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Effect of Light Intensity on Efficiency of Carbon Dioxide and Nitrogen Reduction in *Pisum sativum* L.[1]

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ABSTRACT

Photosynthetic efficiency, primary productivity, and N_2 reduction were determined in peas (*Pisum sativum* L. var. Alaska) grown at light intensities ranging from severely limiting to saturating. Plants grown under higher light intensities showed greater carboxylation and light capture potential and higher rates of net C exchange. Uptake of N_2, computed from measured C_2H_2 reduction and H_2 evolution rates, also increased with growth light intensity, while the previously proposed relative efficiency of N_2 fixation, based on these same parameters, declined. The plot of N/C ratios (total nitrogen content/plant dry weight) increased hyperbolically with light intensity, and the plot of N_2/CO_2 uptake ratios (N_2 uptake rate/net CO_2 uptake rate) increased linearly. Both plots extrapolated to the light compensation point. The data indicate that the relative efficiency of N_2 fixation is not necessarily correlated with maximum plant productivity and that evaluation of a plant's capacity to reduce N_2 is related directly to concurrent CO_2 reduction. A measure of whole plant N_2 fixation efficiency based on the N_2/CO_2 uptake ratio is proposed.

Symbiotic N_2 reduction in legumes has long been known to be a function of photosynthesis (21). Both long term (13) and short term (18) CO_2 enrichment studies have suggested that the availability of this molecule limits N_2 fixation in legumes, presumably through an effect on photosynthesis. The importance of suitable light intensity for optimum plant productivity is an elementary fact, which has led to sophisticated considerations of the role of leaf displays in the productivity of plant communities (15). Growth light intensity has been shown to affect symbiotic N_2 reduction (9, 12) and the inhibition of N_2 fixation by combined N (4). Thus, it seems likely that growth light intensity may cause changes in competitive interactions involving legumes in mixed or pure stands by altering the efficiency with which both CO_2 and N_2 are reduced.

This study was undertaken to correlate the rate of NCE[2] and the amount of C fixed with the rate and amount of N_2 fixation as a function of light intensity, to provide comparisons between different measures for the efficiency of photosynthesis, such as CE (20), NCE (22), and PE (11) with RE which measures N_2 fixation efficiency of nitrogenase (19), and to derive an expression for whole plant N_2 fixation efficiency which incorporates photosynthetic parameters. The effect of growth light intensity on N_2 fixation may be an important consideration in mixed crop and pasture ecology, where selection of plants, whose efficiency to fix N_2 is not imparied under conditions of partial shade, may be desirable to maximize fixed N input into the soil.

MATERIALS AND METHODS

Growth Conditions. Pea plants were maintained in a growth chamber as described previously (2), but at growth light intensities of 200, 400, 600, or 800 μEi. Initial seed weight was 0.22 to 0.26 g. Plants were assayed for N_2 fixation after a 5 hr light period; photosynthetic assays at each light intensity spanned the entire light period.

Carbon Dioxide Fixation. Assimilation of CO_2 by attached leaves was measured in a flow-through gas exchange system with apparatus and data-handling procedures as described by Augustine *et al.* (1). Two plants were selected from uniform stands of six replicates for photosynthetic measurements. Design of the CO_2 assimilation chamber permitted parts of the plant to be inserted in sequential steps without damaging the plant. NCE by the whole plant was determined at the growth light intensity with a CO_2 concentration of 300 μl/l by sequential insertion of different segments of the shoot. PE was measured on the sixth leaf at the saturating light intensity of 1,200 μEi, and the appropriate growth light intensity. PE was calculated as 100 × the ratio of NCE at growth light intensity/NCE at 1,200 μEi. NCE was determined in the fifth leaf at four CO_2 concentrations (50, 100, 150, and 300 μl/l) and a light intensity of 1,200 μEi regardless of growth light intensity. Internal leaf CO_2 concentrations were calculated from physical parameters measured at each of the four external CO_2 concentrations (1). A second order regression line was then computer-plotted through the data points determined by the CER and internal CO_2 concentration values (1). The light compensation point was established as the light intensity intercept of an extrapolated regression line computer-plotted through data points defined by the growth light intensities and the corresponding NCE values. The internal CO_2 compensation point (*x* intercept) and the slope of the regression line at the compensation point were determined by extrapolation. This slope, an indication of the leaf's capacity to respond to changes in ambient CO_2 concentrations, was used as a measure of carboxylation efficiency as proposed by Tregunna *et al.* (20). Leaf temperatures were maintained at 21 C in all cases. Leaf area was measured with a Lambda Instruments LI-3000 area meter.

