The Green Revolution of the last decade has seen the yield of some food crops at least doubled by genetic alterations of plant stature and the ability of plants to respond to increased fertilizer. Since only 5–10% of the dry weight of plants comes from minerals and nitrogen in the soil, it is becoming more difficult to obtain further increases in productivity by this approach. Even scientists associated with the Green Revolution believe they have reached a plateau by these methods (1). Therefore, the next large increases in productivity must come from increasing the 90–95% of the dry weight that comes from the assimilation of airborne CO2 during photosynthesis.

The productivity of plants (dry weight per unit of ground area) is determined by the gross CO2 assimilation during photosynthesis minus the CO2 released during respiration. The “dark” respiration processes of green plants, which probably also occur as well in the light, are biochemically similar to those found in animal tissues and many microorganisms, and are essential for the growth and maintenance of plant cells. It is not certain whether all of the dark respiration is essential, or whether some portion of it may sometimes be uncoupled from ATP production. This wasteful portion of respiration could cause diminished productivity (2). However, it is well documented that many plant species and varieties respire away large portions of their recently fixed CO2 during illumination by an entirely different biochemical process of respiration, known as photorespiration. Since photosynthesis is often much faster in terms of CO2 production than dark respiration, photorespiration greatly lowers plant productivity where this occurs. Some biochemical and plant breeding experiments will be described that hold promise for retention of much of the photorespired CO2, leading to large increases in crop yields.

Relation of net photosynthesis to productivity among species

Crop species vary greatly in their yields of dry weight. For example, from statistics of average market yield in the United States (Table 1), one can calculate that the crop growth rate for maize silage, sorghum silage, and sugarcane (cane) is at least double that for spinach, tobacco (leaf plus stalk), and hay grasses (3). The higher-yielding leafy species all have low fluxes of photorespiration compared with the less efficient species.

Table 2 contains typical values of CO2 assimilation taken from the literature; it shows that much faster rates are usually found in the higher-yielding tropical grasses and in some weeds, as compared with many common crop plants—including spinach, tobacco, and orchard grass—that are lower yielding. A large part of the differences in net photosynthesis between the efficient and nonefficient species can be explained by the much slower rate of photorespiration that is encountered naturally only in the efficient plants.

Characteristics of photorespiration

Since photorespiration is such an important process, it is interesting to speculate on why its discovery was so long delayed. Earlier attempts to detect it failed largely because experiments were unwittingly performed under conditions that tended to eliminate or obscure it—high concentrations of CO2, low light intensities, or low concentrations of O2. Photorespiration occurs by a biochemical mechanism very different from dark respiration, since the primary substrate glycolate is produced rapidly in inefficient photosynthetic species only under the opposite conditions of those just mentioned. Moreover, the absolute measurement of photorespiration (loss of fixed CO2) is difficult because it must be detected while the main flux of CO2 is towards assimilation in the leaf chloroplasts. Hence, the assay of photorespiration by any procedure that requires an analysis of the ambient atmosphere must underestimate it to some degree because of the competition for the released CO2 by the chloroplasts. Nevertheless, rates of photorespiration of more than 50% of net photosynthesis are often encountered in leaves, or at least 100 μmol of CO2 are evolved per mg of chlorophyll per hr (13 mg of CO2 dm⁻² hr⁻¹).

“Photorespiration” was first used in its present sense by Decker and Tió in 1959 (4) to explain the CO2 outburst after illumination shown by leaves of many species that results as an aftermath of photorespiration. About this time, studies on the inhibition of glycolate oxidation in vivo also showed that a large part of the CO2 assimilated during photosynthesis could be normally metabolized through glycolate, and sug-
Statistics, the that some of photosynthesis has been studied in experiments to consult a book with olism (3).

Photorespiration may be detected and assayed in several different ways; each method has certain advantages and pitfalls associated with it (3). An assay of photorespiration used most often in my experiments (16, 17) consists of labeling leaf disks under constant illumination with $^{14}$CO$_2$ after the disks have had a preliminary period in normal air at 30°-35°. The $^{14}$CO$_2$ is first released into a closed system under conditions where the $^{14}$CO$_2$ is completely fixed in about 15 min. The system, however, remains closed for 45 min to allow steady-state conditions to prevail. Then, at zero time, CO$_2$-free air is rapidly swept over the leaf disks (at least three flask volumes per min) and the $^{14}$CO$_2$ released from the tissue is collected and measured.

