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Improving photosynthesis and yield potential

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1. Introduction

Crop yield is fundamentally related to the (a) amount of solar radiation absorbed; (b) efficiency of solar energy use in photosynthesis; (c) translocation of photosynthate to sinks, especially sinks later harvested; (d) capacity for growth in sinks; (e) efficiency of converting photosynthate to new biomass; and (f) metabolic cost of maintenance. *Yield potential* has been defined as the yield of a cultivar grown in an environment to which it is suited, with ample nutrients and water, and with pests, diseases, weeds, lodging, and other stresses effectively controlled (Evans and Fischer 1999). In principle, it integrates the genetic limitations on (a)–(f) as expressed in yield. It is an upper limit to on-farm yield of a cultivar, based on empirical study of that cultivar. As distinct from yield potential, *potential yield* is the yield theoretically possible from a given amount of absorbed solar energy and a specified crop biochemical composition. It is a theoretical construct based on known stoichiometries of biochemical reactions.

Population growth, increased standards of living, and expanding uses of crop products in industrial processes may require significant increases in yield potential of major crops. In recent years, however, yield potential has increased slowly or not at all (Cassman et al. 2003). Genetic engineering offers opportunities to more rapidly increase yield potential — limited ultimately by potential yield — because genes from any organism, not just

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from sexually compatible plants, can be spliced into a crop's DNA. Moreover, genes might be improved by site-directed mutagenesis.

An idea underlying the yield potential concept is that there are genes related to yield under favorable conditions and other genes related to stress tolerance or avoidance (Evans and Fischer 1999). While improving yield potential is the focus herein, improving avoidance or tolerance of abiotic and biotic stresses is also critical to overall crop genetic improvement. In this regard, it is important that increased yield potential may enhance yield in stressful environments (Richards 2000).

This chapter suggests tactics, based in part on the possibilities of genetic engineering, for closing the gap between potential yield and yield potential. Photosynthesis is emphasized because a large fraction of the possible gain in yield potential is associated with greater photosynthesis, but improvements to any of items (a)–(f) might enhance yield potential.

2. Absorbing solar radiation

Solar irradiance at earth's surface (I_S, J m^{-2} s^{-1}) is the energy source for photosynthesis, but not all wavelengths of solar radiation are equally useful. Only radiation in the approximately 400–700 nm wave band contributes significantly to crop photosynthesis (Fig. 2.1), so that radiation is called photosynthetically active radiation, or PAR (McCree 1981). Moreover, the rate of photosynthesis is related better to the number of photons (or photon flux) of PAR than to the amount of energy in that PAR. This is because photosynthetic pigments absorb radiation one photon at a time, and each photon excites only one electron in a primary photochemical reaction.

Photosynthetic solar irradiance (I_P, J m^{-2} s^{-1}) is the part of I_S in the PAR wave band. Typically, $I_P = 0.50\ (\pm 0.02)\ I_S$. At a given place and time, I_P (and the associated photon flux of PAR) depends on earth-sun geometry and state of the atmosphere. In the tropics I_P varies little from month to month. Subtropical regions with limited cloud cover receive more solar radiation annually than many tropical locations, and have a modest seasonal cycle. While annual totals are smaller at midlatitudes than they are in the tropics, monthly totals of I_P at midlatitudes in summer can be larger than they are at tropical locations.

Only the fraction of I_P absorbed by a crop ($a_{P,crop}$) can be used for crop photosynthesis, so the product $a_{P,crop}\ I_P$ places an upper limit on the amount of crop photosynthesis possible. The remainder of I_P is reflected up to the sky ($r_{P,sky}\ I_P$) or absorbed by weeds or soil ($a_{P,noncrop}\ I_P$). For a sparse canopy, $a_{P,crop}$ is small, but for a healthy, dense, weed-free crop canopy, it might be about 0.93 (corresponding to $r_{P,sky} \approx 0.06$ and $a_{P,noncrop} \approx 0.01$).

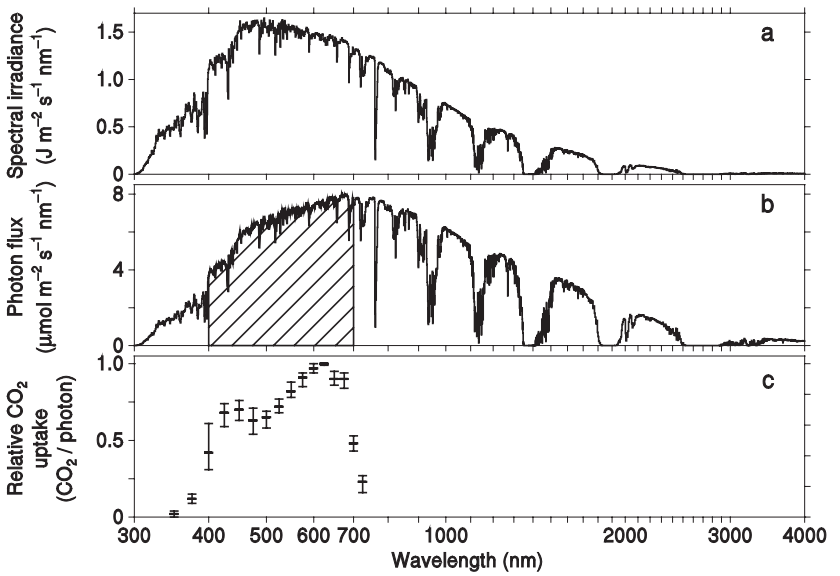

Fig. 2.1. (a) American Society for Testing and Materials G173-03 reference global solar spectral irradiance beneath the 1976 U.S. Standard Atmosphere with air mass = 1.5 (from http://rredc.nrel.gov/solar). This spectrum does not apply to any specific time or place. Dips in the spectrum are caused by atmospheric absorption. **(b)** Spectral photon flux density derived from (a), with the 400–700 nm wave band hatched. There are about 4.6 μmoles of photons per joule in the 400–700 nm wave band [energy in a photon (E, J) is given by $E = hc/\lambda$, where h is the Planck constant (662.6×10^{-36} J s), c is speed of light (299.8 Mm s^{-1}), and λ is wavelength (m)]. **(c)** *Relative* photosynthesis (CO_2 uptake) per photon (in 25-nm intervals) *incident* on leaves of eight field-grown crop species. Bars show minimum, mean, and maximum values among the species (tabulated in McCree 1972)

Some of $a_{P,crop}\ I_P$ is absorbed by entities other than photosynthetic pigments and is thus not available for photosynthesis. That fraction can be called *inactive absorption* as contrasted with *active absorption* by photosynthetic pigments. Inactive absorption might account for 10% of absorbed PAR in green leaves (Seybold 1933); the fraction in whole crops is poorly quantified. Reducing inactive absorption could enhance photosynthesis. A tactic is to quantify inactive absorption in crop leaves, determine if it is beneficial or unavoidable, and if not, reduce that absorption.

Although the 400–700 nm wave band is usually used to define the limits of PAR, photons with wavelength shorter than 400 nm can drive photosynthesis, but the epidermis usually absorbs them before they reach chloroplasts (McCree 1981). If epidermal transmittance of 330–400-nm photons could be increased without detriment to a crop, the PAR wave band for that crop would be increased. The potential increase in photosynthesis might be a few percent (see McCree and Keener 1974).

Whole-season photosynthesis and yield are related to whole-season PAR absorption, which depends on canopy duration (Watson 1958; Shibles and Weber 1965; Warren Wilson 1967; Monteith 1977; Lawlor 1995). Factors that can increase whole-season PAR absorption are rapid emergence and leaf expansion to cover the ground early in a crop cycle (leaf growth rate may be limited by low temperature early in the season) and increased leaf and canopy longevity. Rapid leaf expansion would be at the expense of leaf thickness. This might reduce early-season photosynthesis per unit leaf area, but it would normally be offset by increased photosynthesis per unit ground area. Traits for rapid leaf growth and PAR interception were discussed by Richards (2000).

