

Can improvement in photosynthesis increase crop yields?

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ABSTRACT

The yield potential (Y_p) of a grain crop is the seed mass per unit ground area obtained under optimum growing conditions without weeds, pests and diseases. It is determined by the product of the available light energy and by the genetically determined properties: efficiency of light capture (ϵ_i), the efficiency of conversion of the intercepted light into biomass (ϵ_c) and the proportion of biomass partitioned into grain (η). Plant breeding brings η and ϵ_i close to their theoretical maxima, leaving ϵ_c , primarily determined by photosynthesis, as the only remaining major prospect for improving Y_p . Leaf photosynthetic rate, however, is poorly correlated with yield when different genotypes of a crop species are compared. This led to the viewpoint that improvement of leaf photosynthesis has little value for improving Y_p . By contrast, the many recent experiments that compare the growth of a genotype in current and future projected elevated $[\text{CO}_2]$ environments show that increase in leaf photosynthesis is closely associated with similar increases in yield. Are there opportunities to achieve similar increases by genetic manipulation? Six potential routes of increasing ϵ_c by improving photosynthetic efficiency were explored, ranging from altered canopy architecture to improved regeneration of the acceptor molecule for CO_2 . Collectively, these changes could improve ϵ_c and, therefore, Y_p by c. 50%. Because some changes could be achieved by transgenic technology, the time of the development of commercial cultivars could be considerably less than by conventional breeding and potentially, within 10–15 years.

Key-words: Photorespiration; Harvest index; Global change; Global food supply; Rubisco; Plant canopy architecture; Genetic transformation; Plant breeding; Crop improvement; Yield potential

INTRODUCTION

The world's most important crops in terms of total yield in 2004 were maize (*Zea mays*), 823 Mt; rice (*Oryza sativa*), 725 Mt; wheat (*Triticum aestivum* and *Triticum durum*),

555 Mt; soybean (*Glycine max*), 186 Mt; barley (*Hordeum sativum*), 142 Mt; and sorghum (*Sorghum bicolor*), 59 Mt (USDA 2005). These are all grain crops and are expected to remain the major sources of nutrition for people and for their farmed animals into the foreseeable future. Genetic improvement, increased use of nitrogen fertilizer and improved management since the mid-1950s have produced remarkable worldwide increases in the potential and realized yields of these crops (Evans 1997). Yields rose from an average 1.2 t ha⁻¹ in 1951 to 2.3 t ha⁻¹ by 1993 for the major grain crops (Beadle & Long 1985; Evans 1993; Dyson 1996). Yield potential (Y_p) is defined as 'the yield of a cultivar when grown in environments to which it is adapted, with nutrients and water non-limiting, and with pests, diseases, weeds, lodging and other stresses effectively controlled' (Evans & Fischer 1999). Using maize as an example, this increase over the past 50 years may be attributed roughly 50% to genetic improvement (i.e. increased Y_p) and 50% to improved management (Duvick 2005). As the use of nitrogen and other chemicals may also be reaching a maximum, increasing Y_p may be even more important as the means to increase actual yields over the next 50 years. Given environmental and economic restraints, it may be argued that further increases in Y_p cannot be ones that can only be realized with the addition of further nitrogen. This review examines opportunities for further improvements in Y_p . It shows that (1) photosynthesis is the only remaining major trait available for any further increases in Y_p on the scale of the past 50 years; (2) increasing leaf photosynthesis will increase yield when other factors are held constant; (3) analysis of theoretical limits to the efficiency of the photosynthetic process can reveal the key targets for improvement; and (4) a range of specific options for engineering improved leaf photosynthesis and crop yield might be realized on a relatively short time scale.