The CO$_2$-free air is used in the assay to change the main flux of CO$_2$ from the normal uptake during photosynthesis to a greater release of photosynthetic CO$_2$ to the atmosphere. Independent experiments have established that photorespiration is fairly constant between the range of "zero" CO$_2$ and 300 ppm (normal concentration) in the ambient atmosphere (18, 19). If a shorter time than 45 min is used for the period of $^{14}$CO$_2$ fixation in the assay, the light to dark ratio (see below) is little affected (17).

After a period in the light, the $^{14}$CO$_2$ released in a subsequent period of darkness is also measured. This procedure permits a calculation of the ratio of the $^{14}$CO$_2$ released during a fixed time, usually 30 min, after a constant rate is achieved in the light and dark (the light/dark ratio). Fig. 1 shows a typical assay performed with a standard tobacco variety and with hybrid maize. The light/dark ratio for tobacco is usually between 3 and 5, indicating that photorespiration exceeds dark respiration by about 3- to 5-fold. By comparison, this ratio for maize is usually less than 0.1, showing that this species has a very much slower rate of photorespiration than tobacco.

Glycolate biosynthesis and photorespiration

It has already been indicated that the biosynthesis and further metabolism of glycolate have many characteristics in common with those of photorespiration; this similarity suggested that photoreceptor CO$_2$ might be derived from the breakdown of glycolate. Direct evidence for the role of glycolate in photorespiration comes from experiments on conditions for $^{14}$CO$_2$ release by leaf tissue supplied with $^{14}$C-labeled glycolate, and by use of metabolic inhibitors of the oxidation of glycolate in vivo.

Although glycolic acid is a simple two-carbon compound,
CH_3OH—COOH, there is still uncertainty about the mechanism of its biosynthesis in photosynthetic tissues. It is well established that rapid synthesis occurs in light (probably in the chloroplasts), and in the relatively low concentrations of CO_2 and high concentrations of oxygen normally found in the atmosphere. These characteristics consistent with its role as the primary substrate of photosynthesis.

Several biochemical mechanisms for glycolate biosynthesis have been proposed (3); all of them have some merit and plausibility, although none can account for all of the known properties of the biosynthetic pathway that produces this substance. The older proposals suggest that glycolate arises from a two-carbon fragment derived from the Calvin photosynthetic carbon reduction cycle, that it is produced from a novel carboxylation reaction, or that it is synthesized by the reduction of glyoxylate. The major obstacle to agreement on the importance of any one mechanism is that none of the reconstructed biochemical systems can synthesize glycolate at rates anywhere near those required of a substrate of photosynthesis; that is, at rates that are 50% or more of net photosynthesis.

Most recently, Ogren and his colleagues (20, 21) have discovered that phosphoglycolate is synthesized from ribulose diphosphate in the presence of purified ribulose diphosphate carboxylase in an atmosphere of O_2. Since an active phosphoglycolate phosphatase is present in leaves (22), this reaction would provide a reasonable pathway for glycolate biosynthesis. However, under optimal conditions (100% O_2), the rate of phosphoglycolate formation was only 15% of the rate of CO_2 fixation under optimal conditions by the enzyme (23). Hence, these rates also appear to be too slow to account for the rate of glycolate biosynthesis needed for photosynthesis.

There is no reason for believing that CO_2 is fixed by only one carboxylation reaction in any photosynthetic system (3). Neither is there any reason for believing that one pathway of glycolate synthesis is dominant in all tissues. Experiments with labeled precursors have shown that multiple pathways of glycolate synthesis exist simultaneously in Chlorella (24, 25) and also in higher plants (26). Glycolate is synthesized rapidly in light from ^14CO_2 in leaves with high rates of photosynthesis, such as tobacco (and the carbon atoms of the glycolate produced have a specific radioactivity similar to that of the ^14CO_2 supplied). It is synthesized slowly—but more readily from organic acids (pyruvate, phosphoenolpyruvate)—in leaves with slow rates of photosynthesis such as maize, as compared with tobacco (26).

Specific inhibitors of glycolate synthesis that would not inhibit CO_2 assimilation during photosynthesis would be most useful for study of the mechanism of biosynthesis, as well as for an evaluation of glycolate metabolism in photosynthesis. Such inhibitors are being sought. Even though the biosynthetic pathway is uncertain, it is possible to assay for potential inhibitors of glycolate biosynthesis by floating leaf disks on solutions of the compound to be tested and then measuring the initial rate of glycolate accumulation after adding an effective inhibitor of glycolate oxidase. In this way, I found that isonicotinic acid hydrazide (INH), which Goldsworthy (16) had shown to be an inhibitor of photosynthesis, slows the rate of glycolate synthesis under conditions similar to those used to inhibit photosynthesis (26).