Breeding for longer leaf duration apparently contributed to greater yield potential in several crops (Evans 1993; Tollenaar and Wu 1999), and durum wheat mutants with delayed leaf senescence out-yielded their parental line in a glasshouse environment[1] (Spano et al. 2003). Effects of delayed leaf senescence on yield may be particularly important during grain and tuber filling periods. There is genetic variation in leaf longevity in crop species, and lengthening canopy duration may be the simplest way to increase whole-season photosynthesis through genetic changes (Richards 2000). A potential tradeoff exists between delayed leaf senescence and crop nitrogen use. Maintaining green leaves with large protein contents to the time of crop maturity implies significant nitrogen in crop residue. This can be thought of as inefficient nitrogen use.

Where cold winters occur (i.e., locations with extended periods of mean 24-hour temperature below about 10°C, corresponding roughly to latitudes greater than 30°), crop growth is limited to summers. A goal for such locations is to grow and maintain a healthy canopy during as much of the summer as possible, which corresponds to the period of greatest I_P. Where all or most of the year is favorable for crop growth (i.e., locations that are warm year-round without significant dry periods, or that are irrigated), most of the annual integral of I_P can be used for crop photosynthesis by (a) using crops such as sugarcane or sugarbeet that can grow up to a full year before harvest, or such as alfalfa, which can be harvested repeatedly, or (b) planting a series of crops on the same land during a year. The latter may depend on early maturity by each crop in the series rather than extended growth duration (Evans and Fischer 1999; Dobermann 2000).

[1] Glasshouse experiments can provide important insight into relationships between crop physiology, genetics, and yield. To relate genetic changes to yield potential, however, field trials that include the highest-yielding cultivars are required.

3. Quantum requirement for photosynthesis

The number of photons of PAR that must be absorbed to assimilate a molecule of CO_2 is the *quantum requirement* for photosynthesis. The number of photons of PAR absorbed by a crop, divided by the quantum requirement, is the maximum amount of photosynthesis possible. The minimum theoretical quantum requirement is therefore a central aspect of the determination of potential yield. It differs between C_3 and C_4 crops[2].

The inverse of the quantum requirement is called quantum yield.

3.1 C_3 photosynthesis

A commonly stated theoretical minimum quantum requirement for C_3 photosynthesis is eight photons per CO_2 molecule. This is based on (a) operation of the Q-cycle in chloroplasts (Berry and Rumberg 1999); (b) transport of four protons through a chloroplastic ATP synthase per ADP phosphorylated (Pänke and Rumberg 1997); (c) use of two NADPH and three ATP in the reductive pentose phosphate cycle (RPPC) per CO_2 assimilated [(a) and (b) together imply that exactly eight photons can reduce two $NADP^+$ and phosphorylate three ADP]; and (d) fructose 6-P as end product. But fructose 6-P is not the end product of photosynthesis. Assimilated carbon is not accumulated or transported in that form. Rather, sucrose, starch, raffinose, and related compounds are the main end products.

To synthesize sucrose from fructose 6-P, 0.083 UTP (taken to be equivalent to 0.083 ATP) are needed per carbon. To produce raffinose and starch, 0.11 and 0.17 ATP/carbon are needed, respectively. Cyclic photophosphorylation might supply that ATP with a stoichiometry of two photons per ATP (i.e., assuming transport of two protons across a thylakoid membrane per photon absorbed by that thylakoid and a four proton requirement for ADP phosphorylation). A theoretical minimum quantum requirement for C_3 photosynthesis is thus 8.17 photons/CO_2 with sucrose as end product. This would represent about 26.5% efficiency of PAR use[3].

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[2] The C_3 crops include alfalfa, barley, cassava, chickpea, clover, coconut, cotton, cowpea, faba bean, field bean, flax, oat, palm, pea, peanut, pigeonpea, rapeseed (canola), rice, rubber, ryegrass, soybean, sugarbeet, sunflower, wheat, and yam. The C_4 crops include amaranth, maize, millet, sorghum, and sugarcane.

[3] With 4.6 mol photons MJ^{-1} in the PAR wave band (Fig. 2.1), 8.17 mol photons (PAR) contain 1.776 MJ. Sucrose contains 0.470 MJ $(mol\ C)^{-1}$, and $0.470/1.776 \approx 0.265$. The other 1.306 MJ in PAR would be converted to some combination of sensible heat and latent heat.

A key property of the C_3 photosynthesis carboxylating enzyme ribulose-1,5-P_2 carboxylase/oxygenase (rubisco) is that CO_2 and O_2 are competitive substrates. Rubisco's oxygenase activity initiates photorespiration. With a closed cycle of photorespiration (including regeneration of ribulose 1,5-P_2 from 3-phosphoglycerate) each oxygenation catalyzed by rubisco results in the release of 0.5 CO_2, the use of 3.4 ATP, and the oxidation of two NADPH equivalents (i.e., one NADPH and two reduced ferredoxin). The CO_2 release represents a loss of solar energy previously converted into chemical bonds. Eight photons can produce two NADPH and three ATP (as above), but additional energy is needed for the "extra" 0.4 ATP per oxygenation. Using cyclic photophosphorylation, 0.8 mol photons can produce 0.4 mol ATP (as above), so a theoretical minimum quantum requirement by the photorespiratory cycle is 8.8 photons per oxygenation (17.6 photons per CO_2 released).

The relative rates of ribulose 1,5-P_2 oxygenation (v_o) and carboxylation (v_c), with each reaction being catalyzed by rubisco, determines the theoretical quantum requirement for net CO_2 assimilation by C_3 photosynthesis as follows: quantum requirement = $0.17 + (8v_c + 8.8v_o)/(v_c - 0.5v_o)$. This applies to sucrose as the end product. The ratio v_o/v_c, and therefore quantum requirement, is positively related to temperature and negatively related to [CO_2] (Fig. 2.2).

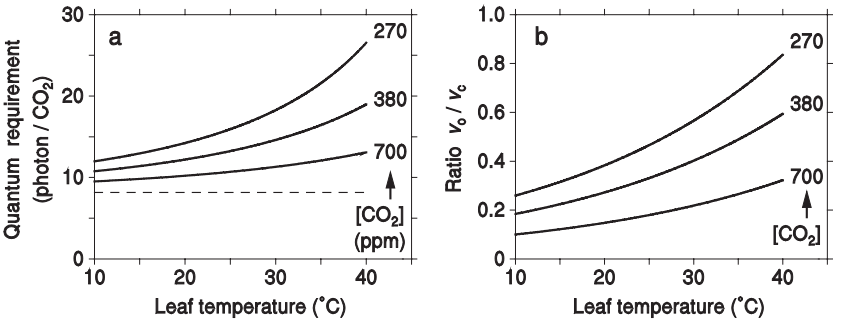

Fig. 2.2. (a) Theoretical quantum requirement for C_3 photosynthesis producing sucrose (—) as a function of temperature for three ambient [CO_2]s with only active absorption of PAR. The lines indicate the balance of rubisco-catalyzed carboxylations and photorespiratory decarboxylations. The 270 ppm [CO_2] corresponds to the preindustrial atmosphere, the 380 ppm [CO_2] corresponds to the contemporary atmosphere, and 700 ppm is a possible atmospheric [CO_2] 90–100 years in the future. A typical ratio of intercellular to ambient [CO_2]s in C_3 leaves was assumed (i.e., 0.7). The dashed line (- - -) is the theoretical minimum quantum requirement without photorespiration (i.e., 8.17 photons/CO_2). **(b)** Theoretical ratio of rubisco oxygenations to carboxylations (v_o/v_c) for conditions as in (a). The fraction of CO_2 assimilated that is subsequently released by photorespiration is $0.5v_o/v_c$.

3.2 C_4 photosynthesis

The C_4 system involves specialized leaf anatomy and adds biochemical reactions (i.e., C_4 cycles) upstream of the RPPC. The system acts to concentrate CO_2 in bundle sheath cells, the location of rubisco in C_4 leaves. This greatly reduces photorespiration in C_4 leaves. Three types of C_4 photosynthesis are usually distinguished, based on the enzyme releasing CO_2 from the C_4 cycle within bundle sheath cells, though the differences between types are not absolute (Leegood 2002). Those enzymes are NADP-malic enzyme (NADP-ME), NAD-ME, and phosphoenolpyruvate carboxykinase (PCK). Maize, sorghum, and sugarcane are NADP-ME-type C_4 crops.