THEORETICAL FRAMEWORK FOR ANALYZING YIELD INCREASE

What are the physiological bases of increases in Y_p ? Following the principles of Monteith (1977) the Y_p of a crop and the primary production (P_n) at a given location are determined by

$$P_n = S_i \cdot \epsilon_i \cdot \epsilon_c / k \quad (1a)$$

$$Y_p = \eta \cdot P_n \quad (1b)$$

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where S_i is the annual integral of incident solar radiation (MJ m^{-2}); ϵ_i is the efficiency with which that radiation is intercepted by the crop; ϵ_c is the efficiency with which the intercepted radiation is converted into biomass; k the energy content of the plant mass (MJ g^{-1}); and η is the harvest index or the efficiency with which biomass is partitioned into the harvested product. S_i is determined by the site and the year. Although k varies very little between vegetative organs, typically averaging 17.5 MJ kg^{-1} , grain with significant oil content may have significantly higher energy contents that should be taken into account in computing yield from eqn 1 (Monteith 1977; Roberts *et al.* 1993). P_n is the primary productivity (i.e. the total plant biomass produced over the growing season). Y_p is therefore determined by the combined product of three efficiencies, each describing broad physiological and architectural properties of the crop: ϵ_i , ϵ_c and η . ϵ_i is determined by the speed of canopy development and closure, canopy longevity, size and architecture. ϵ_c is determined by the combined photosynthetic rate of all leaves within the canopy, less crop respiratory losses. Because reported P_n for annual crops is commonly the total above-ground biomass, measured ϵ_c is also lowered by the fact that some shoot tissues are shed before harvest and that root mass is not included. These factors lower apparent ϵ_c on the order of about 20% for annual grain crops (Monteith 1977; Beadle & Long 1985).

With reference to eqn 1, how has increase in Y_p been achieved and what scope is there for further increases? Over the past 50 years, increase in Y_p has been successfully achieved largely through increase in η . Grain in the modern cultivars of cereals can represent 60% of the total above-ground biomass at harvest (Evans 1993; Hay 1995). A minimum quantity of biomass must remain in the plant body, however, to ensure that vital nutrients and reserves can be translocated into the maturing grain, and to account for cell wall materials that cannot be degraded. While some opportunities for further increase in η remain (Evans 1997), particularly through molecular suppression of the shade-avoidance response in monotypic crop stands (Shlumukov *et al.* 2001), it seems unlikely that a η much greater than 0.6 may be realized.

Increased Y_p also results from increased ϵ_i through earlier canopy development and ground cover, and selection of cultivars able to respond to additional nitrogen fertilization without lodging. With these cultivars achieving an ϵ_i of 0.9 over the growing season, again, scope for further improvement is very limited (Beadle & Long 1985).

If η and ϵ_i are approaching an upper limit, further increase in Y_p can only be achieved by an increase in ϵ_c which is determined by photosynthesis and respiration. In theory, ϵ_c depends on the efficiency with which the absorbed light energy can be transduced into biomass (i.e. the efficiency of photosynthesis corrected for respiratory losses). This review considers the limitations to, and opportunities for, increasing net photosynthesis in crops. It is first necessary, however, to establish whether photosynthesis limits crop production and whether an increase in photosynthesis actually results in increased crop yields.

LEAF PHOTOSYNTHESIS: A TARGET FOR IMPROVEMENT?

The arguments against

The advent of transportable infrared CO_2 analysers opened the opportunity for selecting crop genotypes on the basis of leaf photosynthetic rates (Long, Farage & Garcia 1996). Influential studies, however, question the idea that leaf photosynthesis limits crop production. Evans and Dunstone (1970) show that modern bread wheat cultivars have lower leaf photosynthetic rates than their wild ancestors. This lack of correlation between crop yield and leaf photosynthetic rate is noted frequently in other studies (reviewed by Evans 1993, 1998). The lack of correlation between leaf photosynthetic rate and yield in such studies should have been no surprise because these plants differ genetically in many respects beyond photosynthesis. While it is implicit in eqn 1 that photosynthetic efficiency is critical to crop yield, this is the photosynthetic efficiency of the whole crop averaged over time. Many surveys of leaf photosynthesis are based on the light-saturated rate of a single leaf at a single stage in crop development (Long 1998). The relationship between single-leaf measurements and the whole crop will be complex, and not intuitive. Firstly, as much as 50% of crop carbon may be assimilated by leaves under light-limiting conditions in which very different biochemical and biophysical properties determine photosynthetic rate (Long 1993). Secondly, increases in leaf area may often be achieved by decreased investment per unit leaf area; thus, light-saturated photosynthetic rate is commonly lower in species with thinner leaves, quite simply because the apparatus is spread more thinly (Beadle & Long 1985). If crop improvement results in increased leaf area, mean leaf photosynthetic rate may decline because of increased self-shading, and maximum leaf photosynthetic rates may decline because resources are spread more thinly across the larger leaf area (Evans 1993).