**Glycolate oxidation and photorespiration**

Since glycolate is synthesized rapidly by leaves with high rates of photosynthesis (see below), yet is normally found in low concentrations (less than 0.4 µmol/g of fresh weight), it must turn over very rapidly. The oxidation of glycolate in leaves is catalyzed by an active enzyme, glycolate oxidase, which was first described by Kolesnikov (27) and Clagett et al. (28). The enzyme is a flavoprotein (29, 30) that catalyzes the reaction:

\[ \text{CH}_3OH—\text{COOH} + \text{O}_2 → \text{CH}_2—\text{COOH} + \text{H}_2\text{O} \]  

[1]

In the absence of catalase, the glycolate formed is oxidized nonenzymatically by the H_2O_2 to produce formate and CO_2, the CO_2 arising from the carboxyl-carbon of glycolate:

\[ \text{CH}_2—\text{COOH} + \text{H}_2\text{O} → \text{H}_2\text{COOH} + \text{CO}_2 + \text{H}_2\text{O} \]  

[2]

Reaction [1] has been found localized in the peroxisomes of leaf cells (31), and these organelles contain a large excess of catalase activity. Thus, if the production of CO_2 from glyoxylate occurs in a manner similar to Reaction [2], it must occur somewhere in the cell other than the peroxisomes.

**Table 3. Requirements for the enzymatic decarboxylation of [1-^14C]glyoxylate by spinach chloroplasts (ref. 35)**

<table>
<thead>
<tr>
<th>Reaction mixture</th>
<th>^14CO_2 produced (µmol/mg of chlorophyll per hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete</td>
<td>61.0</td>
</tr>
<tr>
<td>No Mn</td>
<td>3.1</td>
</tr>
<tr>
<td>Dark</td>
<td>14.6</td>
</tr>
<tr>
<td>N_2 atmosphere</td>
<td>4.8</td>
</tr>
<tr>
<td>Heated chloroplasts</td>
<td>7.9</td>
</tr>
<tr>
<td>No chloroplasts</td>
<td>8.6</td>
</tr>
</tbody>
</table>

The complete mixture consisted of [1-^14C]glyoxylate (10 mM), MnCl_2 (10 mM), chloroplast suspension (32 µg of chlorophyll), and HEPES buffer at pH 7.6 (20 mM) in a final volume of 1.0 ml; 30º; white light (0.14 cal cm^{-2} min^{-1}; 400–700 nm).
Leaf disks 1.6 cm in diameter were cut with a sharp punch, floated on water at 30°, and illuminated with 16,000 lux in air. After 90 min, the water was withdrawn and replaced with 10 mM α-HPMS for the times shown; the leaf disks were then killed (six disks with a fresh weight of 240 mg in each sample) and analyzed for glycolate accumulation. The different values shown for some experiments were obtained from leaves excised from different plants.

Some investigators believe that glyoxylic acid must first be converted to glycine, and that the CO₂ in photosynthesis is produced during the complex reaction by which two glycine molecules condense to produce serine, as shown in Fig. 2 (32, 33). Recent experiments comparing tissues supplied with [1-14C]glycolate and [1-14C]glycine suggest that the condensation of glycine to serine does not produce sufficient CO₂ to account for photosynthetic rates (34), and that a direct photooxidative decarboxylation of glycolate (Fig. 2) is more likely to be the primary source of this CO₂ (35). This view is supported by the demonstration that isolated chloroplasts can perform the rapid decarboxylation of [1-14C]glycolate to form the products shown in Reaction [2] in the presence of manganous ions, light, and oxygen (Table 3). These requirements, and the rates obtained, are consistent with the participation of such a reaction in the photosynthetic process.

**Inhibition of glycolate oxidase**

Bisulfite addition compounds of aldehydes are salts of α-hydroxysulfonates, and have the general structure R—CHOH—SO₂Na. Such compounds are analogs of glycolate and are effective at low concentrations as competitive inhibitors of purified glycolate oxidase (36). The most effective inhibitor of glycolate oxidase in vivo is α-hydroxy-2-pyridinemethanesulfonic acid (α-HPMS) (5). The uptake of [14C]CO₂ was not inhibited in tobacco leaf disks floated on 10 mM α-HPMS, and glycolate accumulated at an initial rate of 40 μmol/g of fresh weight per hr at 30° during exposure to the inhibitor for up to 10 min in light (37).