All three types of C_4 photosynthesis involve coordinated activities and interconnections between mesophyll cells (where CO_2 is assimilated in the C_4 cycle) and adjacent bundle sheath cells (where the C_4 cycles release CO_2 and where the RPPC takes place) that encircle leaf veins. The C_4 bundle sheath system is known as Kranz (wreath or ring) anatomy.

The C_4 system requires the production of two NADPH and three ATP per CO_2 assimilated in the RPPC (as for C_3 photosynthesis), and two more ATP per CO_2 moving through the C_4 cycle. The "extra" ATP might come from cyclic photophosphorylation, in the ratio 0.5 ATP/photon (as above), so absorption of four photons could supply the two extra ATP for the C_4 cycle. To synthesize sucrose from CO_2 with the C_4 system would therefore require a minimum of 12.17 photons/CO_2. Some CO_2 leakage from the bundle sheath occurs, however, so the C_4 cycle operates more rapidly (C_4 overcycling) than the RPPC. The amount of C_4 overcycling in C_4 crop species may be of order 0.15 (i.e., 15%) (Hatch et al. 1995), increasing the theoretical quantum requirement of order 0.6 photons/CO_2.

As distinct from C_3 photosynthesis, the quantum requirement for C_4 photosynthesis is largely insensitive to [CO_2] and temperature.

3.3 Comparing C_3 to C_4 and potential to actual

Although C_3 photosynthesis without photorespiration has a smaller quantum requirement than C_4 photosynthesis, once v_o/v_c exceeds about 0.35 in a C_3 leaf, the theoretical quantum requirement for C_4 photosynthesis becomes superior (Table 2.1). This may occur around 25°C (Fig. 2.2).

Observed (i.e., measured or apparent) quantum requirements for CO_2 uptake in unstressed C_3 leaves are larger than theoretical values. For example, sunflower leaf quantum requirement measured in low light (where efficiency is highest) was about 11.5 photons/CO_2 when photorespiration was suppressed by low [O_2] or high [CO_2] (Table 2.2). This is considerably larger than the theoretical value of about 8.2 photons/CO_2. Observed

quantum requirements in C_4 leaves also exceed theoretical values, even after allowing for overcycling of 0.2 (20%) (compare Tables 2.1 and 2.2).

Observed quantum requirements exceed theoretical ones in part because of inactive absorption. With 10% inactive absorption, a theoretical quantum requirement of 8.2 photons/CO_2 (about the minimum for C_3 photosynthesis) increases to 9.1 photons/CO_2. Similarly, a 13.1 photon/CO_2 theoretical value (C_4 photosynthesis with 10% overcycling; Table 2.1) would increase to 14.6 photons/CO_2 with 10% inactive absorption. Also, crop photochemistry is not 100% efficient, and the two photosystems are unlikely to be perfectly coordinated, both of which contribute to differences between potential and actual quantum requirements.

The use of photosynthetically produced NADPH and ATP to support processes in addition to CO_2 assimilation can also affect the measured quantum requirement. An example is daytime NO_3 assimilation in leaves. Effects of light-driven NO_3 assimilation on measured quantum requirement will vary among crops (i.e., depending on how much NO_3 a species assimilates in irradiated leaves), developmental stage, environmental conditions, and the products of NO_3 assimilation (see, e.g., Noctor and Foyer 1998).

Table 2.1. Theoretical minimum quantum requirements for C_3 and maize-type C_4 photosyntheses (synthesis of sucrose with only active absorption of PAR).

System	v_o/v_c of rubisco [oxygenations per carboxylation]	C_4 overcycling [fraction]	Quantum requirement [photons/CO_2]
C_3	0.00[a]	–	8.2
	0.10	–	9.5
	0.20	–	11.0
	0.30	–	12.7
	0.35	–	13.6
	0.40	–	14.6
	0.60	–	19.1
C_4	0.00[a]	0.0[b]	12.2
	0.04[c]	0.0[b]	12.7
	0.04[c]	0.1	13.1
	0.04[c]	0.2	13.5
	0.04[c]	0.3	13.9
	0.04[c]	0.5	14.7

[a]The theoretical lower limit for v_o/v_c is zero (i.e., no photorespiration).
[b]Potential value if CO_2 leakage from bundle sheaths was reduced to zero.
[c]C_4 plants carry out limited photorespiration; the 0.04 value was chosen as representative (e.g., de Veau and Burris 1989; Jenkins et al. 1989).

Table 2.2. Measured leaf-level quantum requirements for C_3 and C_4 photosyntheses (in low light, including any inactive absorption of PAR).

Plant(s)	Quantum requirement [photons/CO_2]	Data source[a]
C_3 *(normal photorespiration)*		
Oat and barley (16°C)	13.7	A
Sunflower (20°C)	13.9–14.3	B
Nine grasses (30°C)	17.9–19.2	A
C_3 *(suppressed photorespiration)*		
Sunflower (20°C)	11.5–11.6	B
Eleven species (28°C)	10.2–11.2	C
C_4		
Eight NADP-ME-type grasses (30°C)	14.5–16.4	A
Three NAD-ME-type grasses (30°C)	16.4–16.7	A
Five PCK-type grasses (30°C)	14.9–16.7	A

[a]A, Ehleringer and Pearcy (1983); B, Sharp et al. (1984); C, Long et al. (1993).

The use of photosynthetic NADPH and ATP to assimilate NO_3 instead of CO_2 during daytime in leaves should not be considered an inefficiency of photosynthesis, but rather an alternative, beneficial use of absorbed PAR. Nonetheless, it is not quantified by CO_2 uptake measurements. Herein, the metabolic cost of NO_3 assimilation is considered part of the substrate requirement for biosynthesis (section 6); i.e., it is considered separately from photosynthesis, even though it may not be so in reality.

Light saturation also increases apparent quantum requirement, often dramatically. For individual C_3 leaves that develop in full sun, photosynthesis can approach light saturation at 15–25% of full sun. Electron transport or carbon metabolism reactions may be near their capacities then. Transport of assimilated carbon out of leaves might also be near its capacity then, which has the potential to cause feedback inhibition of photosynthesis. No matter what causes light saturation of C_3 photosynthesis, it results in a significant increase in the number of photons absorbed per CO_2 molecule assimilated at moderate to high light in isolated C_3 leaves. Light saturation also occurs in C_4 leaves, but the light levels required are higher. This gives a potential advantage to C_4 photosynthesis with high I_p (Fig. 2.3).

Because crop canopies are not usually composed of horizontal leaves, whole-crop photosynthesis is light-saturated only at much higher I_p, and even then the degree of light saturation can be weak (Loomis and Connor 1992).

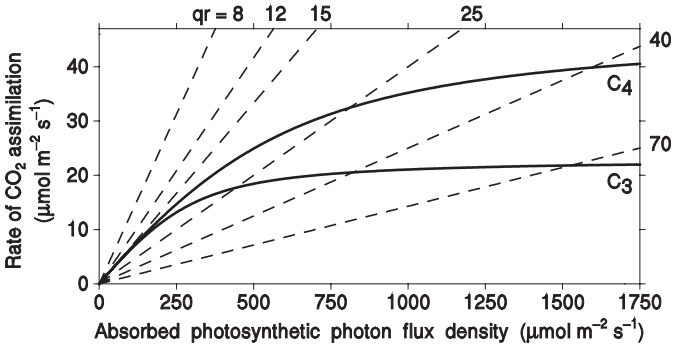

Fig. 2.3. Generalized light response curves on an absorbed photon (PAR) basis for CO_2 assimilation (photosynthesis less photorespiration, but without respiration) by a C_3 and a C_4 leaf. The initial slopes correspond to quantum requirements of 15 photons/CO_2 for both the C_3 and C_4 leaves. The dashed lines indicate CO_2 uptake rates for constant quantum requirements (qr) of 8 (a minimum for C_3 photosynthesis), 12 (a minimum for C_4 photosynthesis), 15, 25, 40, and 70 photons/CO_2. Unshaded horizontal leaves under clear skies can absorb as much as 1750 μmol photons (PAR) m^{-2} (leaf) s^{-1} at midday.