Photosynthesis can be limited by sink capacity (i.e. ability to use photosynthate). After flowering, the major sink in grain crops is the number and potential size of the seed formed. Decreased sink capacity, as may be induced by removing filling grains, can feedback to decrease photosynthetic capacity (Peet & Kramer 1980). It may be expected, however, that breeding selects for the cultivars that are able to make maximum use of photosynthetic capacity. For example, if weather favours increased photosynthesis, an effectively selected cultivar should have sufficient capacity for formation of grain to use the additional photosynthate. However, a recent detailed analysis that reviewed the magnitude of seed dry weight changes in response to manipulations in assimilate availability during seed filling for wheat, maize and soybean has concluded that in all three crops, yield is usually more limited by sink than by source (i.e. photosynthesis) (Borrás, Slafer & Otegui 2004).

Contrary to the finding of Evans and Dunstone (1970), Watanabe, Evans and Chow (1994) show a strong positive correlation between leaf photosynthetic rate and date of release of Australian bread wheat cultivars. This difference

may be explained by the fact that the latter study is limited not only to a single species, but to a narrow range of germ plasm within that species. Here, variability in leaf area per plant and its distribution would be smaller, and variation in leaf photosynthetic rate is not confounded with large differences in total or specific leaf area. The potential of leaf photosynthetic rate in improving potential crop yield can only be evaluated when other factors, in particular leaf canopy size and architecture, are held constant. Sinclair, Purcell and Sneller (2004) reason from theory, however, that even in these circumstances, a 33% increase in leaf photosynthesis may translate into an 18% increase in biomass and only a 5% increase in grain yield, or a -6% change in grain yield in the absence of additional nitrogen. These conclusions that leaf photosynthesis has little potential in increasing crop yields are based on comparisons of different genotypes in which differences in leaf photosynthesis are confounded with many other genetic differences (Evans & Dunstone 1970; Borrás *et al.* 2004) or are limited to untested theoretical analyses (Sinclair *et al.* 2004).

In summary, lack of correlation between leaf photosynthesis and yield, coupled with evidence that yield is sink rather than source limited have led to a pervasive view that crop yields cannot be improved by increasing leaf photosynthetic rates. A true practical test of the question of whether increased leaf photosynthesis increases yield would ideally use the same genotype. Fortuitously, the focus on atmospheric CO₂ concentration [CO₂] increase has provided such tests in abundance.

What do elevated [CO₂] experiments tell us about the link between photosynthesis and yield?

Increase in [CO₂] has two effects on C₃ plants: an increase in leaf photosynthesis and a decrease in stomatal conductance to water vapor (g_s) (reviewed, Drake, Gonzalez-Meler & Long 1997; Long *et al.* 2004). Elevated [CO₂] increases net leaf photosynthetic rate primarily by (1) competitive inhibition of the oxygenase activity of ribulose-1, 5-biphosphate carboxylase/oxygenase (Rubisco) and therefore photorespiration; and (2) acceleration of carboxylation because the CO₂ binding site is not saturated at the current [CO₂]. The European Stress Physiology and Climate Experiment (ESPACE) project grew a single genotype of spring wheat (cv. Minaret) in similar open-top chambers under ambient (350 $\mu\text{mol mol}^{-1}$) and elevated [CO₂] (650 $\mu\text{mol mol}^{-1}$) at seven sites in Germany, Ireland, the UK, Belgium and the Netherlands, over three consecutive growing seasons (Mitchell *et al.* 1999). Across these sites, photosynthesis of the flag leaf – the major source of assimilate for the grain – was on average increased by 50%, and grain yield was increased by 35% (Bender, Hertzstein & Black 1999; Mitchell *et al.* 1999). ESPACE is particularly valuable because it used the same genotype in a range of environments. While limited to one genotype, it agrees well with conclusions that may be drawn from surveys of the several hundred paired treatments in which one genotype of a crop has been grown at both current ambient and elevated