Under optimal conditions in air at 30°, with 2- to 3-min treatments with α-HPMS, average initial rates of glycolate accumulation, or synthesis, were observed of 67 μmol for illuminated tobacco and 80 μmol/g of fresh weight per hr for sunflower (Table 4). Both of these species show rapid rates of photosynthesis, and the quantities of glycolate synthesized are sufficiently rapid to sustain photosynthesis if this amount of substrate is normally oxidized. The average initial accumulation for maize leaf disks under the same conditions during the linear phase of increase was 8.1 μmol/g of fresh weight per hr. Thus, the slow rate of photorespiration commonly observed in maize (Fig. 1) may be explained to a great extent by the much slower rate of glycolate production in this tissue.

Upon addition of α-HPMS to tobacco leaf disks under the conditions of the 14C assay for photosynthesis, the 14CO₂ released during illumination is severely inhibited as the glycolate concentration builds up in the leaf, while the rate of 14CO₂ output in darkness is unaffected (Fig. 3). These results provide some of the evidence that the substrate for photorespiration, unlike dark respiration, is glycolate.

Another characteristic of an efficient photosynthetic species — such as maize — is that leaf CO₂ assimilation increases greatly between 20° and 35°, while in less efficient species — such as wheat and tobacco — photosynthesis changes little over this range of temperature (Fig. 4). These differences on the effect of temperature on net photosynthesis are attributable to differences in photorespiration in the several species.

Treatment of tobacco tissue with α-HPMS at 35° increased the 14CO₂ uptake about 3-fold in short-time experiments (Table 5) when glycolate oxidation was inhibited. The inhibitor had little effect at 25° (38). Therefore, inhibition of the production of photorespiratory CO₂ temporarily converted normal tobacco into a tissue that mimics the photosynthetic rates of the more efficient maize. This result means that sufficient biochemical acceptors for CO₂ and an adequate photochemistry are not lacking in tobacco, but that the photosynthetic production of CO₂ must largely account for the differences, as cited in Table 2 and Fig. 4, that naturally occur in net photosynthesis between these species. Gross photosynthesis must normally also increase at warmer temperatures in species like tobacco, but it is usually masked by the rapid flux of photorespiration.

To account for these results of increased photorespiration masking increased CO₂ uptake, a model balance sheet of CO₂ uptake and evolution has been prepared (Table 6). The budget (3) suggests that the elimination of photorespiration in an inefficient species may increase net photosynthesis by 47% at 25°, and by as much as 170% at 35°.

**Control of photorespiration within a species**

Thus far, differences in photorespiration have been discussed that result in differences in CO₂ assimilation and plant pro-

**Table 3. Initial rates of glycolate accumulation in tobacco, maize, and sunflower leaf disks when glycolate oxidase is inhibited by α-HPMS (ref. 26)**

<table>
<thead>
<tr>
<th>Exp. no.</th>
<th>Time in 10 mM α-HPMS (min)</th>
<th>Glycolate accumulation (μmol/g fresh wt per hr)</th>
<th>Tobacco</th>
<th>Maize</th>
<th>Sunflower</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3</td>
<td>50.0</td>
<td>4.2</td>
<td></td>
<td>61.6</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>48.8</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>82.6</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>72.6</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>62.6</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>70.0</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>90.0</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>85.0</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>92.5</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>—</td>
<td>13.0</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>—</td>
<td>7.1</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

**Fig. 3.** 14CO₂ released by tobacco leaf disks in the presence of α-HPMS at 35°. The inhibitor affects the rate of light, but not dark, respiration. At zero time (see Fig. 1), the fluid was replaced with water or 10 mM α-HPMS. Stomatal widths were not affected by the treatment (17).
TABLE 5. Effect of α-HPMS on $^{14}$CO$_2$ uptake by tobacco leaf disks in light at 35° (ref. 38)

<table>
<thead>
<tr>
<th>Exp. no.</th>
<th>Min in $^{14}$CO$_2$</th>
<th>Disks in water</th>
<th>Disks in inhibitor</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>31</td>
<td>66</td>
</tr>
<tr>
<td>2</td>
<td>2.5</td>
<td>45</td>
<td>268</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>47</td>
<td>203</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>61</td>
<td>181</td>
</tr>
<tr>
<td>5</td>
<td>2.5</td>
<td>58</td>
<td>128</td>
</tr>
<tr>
<td>6</td>
<td>2.5</td>
<td>37</td>
<td>188</td>
</tr>
</tbody>
</table>

After leaf disks on water were kept for a preliminary period at 21,400 lux of illumination, the fluid in Warburg vessels was replaced by 10 mM α-HPMS (5 mM in Exp. 6). After 2 min, 3 μmol of $^{14}$CO$_2$ (initial concentration about 0.1%) was liberated into the gas phase for the time shown.