Nitrogen Fixation. Production of H_2 and C_2H_2-dependent C_2H_4 by root nodules was determined by techniques and equipment described previously (2). Total N/plant was determined by Kjeldahl analysis (8).

RESULTS

Three measures of photosynthetic efficiency used in this study to evaluate plant response to different light intensities increased with increasing illumination. CE of the fifth leaf, an indicator of

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[2] Abbreviations: NCE: net carbon exchange; CE: carboxylation efficiency; μEi; microeinstein $\cdot m^{-2} \cdot sec^{-1}$; PE: photoefficiency; RE: relative efficiency of nitrogen fixation.

the plant's ability to respond to different CO_2 concentrations, increased linearly with growth light intensity (200, 400, 600 and 800 μEi) when plants were assayed under the same saturating (1,200 μEi) light intensities (Fig. 1). Concurrent measurements of NCE/leaf area in the same leaf (fifth) did not show saturation at the highest growth light intensity used, while the CO_2 compensation point was lowest at 600 μEi (Fig. 2). Photoefficiency was determined to measure the capacity of plants grown under different light intensities to respond to saturating light intensity. Plants grown under low light intensity showed a greater percentage increase in NCE when exposed to saturating light than did plants grown under high light intensity (Fig. 1). Specific activity of NCE (NCE/total plant leaf area) (Fig. 3) and the net CO_2 uptake rate of the whole plant (Fig. 4) reached maximum values near the growth light intensity of 600 μEi. The light compensation point was 5.1 μEi, a value within the range previously established (14) for photorespiring plants.

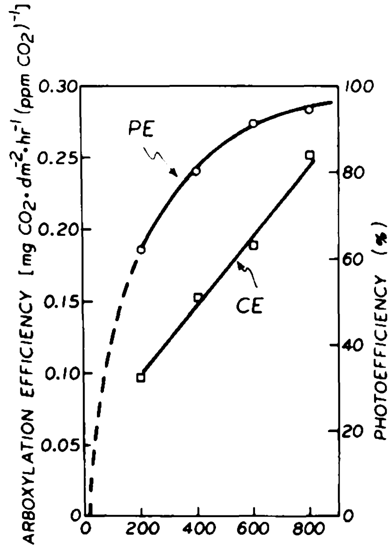

FIG. 1. Carboxylation efficiency (CE) and photoefficiency (PE) of peas grown at different light intensities. CE was measured on the fifth leaf and PE on the sixth leaf of 24-day-old pea plants. Each point on the CE curve represents the slope at the CO_2 compensation point of a regression line through data points obtained for net C exchange at four CO_2 concentrations. Measurements were made at 1,200 μEi regardless of growth light intensity. The PE curve (extrapolated to the light compensation point) indicates the response of plants grown at lower light intensities to a saturating light intensity (1,200 μEi). PE data were calculated as 100 × NCE at growth light intensity/NCE at 1,200 μEi.

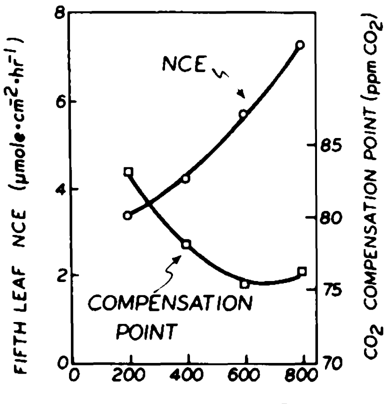

FIG. 2. Effect of growth light intensity on net C exchange rate and internal CO_2 compensation point in peas. Measurements were made on the fifth leaf at the saturating light intensity of 1,200 μEi.