[CO₂]. On average, an approximate doubling of the current [CO₂] in field or laboratory chambers caused no increase in leaf area, a 23–58% increase in leaf photosynthetic rate (Drake *et al.* 1997), and an average 35% increase in crop yield (Kimball 1983). More recent statistical meta-analyses reveal parallel increase in photosynthesis and yield under elevated [CO₂] in soybean (Ainsworth *et al.* 2002) and across the free-air carbon dioxide enrichment (FACE) studies that have grown crops at elevated [CO₂] under fully open air conditions (Ainsworth & Long 2005). These findings provide a very strong indication that a sustained increase in leaf photosynthesis leads to increased crop yield.

It might be argued that these [CO₂]-induced increases can also result from decreased water loss and water stress, or/and from decreased respiration, because elevated [CO₂] decreases g_s and increases net photosynthesis. Evidence that there is an independent increase because of increased leaf photosynthesis comes from two sources: (1) large increases in yield occurred under elevated [CO₂] with little change in leaf area when wheat was irrigated in the field to the level required for maximum yield (Kimball *et al.* 1995; Pinter *et al.* 1996), and when lowland rice was grown in paddy conditions in field chambers (Baker, Allen & Boote 1990); (2) C₄ plants show similar reductions in g_s to C₃ plants when grown at elevated [CO₂], but show no or little increase in net photosynthesis (Drake *et al.* 1997; Long *et al.* 2004, 2005). C₄ crops, compared with C₃ crops, grown under elevated [CO₂], show little or no increase in yield when grown under well-watered conditions (Ghannoum, von Caemmerer & Conroy 2001; Long *et al.* 2004, 2005). This is consistent with the expectation that C₄ photosynthesis is CO₂-saturated in the present atmosphere (Ghannoum *et al.* 2001).

If the Y_p of the major crops are sink rather than source limited, as implied by the analysis of Borrás *et al.* (2004), then again a yield increase should not result under elevated [CO₂]. Ainsworth *et al.* (2004) further analyse this by combining genetic manipulation of sink capacity with growth of soybean at current and elevated [CO₂]. They show a sustained increase in photosynthesis in soybean cv. Williams grown in the field under open-air [CO₂] elevation. However, mutation at the *dt1* locus to make this line determinate decreased potential reproductive sink size, and suppressed the response to elevated [CO₂]. In normal air, photosynthesis of the two lines did not differ significantly; in elevated [CO₂], however, there was a significant increase in non-structural carbohydrates in leaves of the determinate form during seed filling, which corresponded to a decline in photosynthesis, suggesting sink limitation. When a normally determinate line, cv. Elf, was grown in elevated [CO₂], it showed a similar increase in yield to the indeterminate cv. Williams and did not show any loss of photosynthetic capacity. An interpretation of these results is that, at least in soybean, conventional breeding selects for a sink capacity, which normally exceeds photosynthetic capacity. If sink capacity limits yield, then genetically decreasing potential reproductive axes in cv. Williams would decrease

	% Loss at each stage (efficiency at each stage)		% Remaining	
Incident energy outside photosynthetically active wavebands	50.0 (0.5)		50.0	
Reflected and transmitted light	5.0 (0.9)		45.0	
Light absorbed by non-photosynthetic pigments	1.8 (0.96)		43.2	
Photochemical inefficiency	8.4 (0.8)		34.8	
Photosynthetic type	C ₃	C ₄	C ₃	C ₄
Carbohydrate synthesis	22.8 (0.34)	24.8 (0.29)	12.0	10.0
Photorespiration	3.5 (0.7)	0 (1.0)	8.5	10.0
Dark respiration	3.4 (0.6)	4.0 (0.6)	5.1	6.0
Resulting ϵ_c			0.051	0.060

'% loss' shows the proportion of energy lost at each stage from interception to carbohydrate accumulation. Efficiency at each stage is given in parenthesis. '% remaining' shows how much of the energy remains at each stage along the transduction chain. C₃ crops (e.g. rice, wheat, soybean, barley) differ from C₄ (e.g. maize, sorghum). The latter lacks photorespiration, but requires more energy for carbohydrate synthesis; hence there is a different overall ϵ_c . Adapted from Beadle and Long (1985) and Long *et al.* (2005b). ϵ_c , radiation use efficiency.

yield in normal air, and the normal form of cv. Williams cannot increase yield in response to elevated [CO₂]. This is consistent with the fact that large increases in grain yield are achieved for C₃ crops grown in elevated [CO₂], including under fully open-air field conditions (Kimball, Kobayashi & Bindi 2002). This can only be achieved if yield is either source driven, or source and sink activity are coordinated, such that increase in source during early growth, as will occur in elevated [CO₂], stimulates sink capacity to avoid subsequent limitation.

