the longer (30 min) time course of the experiments. The greater percentage of ^{14}C found in citrulline under green light, as well as the higher percentage of ^{14}C found in the non-ninhydrin reactive fraction indicated that *G. verrucosa* under low intensity green light is not highly active in amino acid synthesis. The high percentage of ^{14}C in citrulline may represent a pool of assimilated ammonium and fixed carbon which is not being actively metabolized.

The greater percentage of ^{14}C in citrulline from pre-condition nitrogen limited apical tips under blue and red light may be a result of the physiological nitrogen status of the plants. Nitrogen limited tips incorporated a greater percentage of carbon into carbohydrates and non-ninhydrin reactive metabolites (Tables 1 and 2). While other workers (Kanazawa *et al.*, 1970; Platt *et al.*, 1977; Paul *et al.*, 1978) have shown that nitrogen starved plant cells rapidly shift metabolism towards amino acid synthesis upon exposure to new nitrogen sources, it appears that some lag time may be involved before nitrogen limited *Gracilaria verrucosa* tips show metabolic patterns similar to those cultured in a nitrogen enriched media. This continued "carbohydrate orientation" may result in the accumulation of ^{14}C in citrulline. Under green light, preconditioned nitrogen limited apical tips show the greatest percentages of ^{14}C in non-ninhydrin reactive metabolites (50.9% non-ninhydrin reactive *vs* 49.1% in amino acids). This pattern also suggests that low intensity green light does not stimulate amino acid synthesis in *G. verrucosa*.

Several other differences between nitrogen enriched and limited apical tips were noted in regard to ^{14}C incorporation into amino acids. Under blue light, glycine and serine from nitrogen enriched apical tips contained more ^{14}C than was found in these amino acids under green and red light, while the reverse trend was evident in nitrogen limited tips exposed to blue light (Table 2). Patterns of ^{14}C fixation into these two amino acids by apical tips were similar in their relative importances to ^{14}C incorporation in other amino acids under dark held, respiring conditions (Bird *et al.*, 1980). It is interesting to note that alanine was detected in nitrogen limited thalli, particularly under blue light exposure, while none was detected in nitrogen enriched thalli. The flow of carbon into alanine may be important in responses of nitrogen limited apical tips to the new nitrogen sources in the media. Starved *Chlorella ellipsoidea* cells were also found to show enhanced carbon fixation in alanine upon exposure to blue light (Miyachi *et al.*, 1978).

With regards to the mechanism of blue light effects, Miyachi and co-workers, (Ogasawara and Miyachi, 1970; Kamiya and Miyachi, 1975; Miyachi *et al.*, 1978) have suggested that an effect of low intensity blue light is to stimulate PEP carboxylase activity, an enzyme undetected in *Gracilaria verrucosa* (Bird *et al.*, 1980). Considering the absence of PEP carboxylase and the possibility of non-photosynthetic carbon fixation into citrulline through some form of the urea-ornithine cycle, it appears that the effect of blue light is not to enhance a particular pathway, but rather to stimulate cellular responses favorable to rapid amino acid synthesis. Over longer time periods, enhanced amino acid synthesis and reduced carbon flow into non-ninhydrin reactive substances, such as carbohydrates, could conceivably affect the proportions of carbon fixed in protein and carbohydrate.

While it is difficult to separate the effects of light quality *vs* intensity, there is some evidence that blue light effects may be manifested under ecological conditions. Wallen and Geen (1971) suggested that light quality effects were responsible for differences in carbon allocation in natural populations of phytoplankton, and Dawes *et al.* (1974) observed a higher percentage of protein in *Eucheuma* spp. found in clear, deeper waters. These correlative data suggest that light quality may affect algal carbon allocation *in situ*.

Acknowledgements. The authors wish to thank Dr. A. Michaels for the spectral analyses of the filters. This research was supported in part by N.S.F. Grant OCE 78-14494.