4. Improving photosynthesis

4.1 More rubisco or better rubisco in C_3 crops

If more rubisco — and other photosynthetic enzymes — can be added to C_3 leaves it might allow faster photosynthesis in high light. It might also increase the supply of nitrogen (via mobilization) to growing storage organs during grain or tuber filling periods. This would require greater nitrogen uptake and assimilation, and might require increased nitrogen availability. But even if more nitrogen can be assimilated, there may be limits on how much additional photosynthesis could be obtained in this way. Rubisco contains 20–30% of the nitrogen in C_3 crop leaves, and even more in rice (Evans 1989). It typically accounts for half the *soluble* protein in leaves, and whether much more rubisco could be added to leaves of well-managed crops is unresolved. It was even suggested that there may not be physical capacity to add more rubisco to some leaves (Zhu et al. 2004).

Improving existing rubisco is another possibility for enhancing photosynthesis. Rubisco is an inefficient catalyst of CO_2 assimilation because of its slow turnover number (k_{cat}; a few per second per catalytic site) and low affinity for CO_2. In the present atmosphere, it catalyzes oxygenation of ribulose 1,5-P_2 about 20–60% as rapidly as it catalyzes its carboxylation (Fig. 2.2). This is characterized by the rubisco specificity for CO_2 relative

to O_2, symbolized by τ and equal to $(V_{C,max}K_O)/(V_{O,max}K_C)$. $V_{C,max}$ is the maximum rate of carboxylation, $V_{O,max}$ is the maximum rate of oxygenation, K_O is the Michaelis-Menten constant with respect to dissolved O_2, and K_C is the Michaelis-Menten constant with respect to dissolved CO_2.

An improved rubisco would assimilate more CO_2 per unit mass of rubisco per unit time. Rubiscos from different organisms have (slightly) different amino acid sequences and different properties, including values of τ and k_{cat}. For example, rubiscos from some red algae (Uemura et al. 1997) have much greater τ than found in crops, and C_4 species generally have more efficient rubiscos than C_3 species. Increased τ would reduce quantum requirement and increase C_3 photosynthesis by reducing photorespiration and increasing the rate of carboxylation, even without a change in k_{cat}. A doubling of τ, brought about by increasing $V_{C,max}$ and decreasing $V_{O,max}$ with no change in their sum, might increase wheat leaf photosynthesis 20% at 20°C (Austin 1999). The effect on whole-season photosynthesis could be larger. Faster early-season photosynthesis might increase leaf growth and accelerate canopy closure, thus increasing PAR absorption.

For any given τ, faster k_{cat} could stimulate photosynthesis in high light if cofactors such as P_i were supplied rapidly enough and feedback inhibition of photosynthesis was avoided. The combination of large τ and fast k_{cat} would be most beneficial, but unfortunately, τ and k_{cat} are inversely related across many rubiscos found in nature, including in red algae (Uemura et al. 1997; Spreitzer and Salvucci 2002). Nonetheless, using combinations of τ and k_{cat} observed in nature, simulation modeling indicated that daily crop CO_2 assimilation might be increased 27% simply by replacing the "average C_3 crop rubisco" with the same amount of rubisco from a nongreen-alga, and by 12–17% with rubiscos from C_4 plants (Zhu et al. 2004).

A complication for engineering an improved rubisco is that it is composed of eight large subunits (each of which has a catalytic site) coded for in the chloroplastic genome and eight small subunits coded for in the nuclear genome. Assembling modified subunits in chloroplasts has so far met with difficulty (Parry et al. 2003). The catalytic-site amino acids appear to be conserved and required for maximal activity, implying that engineering an improved rubisco would require non-catalytic-site changes. Also, if a single amino-acid substitution in rubisco could improve photosynthesis, it probably would have been selected already during evolution or created by humans, so it appears that improving rubisco would require multiple changes, perhaps optimized across the whole enzyme complex (Spreitzer and Salvucci 2002). Moreover, the absence of known rubisco mutants with reduced or eliminated oxygenase activity implies that oxygenase activity may be an unavoidable consequence of the reaction mechanism of carboxylation (Leegood 1999). Photorespiration might even benefit a crop during some stresses (see below).

An aggressive tactic for enhancing crop photosynthesis might be to express a high-τ rubisco in lower (shaded) leaves and a high-k_{cat} rubisco in upper (sunlit) leaves, obtained from existing organisms such as algae. A high-τ, fast-k_{cat} rubisco remains the ultimate target for improvement. While improving rubisco will be difficult, the prospects going forward were judged to be "excellent" by Spreitzer and Salvucci (2002), and Parry et al. (2003) wrote that introducing a high-τ rubisco "into crop plants remains a realistic goal". The record on human improvement of rubisco in crops is meager, however, and it might be judged that the probability of significantly increasing yield potential by improving rubisco will be low during the next decade or two.

Improvements might also be made to factors affecting present rubisco. Rubisco activase promotes and maintains the catalytic capacity of rubisco. It reacts with large subunits near their catalytic sites (possibly encircling rubisco as an oligomer), and requires ATP hydrolysis, probably to drive rubisco conformational changes (Portis 2003). The effect of that ATP consumption on the quantum requirement depends on the unknown (perhaps variable) ratio of ATP hydrolyzed to activate rubisco per CO_2 assimilated by rubisco. At high temperature, rubisco activation by activase may not keep pace with rubisco deactivation, possibly limiting photosynthesis above about 30 °C. Increasing activity or stability of rubisco activase at higher temperature might therefore improve the operation of existing rubisco (Crafts-Brandner and Salvucci 2000). Moreover, any engineered change to rubisco may require co-modification of rubisco activase to maintain proper interaction between the two (Spreitzer and Salvucci 2002).

Although the ongoing increase in atmospheric [CO_2] is partly compensating for rubisco's low τ, that [CO_2] increase is slow (atmospheric [CO_2] is now about 380 ppm, increasing about 1.8 ppm/year [Keeling and Whorf 2005]). Modifying rubisco to improve its kinetics should therefore remain a goal. In addition, because [CO_2] inside chloroplasts can be considerably less than [CO_2] in intercellular spaces, increasing the mesophyll conductance (i.e., transfer of CO_2 from intercellular spaces into chloroplasts) could effectively increase CO_2 supply, reduce photorespiration, enhance photosynthesis, and increase yield potential.

4.2 Photoinhibition and photoprotection

Photoinhibition is a reduction of photosynthesis caused by absorption of excess light. It is attributed to thylakoid damage (e.g., damage to the photosystem II reaction center D1 protein) or various "protection" processes (Sage and Reid 1994). Distinguishing effects of actual damage from

effects of protection mechanisms is difficult because both slow photosynthesis. In moderately stressed plants, photoprotection may dominate over thylakoid damage (Demmig-Adams and Adams 1992), but each increases the quantum requirement for photosynthesis.

It is unclear if photoprotection is commonly needed in healthy crops. Young crops with horizontal leaves, or old leaves that become horizontal (Murchie et al. 1999), might be most vulnerable to photodamage. But within developed canopies, leaves are generally not perpendicular to the solar beam, limiting the excess radiation they absorb. Moreover, field crops are "sun" plants, whereas photoinhibition is generally a problem for "shade" leaves. Under stress conditions (e.g., limited soil moisture) photodamage might be common in crops, but the photodamage may be secondary to the stress itself in terms of increased quantum requirement.

If photoprotection is needed and induced in crops, but it persists longer than needed, accelerating the recovery from photoprotection could enhance photosynthesis. The xanthophyll photoprotection system, for example, can be induced by a brief high-light stress but then remain active for hours. More rapid recovery may be desirable, and some higher-yielding rice cultivars may be able to more rapidly recover from artificially induced photoinhibition than traditional cultivars (Wang et al. 2002). It remains unclear, however, whether that faster recovery is related to yield potential. If the recovery rate is important to yield potential, it should be increased, perhaps by genetically modifying the xanthophyll cycle. With respect to engineering changes to C_3-crop rubisco, it is important that photorespiration has the potential to reduce or delay photoinhibition when intercellular $[CO_2]$ is low (e.g., when stomates close because of water deficit). The degree of protection, however, may be modest (Björkman and Demmig-Adams 1994), and because crop mutants with reduced rubisco oxygenase capacity are not known to exist, it is impossible to determine whether rubisco's oxygenase activity per se is beneficial or required by C_3 crops.