Having established that increased leaf photosynthesis will increase crop yields, are there opportunities to increase potential photosynthetic efficiency other than waiting for atmospheric [CO₂] to rise?

WHAT IS THE POTENTIAL RADIATION USE EFFICIENCY OF CROPS (ϵ_c)?

In this section, the limits to maximum conversion efficiency, primarily maximum photosynthetic efficiency, are analysed. This provides a framework for discussing potential routes for improvement. While an ϵ_i of 0.9 and a η of 0.6 are high and probably near maximal, the maximum ϵ_c reported is around 0.024 for C₃ crops and 0.034 for C₄ over a growing season, although higher efficiencies may be observed for brief periods in the life of a crop (Monteith 1977; Beadle & Long 1985). For C₃ crops, the highest short-term efficiencies are about 0.035, and for C₄ about 0.043 (Beadle & Long 1985; Piedade, Junk & Long 1991; Beale & Long 1995). The following explores why these record numbers are apparently so low and shows that photosynthesis in crops is not as inefficient as we may at first assume from such seemingly low numbers.

About 50% of solar energy is in the near infrared wavelengths (> 700 nm). The energy of photons of > 700 nm is

Table 1. Efficiency of the transduction of intercepted solar radiation into plant carbohydrate through photosynthesis of crop leaf canopies

too low to drive charge separation at the photosynthetic reaction centers of land plants, and therefore outside the photosynthetically active waveband (Table 1). Leaves scatter absorbed light, resulting in some photons that reemerge as reflected light. The minimum photon requirement is 8 in C₃ plants, regardless of wavelength below 700 nm (i.e. a red photon has the same effect as a violet photon). A violet photon of 400 nm, however, contains 75% more energy than a red photon of 700 nm. The additional energy of the violet photon is lost as heat, representing an intrinsic photochemical inefficiency (Table 1). Other pigments, such as anthocyanins in the epidermis, absorb some light, but cannot pass this energy on to photosynthesis, resulting in inactive absorption (Beadle & Long 1985). One mole of photons of 690 nm wavelength contains 173.3 kJ, yet when 1 mol CO₂ is released from carbohydrate, it liberates 477 kJ. Because a minimum of 8 mol of photons are required to convert 1 mol of CO₂ to carbohydrate, the synthesis of carbohydrate therefore has a maximum efficiency of 477/(8 × 173.3) = 0.344, a loss of about 66% of energy at this step. Because the C₄ pathway requires more ATP, carbohydrate synthesis here further energy loss in photorespiration, which reoxidizes a portion of this carbohydrate (Beadle & Long 1985); it should be noted though that this cost increases with temperature (Long 1991) and that Table 1 assumes a temperature of 25 °C. Finally, mitochondrial respiration, necessary for synthesis of new tissues and maintenance of existing tissues, consumes about 40% of the remaining energy in all plants (e.g. Monteith 1977; Gifford 1995). In theory, a maximum ϵ_c of about 0.051 is possible in C₃ plants and 0.060 in C₄ plants (Table 1). If we compare this with the observed maxima of 0.035 (C₃) and 0.043 (C₄), then it can be seen that these seemingly low values of ϵ_c are in fact ca. 70% of the theoretical maxima. Clearly, actual yields may be increased by increasing the environmental

tolerance of ϵ_c , but are there opportunities to increase ϵ_c by reference to Table 1 under optimal growth conditions and so increase Y_p ?