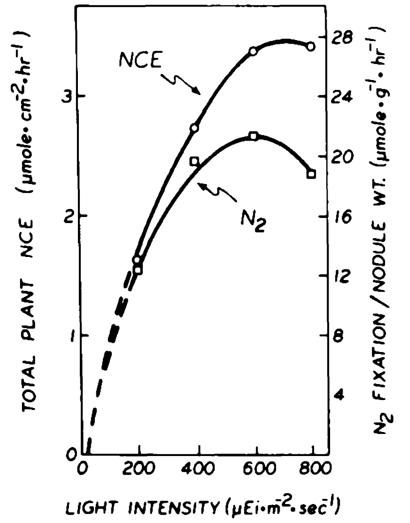

FIG. 3. Correlation of total net C exchange/leaf area with N_2 fixation/nodule wt by pea plants grown at different light intensities. N_2 fixation was computed by the formula (C_2H_2 reduced/3) × RE.

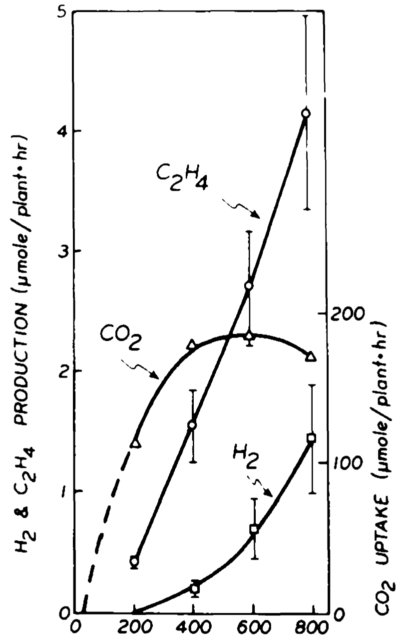

FIG. 4. Net CO_2 uptake by shoots and C_2H_2 reduction or H_2 evolution by root nodules of pea plants grown at different light intensities. Photosynthesis data were determined on two plants by sequential measurement of shoot segments, and root nodule data are means from four plants grown for 24 days at the light intensities indicated.

All plants were at the same stage of development on the basis of leaf numbers.

Both C_2H_2-dependent C_2H_4 production and H_2 evolution increased with increasing light intensity, although at different rates (Fig. 4). As a result, the RE expressed by Schubert and Evans (19) by the formula RE = 1 − H_2 evolved/C_2H_2 reduced, decreased with increasing light intensity (Fig. 5). Analysis of variance showed that differences in RE according to light intensity were highly significant ($P \leq 0.005$). Reduction of N_2, however, computed by the formula (C_2H_2 reduced/3) × RE increased linearly with light intensity (Fig. 5). The specific activity of N_2 reduction peaked at 600 μEi, showing a trend similar to the specific activity of NCE (Fig. 3). Ratios of total N to total plant dry wt were comparable in magnitude to values found in the literature (17) and approached saturation at 800 μEi (Fig. 6). This curve, when extrapolated, intersected the light intensity axis at the light compensation point. The increase in ratio of the rates of N_2 fixation to net CO_2 uptake was linear with light intensity, and the extrapolated x intercept also coincided with the light compensation point (Fig. 6). Nodule and plant dry wt and total N/plant values increased with light intensity (Table I).

DISCUSSION

The data demonstrate that variations in photosynthetic efficiency and plant productivity (Fig. 1 and Table I), which are produced by growth light intensity, are directly correlated with the rate of N_2 fixation (Fig. 5) and N accumulation (Table I). The rate of N_2 fixation and the proposed relative efficiency of N fixation (19) showed opposite trends with variation of growth light intensity (Fig. 5). For these reasons an alternate measure of whole plant N_2 fixation efficiency was derived on the basis of photosynthetic parameters (Fig. 6).