Yield gains in some crops apparently have been related in part to increased stomatal conductance, which allows greater evaporative cooling and CO_2 uptake (e.g., Radin et al. 1994; Fischer et al. 1998; Morrison et al. 1999). Greater conductance can reduce the incidence of photodamage in crops (Wang et al. 2005), a trait most relevant to well-watered fields. Greater conductance might also be indicative of greater photosynthesis, perhaps caused by greater sink activity in newer cultivars (see discussion in Richards 2000).

4.3 Leaf angle

Leaf elevation angles that both minimize high incident irradiance on individual leaves and maximize PAR absorption per unit ground area are best. For leaf area index (LAI) less than about two, horizontal leaves may maximize photosynthesis per unit ground area (Loomis et al. 1967). For high LAI (>4), erect leaves can increase photosynthesis by reducing light saturation (and possibly reducing photoinhibition) in the top of the canopy and enhancing photosynthesis lower in the canopy. In principle, this increases the amount of leaf area carrying out efficient (i.e., not light-saturated) photosynthesis and reduces whole-crop quantum requirement. This effect is expected to be largest with high solar elevation, such as during summer or at low latitude (Duncan 1971).

Erect leaves also allow larger LAI by preventing lower leaves from being shaded to the point of senescence, perhaps by keeping daily photosynthesis greater than daily maintenance respiration. This allows greater plant density, which can enhance yield (Loomis et al. 1967; Duvick 2005). The high LAI possible with erect leaves also increases potential for nitrogen storage in leaves per unit ground area. Because grain crops use leaves as a nitrogen source during grain filling, a large LAI can enhance late-season nitrogen supply to growing seeds (Sinclair and Sheehy 1999). Genes for upright leaves are already in the major cereal crops (Reynolds et al. 2000; Richards 2000), but in other crops there is scope for engineering more erect leaves.

5. Maintenance requirement

Living cells require energy for maintenance, and crops obtain that energy from photosynthate. Processes thought to be quantitatively important to the energy cost of crop maintenance include replacement of degraded proteins resulting from their spontaneous breakdown; active transport of metabolites across membranes to counteract “leaks” (i.e., gradient maintenance); active processes involved in acclimation to environmental changes, such as the replacement of one complement of enzymes with another that is better suited to a new environment; and repair of cellular damage by, for example, oxidants in the environment or produced by the plant.

Turnover of biosynthetic enzymes and RNA associated with growth is called “tool maintenance”. This is distinguished from other maintenance processes, which are collectively called “structure maintenance” (Penning de Vries et al. 1974). “Maintenance” in this section refers to structure maintenance; tool maintenance is part of the substrate requirement for biosynthesis (next section). “Maintenance respiration” is the CO_2 (and heat)

released by maintenance processes and by respiratory processes supplying ATP, NAD(P)H, or carbon skeleton intermediates to the maintenance processes (Penning de Vries 1975).

Rates of maintenance processes are dynamic, affected by both developmental changes in the crop and changes in the environment. Maintenance respiration rate is probably most strongly related to the amount of protoplasm and to temperature. Maintenance processes are slow in cell walls because those structures do not undergo turnover. Maintenance processes are also probably slow in seeds and tubers because macromolecular turnover and gradient losses are limited there (i.e., the products of biosynthesis in those organs are more stable than is vegetative-cell protoplasm). Shaded leaves too have generally slow rates of maintenance respiration, at least in comparison to leaves in high-light environments. One implication of increasing protein amount in leaves is an increase in maintenance expenditures for protein turnover, although the greater photosynthesis could outweigh the greater respiration. Maintenance may be faster in warm environments than cool ones, but some degree of acclimation of maintenance processes to prevailing temperature is to be expected. Hence, acclimation and adaptation will presumably limit (or eliminate) effects of gradual global warming (associated with increasing atmospheric [CO_2]) on crop maintenance processes.

Experimental data and theoretical calculations indicate that maintenance of vegetative tissue, at moderate temperature, consumes photosynthate at a rate equivalent to at least 1% of the energy content of existing biomass each day (Penning de Vries 1975; Amthor 2000). Estimates are imprecise, but over a crop cycle, maintenance respiration may about equal growth respiration, so an engineered reduction in maintenance respiration might increase growth (Amthor 1989; Loomis and Amthor 1999).

Across different selections of perennial ryegrass, mature-leaf respiration rate (one empirical measure of maintenance respiration rate) was negatively correlated with forage production (Wilson 1975, 1982). Causes of the correlation remain unclear, and the correlation can disappear when the plants are grown at low density (Kraus 1992). A negative correlation between mature-leaf respiration rate and biomass production was also observed in different maize hybrids, but the cause of that correlation and whether differences in maintenance contributed to growth differences is also unclear (Earl and Tollenaar 1998). These results hint that there may be variation in maintenance respiration that can affect growth, but this is still unproven. In any case, an ideal crop would carry out only essential maintenance and it would do so with efficient metabolism. It would minimize spontaneous breakdown of macromolecules and metabolite gradient losses.

It is thus important to establish if (a) all existing maintenance processes are necessary in well-managed crops, (b) existing maintenance processes are efficient, and (c) respiration provides maintenance energy efficiently. A minimum energetic cost of turnover of a unit of protein can in principle be calculated (Amthor 2000), but without information on the minimum amount of turnover required in high-yielding crops, it is impossible to estimate the corresponding minimum energy requirements for that minimum required protein turnover. The same is true for metabolite gradient maintenance and acclimation to environmental variation. In the absence of sufficient understanding of the optimal rates and maximum potential efficiencies of required maintenance processes, a theoretical minimum *maintenance requirement* cannot be calculated. Nonetheless, maintenance is essential, and a speculative value of 15% of photosynthesis is adopted herein as the substrate requirement for minimum whole-plant maintenance needs. In consideration of the lower leaf-protein concentration in C_4 crops, the speculative minimum for C_4 crops is 12% of photosynthesis.

One consideration is that rapid rates of maintenance processes may be important to survival and reproductive success in complex and dynamic environments. For example, frequent and rapid physiological acclimation to both environmental fluctuations and the dynamics of interspecies competition may be critical in natural environments. That frequent and rapid acclimation might depend on rapid background maintenance processes. In well-managed monocultures, however, extensive and frequent acclimation processes may be unnecessary. It is unclear whether past crop selection and breeding reduced the rate, or increased the efficiency, of maintenance processes to their optima for a crop's environment. If they have not, genetic improvement is possible, but the potential for any such improvement will remain unknown until more information about optimal and actual maintenance processes in crops is obtained.

6. Substrate requirement for biosynthesis

The *substrate requirement* for biosynthesis is the amount of photosynthate needed to grow a unit of new biomass. This means that an upper limit on the amount of growth possible is the time-integrated amount of photosynthesis, minus the substrate requirement for maintenance, with the result divided by the substrate requirement for biosynthesis.

Biosynthesis includes catabolic and anabolic phases. The catabolic phase breaks down photosynthate to produce carbon-skeleton intermediates, reduce NAD^+ and $NADP^+$, and phosphorylate ADP. The anabolic phase combines carbon-skeleton intermediates into the macromolecules of

biomass, using the NAD(P)H and ATP provided by the catabolic phase. In addition to photosynthate, soil minerals are needed for biosynthesis, especially nitrogen, which must be reduced for incorporation into biomass. The CO_2 released during biosynthesis of new biomass is called "growth respiration". Most of that CO_2 release is associated with the catabolic phase of biosynthesis, but some anabolic pathways also release CO_2.

Because rapid growth requires rapid growth respiration, slower intrinsic respiration could reduce yield potential. The target for yield-potential improvement is reduced substrate requirement in combination with rapid biosynthesis (and therefore rapid growth respiration).

The theoretical minimum substrate requirement for biosynthesis is calculated by tracing the most efficient pathways from photosynthate to the components of biomass (Penning de Vries et al. 1974, 1983). The major components are structural and storage carbohydrates, proteins, lignins, lipids, and organic acids. The pathways are summed in proportion to biomass composition. Inputs of NAD(P)H and ATP to the pathways, and the amount of substrate catabolized to produce that NAD(P)H and ATP are included. The metabolic cost of transporting carbohydrates, minerals, and other substrates from source organs to sites of biosynthesis is also included, as is the cost of assimilating inorganic nitrogen. The chemical forms of photosynthate and nitrogen used, and composition of the biomass grown, are inputs to such calculations.