SPECIFIC OPPORTUNITIES FOR INCREASING PHOTOSYNTHESIS

The maximum ϵ_c that a genotype may achieve under optimum conditions may fall short of the theoretical maximum (Table 1) for two reasons. Firstly, leaves become light saturated and, by definition, energy is wasted and efficiency drops. This may be improved by a canopy architecture that provides a better distribution of light by maintaining the maximum efficiency of photosynthesis under light-limiting conditions and by increasing the photosynthetic rate at light saturation. The latter may be increased by both improved rates of regeneration of the acceptor molecule of CO_2 [ribulose biphosphate (RuBP)] and by higher rates of catalysis of carboxylation. Changes here would bring ϵ_c at 25 °C closer to the theoretical 0.051. Secondly, the theoretical ϵ_c may be increased by decreasing photorespiration (Table 1). This may be achieved by converting C_3 crops to C_4 or by improving the specificity of Rubisco for CO_2 . Conversion of a C_3 to a C_4 crop would theoretically raise the maximum ϵ_c at 25 °C from 0.051 to 0.060. If Rubisco can be engineered to be completely specific to CO_2 , this would raise ϵ_c from 0.051 to 0.073; this larger increase is because no additional energy is required for carbohydrate metabolism in contrast to C_4 photosynthesis. The theoretical bases for each of these changes and practicalities of realizing each are subsequently discussed. It is shown that the most widely promoted strategies – conversion of C_3 crops to C_4 and improved specificity of Rubisco – may be the most difficult to achieve and, from a theoretical basis, might result in lower and not higher ϵ_c .

Modifying crop canopies to increase ϵ_c

Leaf photosynthesis responds non-linearly to increases in solar energy (Fig. 1c). In C_3 crops, leaf photosynthesis is saturated at photosynthetic photon flux densities (PPFD) of about one-quarter of maximum full sunlight; therefore, any PPFD intercepted above this level is wasted. A mature, healthy crop may have three or more layers of leaves (i.e. a leaf area index of ≥ 3). If the leaves are roughly horizontal (Fig. 1a, plant X), the uppermost layer would intercept most of the light at midday, while about 10% may penetrate to the next layer and 1% to the layer below that. With the sun overhead, the PPFD intercepted per unit leaf area by an almost horizontal leaf at the top of a plant canopy would be $1400 \mu\text{mol m}^{-2} \text{s}^{-1}$ or more, about three times the amount required to saturate photosynthesis (Fig. 1c). Therefore, at least two-thirds of the energy intercepted by the upper leaves is wasted. A better arrangement for these conditions would be for the upper layer to intercept a smaller fraction of the light, allowing more to reach the lower layers. This is achieved when the upper leaves are more vertical and the lowermost leaves are horizontal, as in the example of plant

Y (Fig. 1a) (Nobel, Forseth & Long 1993). For a leaf with a 75° angle with the horizontal, the amount of light energy intercepted per unit leaf area would be $700 \mu\text{mol m}^{-2} \text{s}^{-1}$, just sufficient to saturate photosynthesis, but the remaining direct light ($1300 \mu\text{mol m}^{-2} \text{s}^{-1}$) would penetrate to the lower layers of the canopy. By distributing the light energy among leaves in this way, plant Y would have over double the efficiency of light energy use than plant X at midday in full sunlight (Ort & Long 2003). This example oversimplifies the situation, however, because the sun is only directly overhead within the tropics; at all locations, sun angle continually changes. What advantage does this altered canopy architecture have when the daily course of sun angles are taken into account?

A biophysical model of light transmission into leaf canopies was used to determine light flux at different leaves as sun-leaf geometry changed over the course of the day (Humphries & Long 1995). The predicted light fluxes were then used to predict photosynthesis at the individual leaves from the biochemical model of Farquhar, von Caemmerer and Berry (1980), as described by Long (1991). Summing the predicted leaf photosynthesis for the different canopy layers over the course of the day gives the total canopy CO_2 uptake (A_c'). A leaf area index of 3 was assumed for a midsummer day at a mid-latitude (52° lat.) and a canopy temperature of 25 °C. Simulations show that a canopy of type Y had a ϵ_c of 0.046 compared with 0.033 for type X canopy (Fig. 1d). Although this is only about half the increase that would occur if the sun remained directly overhead (Fig. 1d, compared with Ort & Long 2003), it nevertheless suggests considerable improvement may still be achieved by manipulation of canopy architecture. Importantly though, this advantage is lost or is reversed at low leaf area indices (< 1.5).