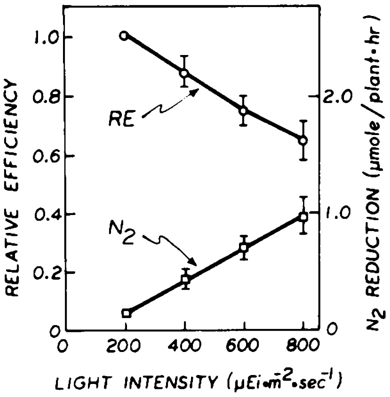

FIG. 5. Symbiotic N_2 fixation by pea plants grown at different light intensities. Reduction of N_2 was computed from C_2H_2 reduction and H_2 evolution data by the formula: (C_2H_2 reduced/3) × RE. Relative efficiency of N_2 fixation (RE), an expression proposed (19) for electron allocation by nitrogenase to reduce N_2 or H^+, is defined as RE = 1-H_2 evolved/C_2H_2 reduced. At the lowest light intensity, H_2 evolution, if present, was not detected by the analytical technique employed. The RE corresponding to the lowest detectable amount of H_2 (at the C_2H_4 level observed) would have been 0.95.

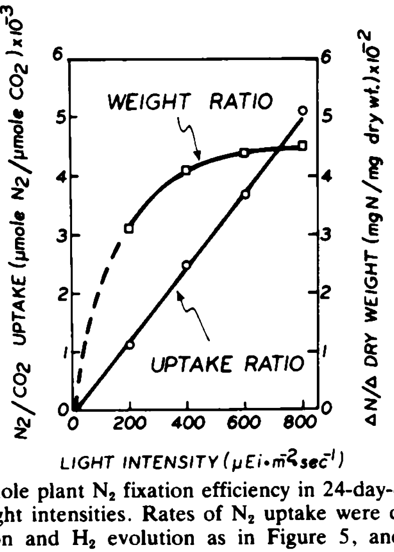

FIG. 6. Whole plant N_2 fixation efficiency in 24-day-old peas grown at different light intensities. Rates of N_2 uptake were calculated from C_2H_2 reduction and H_2 evolution as in Figure 5, and the net CO_2 uptake rate was measured directly (Fig. 4). The weight ratio data, determined as N content/dry wt after subtracting for N and C present in the seed, represent an integration of all N and C metabolism which occurred from the time of planting.

Table I. Leaf area, dry weight and nitrogen content of *Pisum sativum* grown at different light intensities.

Means ± standard error were calculated from 6 replicates, except for leaf area and nodule weight, which are respectively averages of 2 and 4 replicates. Average dry weight and N content of seeds were respectively 200 ± 5 mg and 7.8 ± 0.4 mg.

Light intensity (μEi)	Leaf area (cm^2)	Dry weight (mg)		Nitrogen content	
		Plant	Nodule	Plant (mg)	%
200	52.2	319 ± 16	10.6 ± 1.1	11.5 ± 0.6	3.6
400	65.3	419 ± 10	22.9 ± 1.6	16.8 ± 0.5	4.0
600	54.5	472 ± 47	32.0 ± 3.8	19.7 ± 1.9	4.2
800	49.7	568 ± 65	47.0 ± 6.9	24.5 ± 3.1	4.3

It has been shown that the light capture and carboxylation mechanisms in leaves develop differently under different growth light intensities (5, 6). In shade plants the light absorption function is dominant, whereas in sun-adapted plants carboxylation takes precedence. This concept is supported by data showing increased CE (Fig. 1) and decreased internal CO_2 compensation point (Fig. 2) with increasing growth light intensity. In addition, low PE (Fig. 1) in plants grown in dim light shows that these plants are capable of a greater increase in photosynthesis when exposed to saturating light intensity than plants grown in bright light. To correlate CO_2 reduction efficiency with N_2 fixation in plants grown under light intensities ranging from severely limiting (200 μEi) to saturating (800 μEi) levels, we therefore measured plant responses to changes in both irradiance levels and CO_2 concentration. The two measures of photosynthetic efficiency used, PE and CE, increased with increasing growth light intensity. NCE, a direct expression of the plant's capacity to assimilate CO_2, also increased with light intensity whereas the CO_2 compensation point reached a minimum value near 600 μEi (Fig. 2). The above data were obtained on individual leaves at the same stage of development because CE, NCE, and CO_2 compensation point change during ontogeny (2, 3).