Literature Cited

Bird, K. T., C. J. Dawes and J. T. Romeo: Patterns of non-photosynthetic carbon fixation in dark held, respiring thalli of *Gracilaria verrucosa*. Z. Pflanzenphysiol. Bd. *98*, 359–364 (1980)

Dawes, C. J., J. M. Lawrence, D. P. Cheney and A. C. Mathieson: Ecological studies of floridian *Eucheuma* (Rhodophyta: Gigartinales). III. Seasonal variation of carrageenan, total carbohydrate, protein, and lipid. Bull. mar. Sci. *24*, 286–299 (1974)

DuBois, M., K. A. Gilles, J. K. Hamilton, P. A. Rebers and F. Smith: Colorimetric method for determination of sugars and related substances. Anal. Chem. *28*, 350–356 (1954)

Hanisak, M. D.: Nitrogen limitation of *Codium fragile* subsp. *tomentsoides* as determined by tissue analysis. Mar. Biol. *50*, 333–337 (1979)

Hauschild, A. H. W., C. D. Nelson and G. Kortkov: The effect of light quality on products of photosynthesis in *Chlorella vulgaris*. Can. J. Botany *40*, 179–189 (1962)

Haynes, R. J. and K. M. Goh: Ammonium and nitrate nutrition of plants. Biol. Rev. *53*, 465–510 (1978)

Horváth, I. and K. Szász: Effect of light intensity on metabolic pathways in photosynthesis. Nature, Lond. *207*, 546–547 (1965)

Jerlov, N. G.: Light, general introduction. *In*: Marine ecology, pp 95–103. Ed. by O. Kinne. New York: Wiley 1970

Kamiya, A. and S. Miyachi: Blue light enhanced formation of phosphoenolpyruvate carboxylase in colorless *Chlorella* mutant cells. Plant and Cell Physiol. *16*, 729–736 (1975)

Kanazawa, T., M. R. Kirk and J. A. Bassham: Regulatory effects of ammonium on carbon metabolism in photosynthesizing *Chlorella pyrenoidosa*. Biochim. biophys. Acta *205*, 401–408 (1970)

Kowallik, W.: Über die Wirkung des blauen und roten Spektralbereichs auf die Zusammensetzung und Zellteilung synchronisierter *Chlorellen*. Planta *58*, 337–365 (1962)

Kremer, B. P.: Studies of $^{14}CO_2$-assimilation in marine rhodophyceae. Mar. Biol. *48*, 47–55 (1978)

Kremer, B. P.: Light independent carbon fixation by marine macroalgae. J. Phycol. *15*, 244–247 (1979)

Lowry, O. H., N. J. Rosebrough, A. L. Fan and R. J. Randall: Protein measurements with the folin phenol reagent. J. Biol. Chem. *193*, 265–275 (1951)

Miyachi, S., S. Miyachi and A. Kamiya: Wavelength effects on photosynthetic carbon metabolism in *Chlorella*. Plant and Cell Physiol. *19*, 277–288 (1978)

Morris, I., H. E. Glover and C. S. Yentsch: Products of photosynthesis by marine phytoplankton: the effect of environmental factors on the relative rates of protein synthesis. Mar. Biol. *27*, 1–9 (1974)

Ogasawara, N. and S. Miyachi: Regulation of CO_2 fixation in *Chlorella* by light of varied wavelengths and intensities. Plant and Cell Physiol. *11*, 1–14 (1970)

Paul, J. S., K. L. Cornwall and J. A. Bassham: Effects of ammonium on carbon metabolism in photosynthesizing isolated mesophyll cells in *Papaver somniferum* L. Planta *142*, 49–54 (1978)

Platt, S. G., Z. Plaut and J. A. Bassham: Ammonium regulation of carbon metabolism in photosynthetic leaf discs. Plant Physiol. *60*, 739–742 (1977)

Ryther, J. H. and W. M. Dunstan: Nitrogen, phosphorus, and eutrophication in the coastal marine environment. Science, N. Y. *171*, 1008–1013 (1970)

Steup, M. and V. Ssmark: Photoregulation of ribosomal RNA synthesis in a mutant of *Chlamydomonas reinhardii*. Ber. Deutsch. Ges. Bd. *91S*, 243–250 (1978)

Voskresenskaya, N. P.: Blue light and carbon metabolism. Ann. Rev. Plant Physiol. *23*, 219–234 (1972)

Wallen, D. G. and G. H. Geen: The nature of the photosynthate in natural phytoplankton populations in relation to light quality. Mar. Biol. *10*, 157–168 (1971)