Efficiency of biosynthesis is the inverse of substrate requirement. It can be expressed as either biomass (dry) formed per unit mass of substrate used, or as energy contained in the biomass formed per unit energy in the substrate used. The mass/mass ratio is most often used in crop science, but the energy/energy ratio is more relevant to assessing the efficiency of converting solar energy into chemical bonds in new biomass.

Theoretical efficiencies of converting photosynthate in sources into new biomass in storage organs vary from 0.40 to 0.85 kg kg^{-1} across a range of crops (see Fig. 2.4 for assumptions). The theoretical efficiency of energy use in growing those same storage organs is larger and less variable, i.e., covering the range 0.65–0.88 J J^{-1} (Fig. 2.4). Differences between crops arise from differences in their composition, including differences in protein concentration, which defines a *nitrogen requirement* for biosynthesis. For the organs in Fig. 2.4, the nitrogen concentration varied from about 0.5% (cassava) to over 6% (soybean), a more than 12-fold difference in amount of nitrogen that must be assimilated per unit mass of storage organ grown. Calculated energy contents ranged from 16.4 (sugarbeet) to 27.1 (peanut) kJ g^{-1}. The high lipid concentrations in coconut, oil palm, cotton, sunflower, peanut, and soybean contributed to relatively low mass-based biosynthetic efficiencies. The range of compositions of the storage organs in Fig. 2.4 spans many possibilities, including values for most vegetative

organs. Nonetheless, for new crops producing large amounts of novel compounds, it is possible that theoretical substrate requirements (or potential biosynthetic efficiencies) would be outside the range considered here.

Efficiency of substrate use for biosynthesis could be increased only to the extent that it is now less than its potential. Research in the 1970s and 1980s indicated that biosynthesis in crops may be near the theoretical maximum efficiency allowed with known biochemical pathways (Penning de Vries et al. 1983). That research included uncertainty, however, and the actual efficiency of biosynthesis in crops in the field is imprecisely known.

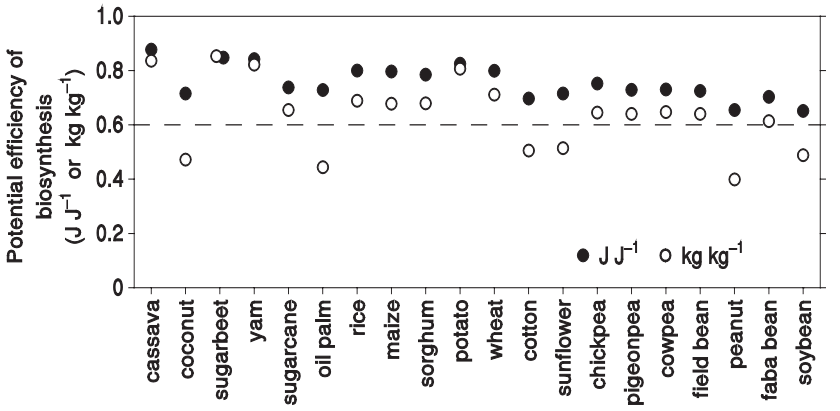

Fig. 2.4. Potential (theoretical) efficiency of biosynthesis (i.e., retention of energy or mass) of crop *storage organs* from sucrose. The organs are tubers and beets (cassava, sugarbeet, yam, potato), sugarcane shoot, fruits (coconut, oil palm), inflorescences with seeds (rice, sorghum, wheat, sunflower), maize cob (70% seed), cotton bole (35% lint), and pods with seeds (chickpea, pigeonpea, cowpea, field bean, peanut, faba bean, soybean). Concentrations of carbohydrates, proteins, lipids, lignins, organic acids, and minerals in the organs are based on Penning de Vries et al. (1983). Organs are arranged from left to right according to increasing protein concentration. Substrate requirements for biosynthesis of each class of biochemical are from Penning de Vries et al. (1983) and Amthor (2003) (for lignins), with small modifications herein for carbohydrates. Tool maintenance estimates are included, and 5% of the sucrose substrate is respired to provide energy to transport the remaining 95% from sources to growing cells in the storage organs. Minerals are supplied as needed, accounting for uptake cost (i.e., sucrose catabolism) in roots. Nitrogen is provided as NO_3 and estimated cost of NO_3 assimilation is included; if some NO_3 assimilation occurs as part of "photosynthesis", biosynthetic efficiency would increase, but the quantum requirement for photosynthesis would increase too. Energy contents of biochemical fractions are based on Appendix. Different assumptions about organ chemical composition or pathways of biosynthesis would result in (generally small) differences in potential efficiencies. The dashed line (– – –) is for visual reference only.

Recent theoretical work revealed potential for variation in the efficiency of biosynthesis of lignins with extant enzymes and pathways (Amthor 2003), implying the possibility for genetic improvement, and the same could be true for other complex biomolecules. To determine if (and how much) improvement is possible for a specific product in a specific crop will require precise measurements of biosynthesis, substrate consumption, and the biosynthetic and respiratory pathways used. If efficiency is not maximal, it could become a target for improvement.

7. Efficiency of PAR use by productive C_3 and C_4 crops: potential and actual

Based on estimates of substrate requirement for biosynthesis and maintenance thought to be representative, the so-called radiation-use efficiency (RUE; grams dry whole-plant biomass grown per joule PAR absorbed) was modeled for various quantum requirements for photosynthesis by wheat at midseason (Loomis and Amthor 1996). Results indicated that potential RUEs would range from 2.3 to 8.6 g MJ^{-1} for quantum requirements ranging from 30 down to 10 photons/CO_2, respectively.[4]

A summary of field measurements of wheat and barley indicated a mean actual RUE of about 3.0 g MJ^{-1}, or 5.1% of energy in absorbed PAR for crops containing 17 kJ g^{-1} (Fischer 1983). A quantum requirement of 25 photons/CO_2 could explain that RUE (Loomis and Amthor 1996). Data on the "high side" of Fischer's (1983) summary indicated that the best crops were about 6.5% efficient at converting absorbed PAR into chemical bonds in new biomass, corresponding to a quantum requirement of 20 photons/CO_2 in the model crop (Loomis and Amthor 1996).

A similar model analysis for maize resulted in potential RUEs ranging from 4.2 to 6.2 g MJ^{-1} for quantum requirements ranging from 18 down to 14 photons/CO_2, respectively (Loomis and Amthor 1999). The more limited range of quantum requirement was used because C_4 quantum requirement is relatively insensitive to the environment and 14 photons/CO_2 may represent a minimum achievable for C_4-crop photosynthesis.

A 5-year field study indicated a mean RUE of about 4.2 g (assumed here to be 10% roots) MJ^{-1} in well-managed maize (Lindquist et al. 2005). That corresponds to the 18-photon-photosynthesis model crop. For model biomass containing 17.6 kJ g^{-1} (Loomis and Amthor 1999), such a crop would be 7.4% efficient at producing chemical bonds in new biomass from absorbed PAR. Data on the "high side" of Lindquist's et al. (2005) summary

[4] Loomis and Amthor (1996, 1999) used 4.4 mol photons MJ^{-1} (PAR) in their analyses. Results herein were recalculated using 4.6 mol MJ^{-1} (Fig. 2.1).

indicated RUEs of about 5.2 g MJ^{-1} (9.1–9.2% efficiency of converting absorbed PAR into biomass energy) during both vegetative and reproductive growth. An RUE of 5.2 g MJ^{-1} would correspond to a quantum requirement of 15.2 photons/CO_2 in the model crop. That quantum requirement is within the range measured for individual C_4 leaves in low light (Table 2.2; in the model crop, the cost of NO_3 assimilation is part of the cost of growth, but in the measured leaves it might be part of photosynthesis).

If the efficiency of biosynthesis is similar in C_3 and C_4 crops, but the observed quantum requirement for photosynthesis is lower in C_4 crops, then C_4 crops should be more productive. (Also, maintenance requirement may be less in C_4 crops because of a smaller protein concentration in leaves.) A summary of standing biomass (not yield) at harvest for highly productive crops supports this supposition [excluding published values likely to be erroneous; see Loomis and Gerakis (1975) and Monteith (1978)], with a qualification about latitude (Fig. 2.5). At latitudes greater than 50° (absolute value), C_3 crops have an apparent advantage, probably because C_4 crops are more sensitive to low temperature than most C_3 crops.