Greater photosynthetic productivity/plant at higher light intensities (Table I) was correlated with greater nitrogenase activity/plant as shown by C_2H_2 reduction and H_2 reduction (Fig. 4) and N_2 reduction as computed from these data (Fig. 5). The specific activities of NCE and N_2 fixation (NCE/leaf area and N_2 reduced/nodule mass), however, did not exhibit a linear increase with light intensities (Fig. 3). The maximization of these parameters at 600 to 700 μEi may have resulted from artificially imposed limiting conditions, such as pot size and a uniform watering schedule, but the interdependence of the two processes is underscored by the coincidence of the peaks and the observation that both curves may be extrapolated to the light compensation point.

The decline in RE with increasing growth light intensity (Fig. 5) is not in contradiction to the opposite trend shown by photosynthetic efficiency, for the decline is not an indication of diminished nitrogenase activity. It can be interpreted as a shift in electron allocation (7) from N_2 to H^+ reduction at higher growth light intensities or as increased H_2 uptake by a hydrogenase (10) which may be preferentially expressed under the energy-deficient conditions at the lower growth light intensities. In the latter case hydrogenase activity would lower the amount of H_2 available for measurement, thus giving the appearance of lower H_2 evolution rates. The contrary trends of RE and of actual nitrogen fixation as computed from C_2H_2 reduction and H_2 evolution data (Fig. 5) as well as total N values (Table I) show that this proposed RE is descriptive only of electron allocation to N_2 or H^+ and not of the actual capacity of the symbiosis to fix N_2. Thus, the N_2/H^+ electron allocation ratio may be higher (*i.e.* RE greater) under conditions less favorable for photosynthesis than under favorable conditions, yet the latter could permit increased nitrogenase activity and greater N_2 uptake even at lower (less efficient) N_2/H^+ ratios.

The direct dependence of symbiotic N_2 fixation on photosynthesis suggests the importance of incorporating photosynthetic parameters into any expression of N_2 fixation efficiency in the *Rhizobium*-legume association. Possible expressions of efficiency include the ratio of change in total N during the growth period to change in biomass as a function of growth light intensity (Fig. 6). This plot shows that the N/C ratio approached a maximum value as growth light intensity increased, a possible indication of sink limitation (16) at higher light intensities. A more useful technique for expressing the relation between N_2 fixation and photosynthesis is to plot N_2/CO_2 uptake rates against growth light intensity (Fig. 6). When extrapolated, both lines plotted in Figure 6 intersect the x axis at the light compensation point, confirming the previously noted (12) dependence of N_2 fixation on the availability of photosynthetic products. It must be considered, however, that the N_2/CO_2 uptake ratio technique is presumably sensitive to short term fluctuations in environmental parameters and to the time of assay during the light/dark cycle. It is reasonable to assume that a plot of the N_2/CO_2 uptake ratio against any environmental parameter will vary with the direct influence of that parameter on the mechanisms and saturation characteristics of both N_2 and CO_2 uptake. The shape of the plot may also be affected indirectly by an interaction of the two processes. The straight line relationship between the N_2/CO_2 uptake ratio and light intensity (Fig. 6) is therefore considered to be a fortuitous result of the environmental parameters employed in this study.

The advantage of the N_2/CO_2 uptake ratio technique over the simpler dry wt ratio method is that N_2/CO_2 uptake ratio measurements provide for a calculation of short term N_2 and CO_2 reduction ratios which is limited only by the time required for new photosynthate to reach the root nodules. Thus, the effect of environmental parameters on the N_2/CO_2 uptake ratios can be determined allowing the system to achieve an equilibrium in a matter of hours. Such experiments are not feasible using N and C mass increments because the amounts of those elements present at the start of the experimental treatment are difficult to determine precisely. For this reason the N_2/CO_2 uptake ratio under different light intensities is proposed as a measure of whole plant N_2 fixation efficiency. On the whole plant level our proposed measure of N_2 fixation efficiency complements other existing measures such as the "electron-allocation coefficient" of Burns and Hardy (7) or the "relative efficiency of nitrogen fixation" of Schubert and Evans (19), which describe constituent phenomena on the enzymic and bacteroid levels. This proposed measure incorporates both interdependent parameters of the symbiotic association, which are of interest here: microbial variation in apparent N_2 fixation, and host plant variation in photosynthetic response to varying growth light intensity. These parameters seem to be particularly important as the search continues for *Rhizobium* and legume symbionts which reduce N_2 and CO_2 efficiently under field conditions where competition for light is often significant (15).

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