Mathematical models have been developed to design optimum distributions of leaves to maximize efficiency, which have been used as a guide for selecting improved crops. This approach has been a major factor in improving the productivity of rice (Beadle & Long 1985). Older varieties with more horizontal leaves such as plant X have been replaced by newer varieties that have been bred to have more vertical leaves in the top layer, such as plant Y (Nobel *et al.* 1993). The advantage of this change in canopy design is greatest when the sun is overhead and diminishes progressively as sun angles decline and is in diffuse lighting conditions, but are still substantial (Fig. 1d compared with Ort & Long 2003).

Relaxing the photoprotected state more rapidly to increase ϵ_c

Figure 1b shows the typical non-rectangular hyperbolic response of photosynthesis to PPFD. As PPFD increases, photosynthesis saturates. However, the leaf continues to absorb photosynthetically active radiation. This additional energy exceeds the capacity for photosynthesis, and without some alternative mechanism to dissipate the energy, it will cause photooxidative damage to the photosynthetic

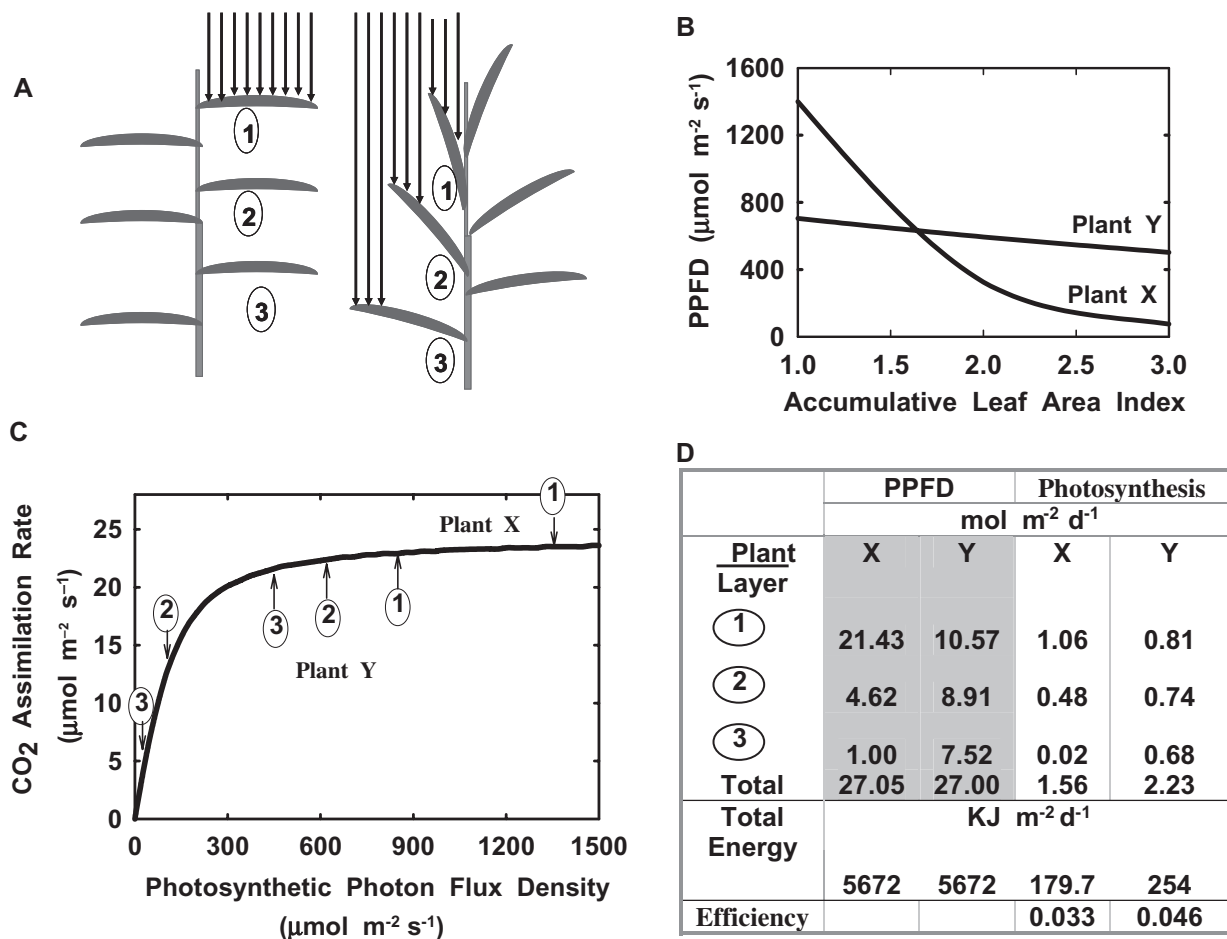


Figure 1. (a) Plant X has mostly horizontal leaves, such that the upper layer (1) intercepts most of the incoming solar energy, shading the lower layers [(2) and (3)]. Plant Y has vertical leaves at the top, becoming more horizontal near the bottom. This arrangement spreads the light more evenly between layers (derived from Ort & Long 2003 and Long *et al.* 2005b). (b) The predicted average photosynthetic photon flux density (PPFD) at noon at different canopy depth represented by accumulative leaf area index for plant X and plant Y. The simulation is done using Windows Intuitive Model of Vegetation response to Atmosphere and Climate Change (WIMOVAC) (Humphries & Long 1995) for the 190th day of year at a latitude of 52° N assuming a leaf area index of 3, and a constant canopy temperature of 25 °C. Parameters and method as detailed in Long (1991). (c) The response of photosynthetic CO₂ uptake rate to PPFD. Arrows below the curve indicate the average PPFD at the three leaf layers of canopy in plant Y, and arrows above the curve indicate the average PPFD for three leaf layers of canopy in plant X. (d) From graph c, the diurnal course of PPFD for three layers in plants X and Y and the diurnal photosynthetic CO₂ uptake rates are calculated and integrated. The amount of solar energy and the photosynthetic CO₂ uptake for each leaf layer and their totals for the two plants are given. The efficiency is calculated as the ratio of solar energy stored in the form of carbohydrate to total intercepted solar energy by the two plants.