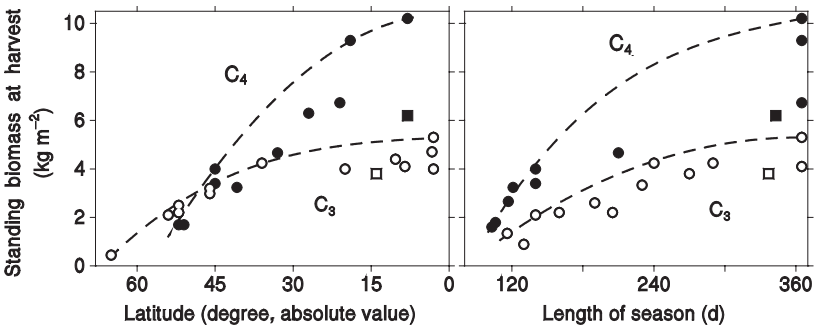

Fig. 2.5. Estimated standing whole-plant biomass at harvest for high-productivity C_3 (○) and C_4 (●) crops as a function of latitude (absolute value) and season length (not all crops shown at left are shown at right and vice versa). Data sources are given in Cooper (1975), Loomis and Gerakis (1975), Loomis (1983), and Smith and Banta (1983). Also shown is the "Comet" soybean crop of Morrison et al. (1999), the 2001 maize crop of Lindquist et al. (2005), a good rice-rice-rice triple-crop at the International Rice Research Institute (□; KG Cassman pers comm), and a good rice-maize-maize triple-crop in East Java (■; A Dobermann pers comm). Total production exceeds values shown because biomass shed prior to harvest is excluded. Lines are drawn by eye to summarize the best C_3 or C_4 crops. Lines in the right panel are concave down in part because average I_P is greater during summer at midlatitudes (about 120-d season) than during the whole year in the tropics

As indicated by Fig. 2.5, the best C_4 crops have faster long-term (i.e., seasonal-average) growth rates than the best C_3 crops. They also have faster short-term, peak growth rates (Loomis and Gerakis 1975; Monteith 1978), at least in warm environments.

The largest standing biomass production estimate in Fig. 2.5 is 10.2 kg m^{-2}, for sugarcane growing all year (Irvine 1983)[5]. A sunny tropical site can receive 3500 MJ (PAR) m^{-2} annually, and a 10 kg m^{-2} sugarcane crop might contain 170–180 MJ m^{-2}, implying about 5.0% efficiency of I_P use averaged over the year. This efficiency is unremarkable compared to the shorter-term efficiencies from Fischer (1983) and Lindquist et al. (2005) above, although it is on an incident rather than absorbed PAR basis.

Based on analysis above, there is large theoretical scope for improving the efficiency of converting absorbed PAR into chemical bonds in C_3 crops. A canopy-scale quantum requirement for C_3 photosynthesis as small as about 11 photons/CO_2 can be contemplated based on reduced photorespiration. This would allow nearly 20% efficiency of converting energy in absorbed PAR into energy in sucrose [i.e., 0.470 MJ (mol C)$^{-1}$/(11 mol photons (mol C)$^{-1}$ / 4.6 mol photons MJ^{-1})≈0.197 J {in sucrose} J^{-1} {in absorbed PAR}]. If the maintenance requirement consumes 15% of the energy in the sucrose product of photosynthesis, and 65–88% of the energy in the remaining sucrose can be retained in new biomass, then an upper limit on converting energy in absorbed PAR into chemical bonds in biomass is 11–15%, depending on biomass composition. For whole wheat plants, the maximum theoretical efficiency of biosynthesis might be about 0.79 J J^{-1}, giving a potential efficiency of converting absorbed PAR into chemical bonds in new biomass of about 13%. This is twice the 6.5% efficiency derived above for the best crops in Fischer's (1983) analysis.

The theoretical scope for improving the efficiency of solar energy use in C_4 crops is smaller because (a) the minimum canopy-scale quantum requirement of C_4 photosynthesis is larger (probably about 14 photons/CO_2) and (b) C_4 crops are already more productive than C_3 crops. Based on composition of whole maize plants, the maximum theoretical energy-based efficiency of biosynthesis may be about 0.82 J J^{-1}. Allowing for a 14-photon/CO_2 minimum canopy-scale quantum requirement and a minimum maintenance requirement of 12% of photosynthesis, the overall potential efficiency of converting energy in absorbed PAR into chemical bonds in new biomass would be about 11%. The measurements by Lindquist et al. (2005) indicate that well-managed maize may now be achieving more than 80% of this potential. To significantly increase biomass accumulation by

[5] Irvine (1983) estimated maximum annual sugarcane biomass production in two other experimental studies at 11.3 and 11.6 kg m^{-2}.

such crops, it may be necessary to increase the amount of PAR absorbed, either through earlier canopy closure or longer canopy duration.

It is noted that potential efficiencies above ignore unavoidable losses, if any, in processes such as leaf senescence, exudation from roots, and leaching from leaves. Also, the maximum measured efficiencies apply to shorter-term periods; they do not, for example, apply to whole years.

8. Engineering C_3 crops to use C_4 photosynthesis

Because C_4 crops are often more productive than C_3 crops at present CO_2 levels, it may be desirable to engineer C_3 crops to use C_4 photosynthesis. It is important, however, that increasing atmospheric [CO_2] is reducing some of the C_4 advantage, although that advantage is unlikely to disappear for many decades. It is also important that the maximum potential efficiency of solar energy use by C_3 crops exceeds the potential by C_4 crops, but if significant improvement to C_3-crop rubisco remains unrealized, C_4 photosynthesis may be preferred. This might not only increase photosynthesis, but could improve water-use efficiency. It could also reduce the nitrogen requirement for photosynthesis, though this could involve a drawback. Using rice as an example, Sheehy (2000) noted that the nitrogen content of C_4 canopies can be insufficient to supply enough nitrogen for high grain yield, at least if root uptake of nitrogen is limited during grain filling.

A number of approaches might be used to engineer C_4 photosynthesis into C_3 crops. They include (see Leegood 2002) introducing into C_3 crops (a) the complete complement of metabolic reactions and structural characteristics of C_4 crops; (b) some form of a single-celled C_4 system; (c) an intracellular CO_2-concentrating compartment, modeled after those found in some algae and cyanobacteria; or (d) a relocation of photorespiratory decarboxylation to the bundle sheath, modeled after that found in C_3–C_4 intermediate species. Expression of genes related to C_4 photosynthesis in C_3 plants can stimulate photosynthesis under some circumstances, but this has so far been unrelated to C_4 photosynthesis per se (e.g., Ku et al. 2001). It is impossible to reliably estimate the length of time required to engineer C_3 crops to use C_4 photosynthesis, but it would probably be decades.

In many cases it would be more expedient to modify existing C_4 crops to produce new products than to convert C_3 crops into C_4 crops. Indeed, the C_4 crop maize has advantages as a production system for industrial products (Stoger et al. 2005) and maize is already a major industrial crop. It has hundreds of nonfood/nonfeed product uses, though they are mostly related to grain starch. Production of different types of products, in desired quantities

and purities, might require considerable research, but advances in genetic engineering would presumably assist in some such efforts.

Improving the low-temperature performance of C_4 crops might allow expansion of their latitudinal range (global warming may contribute modestly to future C_4-crop range expansion too). The NADP-ME-type C_4 plant *Miscanthus* × *giganteus* is relatively tolerant of low temperature and it might serve as a genetic resource for improving the low-temperature tolerance of C_4 crops (Naidu and Long 2004). The time required to make such an improvement in C_4 crops would perhaps be decades.

9. Harvest index and sink activity

A comprehensive definition of harvest index (HI) is the fraction of whole-plant biomass that accumulates in a harvested organ, or the fraction of the whole plant made up of desired biochemicals (see also Irvine 1983). For grain crops, HI generally means the fraction of total aboveground biomass that is contained in grain at the time of harvest. The relationship between HI, biomass accumulation, and yield is simple: HI × biomass = yield.