Productivity between species. Besides attempting to learn how to control photorespiration by biochemical methods, my colleagues and I have also been experimenting with the control of photorespiration within a species that usually shows high rates by the use of pedigree selection and genetic methods. In addition to the results described below, differences in photorespiration have been found in varieties of beans with the $^{14}$C assay (39) and in clones of ryegrass (Lolium) with an $^{14}$O$_2$ uptake assay (40).

Our first studies were concerned with a spontaneous mutation of the tobacco variety John Williams Broadleaf (JWB) possessing yellowish leaves, first described by Burk and Menser (41). The yellow mutant (JWB Mutant) is dominant (Su/su), and is viable only when heterozygous. When a yellow plant is self-pollinated, 25% of the progeny are white and do not survive (Su/Su), 50% are the yellow plants (Su/su), and 25% have dark-green leaves (JWB Wild or su/su). At high concentrations of CO$_2$ (0.45-9%) the mutant performs rapid photosynthesis (42), but at normal CO$_2$ levels it has an inferior CO$_2$ assimilation (43) and grows poorly. The low rate of photosynthesis and poor growth result from an unusually high rate of photorespiration in a normal environment (43).

Fig. 4. Effect of temperature on net photosynthesis in single leaves of several species at 300 ppm of CO$_2$ in air in bright light (3).
of photorespiration and unusually high (above 22 mg dm\(^{-2}\) hr\(^{-1}\)) rates of CO\(_2\) assimilation for tobacco. A similar proportion of efficient plants was obtained on self-pollination of low-photorespiration plants in each of the next two generations.

One of these plants in the third generation had a mean light/dark ratio of 1.7 and a net photosynthesis of 23.4 mg of CO\(_2\) dm\(^{-2}\) hr\(^{-1}\) when sampled over a 2-week period (Table 8). This plant in the third generation was compared with another plant with a high rate of photorespiration that was derived from self-pollinating a plant with a light/dark ratio of 3.8 and a net photosynthesis of 18.8 mg of CO\(_2\) dm\(^{-2}\) hr\(^{-1}\). The mean CO\(_2\) uptake in the high-photorespiration plant was 38% less than that of the low-photorespiration plant (17.0 mg of CO\(_2\) dm\(^{-2}\) hr\(^{-1}\)) over the 2-week period.

Thus far, our work on pedigree selection of plants with high and low rates of photorespiration in tobacco indicates that plants with low photorespiration and high photosynthetic rates when self-pollinated produce many more progeny with these characteristics than does self-pollination of plants with high rates of photorespiration. Our screening methods, based on the \(^{14}\)C assay, appear reliable, but they are cumbersome and, even with recent improvements, we are able to assay only eight plants each day. Reliable and more efficient screening methods permitting the assay of larger populations will be necessary before the genetics of photorespiration can be easily studied in a wide range of crop plants. Nevertheless, the 38% greater net photosynthesis shown in Table 8 in a normal tobacco plant with a lower than usual rate of photorespiration indicates that if this attribute could be fixed in an otherwise suitable genetic background, large increases in plant productivity would be obtained by the control of photorespiration.

Table 8. Variation in photorespiration and net photosynthesis in excised leaves of two tobacco plants in the third generation sampled at different times

<table>
<thead>
<tr>
<th>Low-photorespiration plant, N-7-1</th>
<th>High-photorespiration plant, N-7-4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Date sampled</strong></td>
<td><strong>Photospiration</strong></td>
</tr>
<tr>
<td></td>
<td>((^{14})CO(_2) released light/dark)</td>
</tr>
<tr>
<td>Dec. 31</td>
<td>1.5</td>
</tr>
<tr>
<td>Jan. 4</td>
<td>—</td>
</tr>
<tr>
<td>Jan. 12</td>
<td>—</td>
</tr>
<tr>
<td>Jan. 13</td>
<td>1.4</td>
</tr>
<tr>
<td>Jan. 13</td>
<td>2.2</td>
</tr>
<tr>
<td>Jan. 13</td>
<td>1.6</td>
</tr>
<tr>
<td>Mean 1.7</td>
<td>Mean 23.4</td>
</tr>
</tbody>
</table>

Photorespiration was determined by the \(^{14}\)C assay described in the text. CO\(_2\) uptake was measured at high illuminance in normal air in different leaves excised on the dates shown. There was little difference in the stomatal widths between leaves of the two plants.