Effect of Light Intensity on Efficiency of Carbon Dioxide and Nitrogen Reduction in *Pisum sativum* L.\(^1\)

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GABOR J. BETHELENFAVY AND DONALD A. PHILLIPS
Department of Agronomy and Range Science, University of California, Davis, California 95616

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 CO\(_2\) assimilation and CO\(_2\) 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/C 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\(^1\) 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 impaired 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 \(\mu\)Ei. 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 \(\mu\)l/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 \(\mu\)Ei, and the appropriate growth light intensity. PE was calculated as 100 \(\times\) the ratio of NCE at growth light intensity/NCE at 1,200 \(\mu\)Ei. NCE was determined in the fifth leaf at four CO\(_2\) concentrations (50, 100, 150, and 300 \(\mu\)l/l) and a light intensity of 1,200 \(\mu\)Ei 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\(_2\)H\(_4\) 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; \(\mu\)Ei: microeinstein \(\cdot\) m\(^{-2}\) \cdot\) sec\(^{-1}\); PE: photosynthetic efficiency; RE: relative efficiency of nitrogen fixation.
the plant's ability to respond to different CO₂ 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 CO₂ 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 CO₂ 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 pea 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 CO₂ compensation point of a regression line through data points obtained for net C exchange at four CO₂ 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 CO₂ 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 N₂ fixation/nodule wt by pea plants grown at different light intensities. N₂ fixation was computed by the formula \((C_2H_4\text{ reduced}/3) \times RE\).

Fig. 4. Net CO₂ uptake by shoots and \(C_2H_4\) 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 \(C_2H_4\)-dependent \(C_2H_4\) production and \(H_2\) evolution were 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 \(RE = 1 - H_2\text{ evolved}/C_2H_4\text{ reduced}\), 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 N₂, however, computed by the formula \((C_2H_4\text{ reduced}/3) \times RE\) increased linearly with light intensity (Fig. 5). The specific activity of N₂ 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 
$mEi$ (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).

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 $mEi$) to saturating (800 $mEi$) 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 $mEi$ (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 the CO$_2$ 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 $mEi$ 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 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 the CO$_2$ 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

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

<table>
<thead>
<tr>
<th>Light intensity ($mEi$)</th>
<th>Leaf area (cm$^2$)</th>
<th>Dry weight (mg)</th>
<th>Nitrogen content (%</th>
<th>C / N</th>
</tr>
</thead>
<tbody>
<tr>
<td>200</td>
<td>52.2</td>
<td>319 ± 16</td>
<td>10.6 ± 1.1</td>
<td>3.6</td>
</tr>
<tr>
<td>400</td>
<td>65.3</td>
<td>419 ± 10</td>
<td>22.8 ± 3.4</td>
<td>4.6</td>
</tr>
<tr>
<td>600</td>
<td>54.5</td>
<td>472 ± 17</td>
<td>32.0 ± 3.8</td>
<td>3.9</td>
</tr>
<tr>
<td>800</td>
<td>49.7</td>
<td>568 ± 65</td>
<td>47.0 ± 6.9</td>
<td>3.1</td>
</tr>
</tbody>
</table>

Means ± standard error were calculated from 6 replicates, except for 4 replicates for the leaf 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.

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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 N₂ uptake even at lower (less efficient) N₂/H⁺ ratios.

The direct dependence of symbiotic N₂ fixation on photosynthesis suggests the importance of incorporating photosynthetic parameters into any expression of N₂ 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 N₂ fixation and photosynthesis is to plot N₂/CO₂ 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 N₂ fixation on the availability of photosynthetic products. It must be considered, however, that the N₂/CO₂ 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 N₂/CO₂ uptake ratio against any environmental parameter will vary with the direct influence of that parameter on the mechanisms and saturation characteristics of both N₂ and CO₂ uptake. The shape of the plot may also be affected indirectly by an interaction of the two processes. The straight line relationship between the N₂/CO₂ 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 N₂/CO₂ uptake ratio technique over the simpler dry wt ratio method is that N₂/CO₂ uptake ratio measurements provide for a calculation of short term N₂ and CO₂ 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 N₂/CO₂ 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 N₂/CO₂ uptake ratio under different light intensities is proposed as a measure of whole plant N₂ fixation efficiency. On the whole plant level our proposed measure of N₂ 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 N₂ 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 N₂ and CO₂ efficiently under field conditions where competition for light is often significant (15).

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