While increased HI was often the most important factor in past increases in yield potential, how it occurred is incompletely understood. One critical change to grain crops was reduced stem growth, but exactly how that caused greater yield potential is unclear. Perhaps several mechanisms were involved (Evans 1993). The potential (maximum possible) HI has been speculated about (Austin 1999), but it lacks rigorous determination. Nonetheless, for crops already intensively bred for large HI, the scope for further improvement may be small. In those crops, further yield potential increases must come mainly from greater total biomass production. This would require more photosynthesis or more efficient biosynthesis and respiration. In other crops, there may be significant opportunity to increase yield potential by increasing HI. This should be pursued because history shows that such increases in yield potential can be dramatic.

The total amount of substrate imported by harvested sinks places an upper limit on yield. Substrate supply depends on the rate of assimilation in sources (and/or mobilization in intermediate storage pools) and the rate of translocation into sinks. Either could be a limiting process, but phloem *capacity* does not appear to limit transport (Evans 1993). The developmental stage is important, at least in grain crops. Increased photosynthesis prior to flowering often enhances spike growth and number of seeds set, and seed number is often positively related to yield. Increasing the duration of juvenile spike growth might increase seed number, and this might be brought about through genetic modification of photoperiod perception (Reynolds et al. 2000).

The potential importance of this is highlighted by the fact that past increases in yield potential of grain crops was related strongly to more seeds, rather than larger seeds (Evans 1993; Egli 1998; Reynolds et al. 2000; Richards 2000).

It is well established that slow sink activity can feed back to slow photosynthesis (Evans 1993), but with respect to increasing yield potential it is more important to know if increased sink activity (i.e., growth) can stimulate photosynthesis per unit of absorbed PAR. This is difficult to determine, and evidence indicating both negative and affirmative views can be cited (e.g., Evans 1993; Reynolds et al. 2000; Richards 2000; Egli and Bruening 2003). The issue will not be resolved here, and it may depend on species and other variables. In terms of potential yield, photosynthesis is limited by absorption of PAR and photosynthetic pathways and yield is then limited by the (a) fraction of photosynthate that can be transported into desired sinks and (b) amount of growth possible from that transported photosynthate. In principle, sink capacity might affect yield potential, and increasing the capacity for sink growth may be an appropriate target for crop genetic improvement (Reynolds et al. 2000; Richards 2000). A research goal is to quantify, and then experimentally increase, growth potential of desired sinks to determine if such an increase affects yield potential. Sink strength and growth potential may be related in part to phloem unloading, so possible candidates to increase sink activity are overexpression of invertase and sucrose transporters in sinks.

Whether enhanced sink capacity would increase yield potential or not, source activity now typically limits yield. Increasing photosynthesis by increasing PAR absorption or [CO_2] usually enhances yield if soil nutrients are adequate. And even high-yielding grain crops have unfilled seeds, although this may be related to ear structure, a sink attribute possibly amenable to improvement (Sharma-Natu and Ghildiyal 2005). Such observations indicate that source activity limits yield, yet other considerations indicate that there is unused source capacity in crops (see Richards 2000). Again, timing is important. Photosynthesis may be limiting during one period (e.g., prior to flowering), but sink capacity could be limiting during another period. This is particularly difficult to study quantitatively.

Experiments with transgenic plants indicate that changes in sink biochemistry can affect yield. For example, downregulation of plastidial adenylate kinase increased potato tuber growth in a field trial (Regierer et al. 2002), and expression of ADP-glucose pyrophosphorylase with reduced sensitivity to inhibition by P_i increased seed yield in greenhouse-grown rice and wheat (Smidansky et al. 2002, 2003). It is unknown whether such genomic changes could increase yield potential.

For any average rate of desired-product biosynthesis, yield is proportional to duration of that biosynthesis. Duration of both photosynthate transport to harvested sinks and growth in those sinks may therefore limit yield. In many crops the duration of sink growth is controlled by "thermal time" such that warmth reduces growth duration. Lengthening the duration of sink growth by relaxing the relationship between temperature and development, especially in warm climates, might increase yield potential. If duration of sink activity is maximized by extending it to the end of the possible growing period, yield will be limited by substrate supply, efficiency of biosynthesis, or average rate of biosynthesis. (Conversely, rapid maturity may be required when several crops are grown in series in a year.)

Much of the nitrogen (and some of the carbohydrate) used for grain growth in many crops comes from breakdown of older vegetative tissues, especially leaves. Rubisco in particular can be an important source of grain nitrogen. Proteins synthesized in grain from the breakdown products of proteins in vegetative organs are in essence synthesized twice; once in the vegetative organ and again in the grain. The cost of the second synthesis may be balanced by benefits of multiple uses of the same nitrogen, such as in photosynthetic enzymes early in a crop cycle followed by grain protein storage later. This reduces the total amount of nitrogen that must be assimilated. It may also be a limitation on yield — perhaps a vestige of evolution in competitive environments with limited nutrients. Increasing the capacity to acquire and assimilate nitrogen during grain filling might increase yield potential (Austin et al. 1977; Tollenaar and Wu 1999). This might require modified root mass, depth, longevity, or functioning.

10. Final comments

To obtain high yields, crops must assimilate environmental resources (solar radiation, CO_2, and nitrogen are key) at fast rates for significant duration. For this to occur, effective root and canopy systems (including stem structure for leaf display) must be grown, maintained, and protected from pests and stressors. Soil water must be adequate to allow high stomatal conductance and transport of CO_2 into leaves, especially in C_3 crops. Mineral nutrients (especially nitrogen) must be available and assimilated to allow large capacities for photosynthesis and growth.

Modest gains in yield potential might be achieved by reducing inactive absorption of PAR, causing more erect leaf display following canopy closure, or increasing the capacity for stomatal conductance or mesophyll conductance. Even larger yield potential gains might be possible by accelerating early season leaf expansion, increasing leaf or canopy longevity, increasing uptake and assimilation of nitrogen during grain or tuber filling,

reducing photorespiration in C_3 crops, engineering C_3 crops to use C_4 photosynthesis, or increasing HI in crops not previously bred for large HI. More research is needed to better understand other possibilities for improving yield potential. For example, research to quantify actual efficiency of biosynthesis, actual rates and efficiencies of maintenance processes, the minimum maintenance requirements of well-managed crops, and the necessity and extent of photoprotection processes is needed. If any parts of those processes are found to be unnecessary or inefficient, those parts should become targets for elimination or improvement. Although many of the above crop processes and characteristics have been improved in some crops, there remains scope for additional enhancement of yield potential in most cases. An overriding consideration is that multiple factors may co-limit yield potential and the relative importance of those factors can change during the course of a crop cycle and can differ between crop species and environments. So although "no one process provides the master key to greater yield potential" (Evans 1993 p. 169), by understanding factors determining potential yield, the gap between it and yield potential might be closed more rapidly and more efficiently.

By supplementing crop breeding with genetic engineering, not only might the yield potential of present crops be improved more rapidly, but entirely new crops and crop products can be considered. That is, in addition to starch, sugars, rubber, cellulose, lipids, and other traditional industrial-crop products, new products as diverse as biodegradable plastics (e.g., Poirier 1999; Scheller and Conrad 2005) and vaccines (e.g., Santos and Wigdorovitz 2005) can be grown by crops. The possibilities are legion, and the details will vary with each product-crop combination. Because grain crops have advantages as production systems for many products (e.g., Stoger et al. 2005) they will remain valuable for both present and future industrial uses.

Appendix: crop energy content

Plant dry mass may typically contain about 17.8 kJ g^{-1} (Whittaker 1975 p.193). Crop vegetative organs may average 17.0–17.5 kJ g^{-1} (Loomis and Connor 1992 p. 15). Those are heats of combustion (ΔH_C), measured in bomb calorimeters, relative to CO_2 (gas) and H_2O (liquid). Representative ΔH_C values for different classes of biochemicals include (e.g., Loomis and Connor 1992): organic acids, 4–11; carbohydrates, 15.6–17.5 (glucose = 15.6, sucrose = 16.5, starch = cellulose = 17.5); proteins, 22–25; lignins, 26–30; and lipids, 35–40 kJ g^{-1}. Because different compounds have different ΔH_C values, differences in crop composition can cause differences in whole-organ and whole-crop ΔH_Cs. For example, measured ΔH_C values were

16.8, 17.3, 17.6, 18.2, 21.1, and 26.9 kJ g^{-1} for potato tubers, wheat ears, rice ears, maize seeds, soybean pods, and sunflower seeds, respectively (Shinano et al. 1993).

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