apparatus, especially the photosystem II (PSII) reaction center. This is largely avoided by an induced increase in thermal dissipation of energy via the formation of epoxidated xanthophylls (Long, Humphries & Falkowski 1994; Havaux & Niyogi 1999; Baroli & Niyogi 2000). This process increases thermal dissipation of absorbed light energy within the PSII antenna system and protects PSII from damage in high light. This reversible increase in thermal quenching is termed photoprotection, and it decreases the maximum quantum yield of PSII (F_v/F_m) and CO₂ uptake (Φ_{CO_2}), that is, the initial slope of the response of photosynthetic CO₂ uptake rate to PPFD (Fig. 1b) (reviewed, Zhu *et al.* 2004a). In addition, it decreases the convexity (θ) of the non-rectangular hyperbolic response (Fig. 1b). At

high light, decreases in Φ_{CO_2} and θ have minimal impact on carbon gain, while the increased thermal energy dissipation protects PSII against oxidative damage. However, the decrease in Φ_{CO_2} and θ , decrease carbon gain at low light. A finite period of time is required for recovery of Φ_{CO_2} and θ when solar radiation drops, as for example when a cloud obscures the sun or change in sun angle places one leaf in the shade of another. Given that light in leaf canopies in the field is continually fluctuating, what is the cost of this delayed recovery to potential CO₂ uptake by a canopy in the field?

Zhu *et al.* (2004a) use a reverse ray-tracing algorithm for predicting light dynamics of 120 randomly selected individual points in a model canopy to describe the discontinuity

and heterogeneity of PPFD within the canopy. Because photoprotection is at the level of the cell, not the leaf, light is simulated for small points of $10^4 \mu\text{m}$ rather than as an average for a leaf. The predicted light dynamics are combined with empirical equations simulating the dynamics of the light-dependent decrease and recovery of ΦCO_2 and θ , and their effects on the integrated daily canopy carbon uptake (A_c'). The simulation was for a model canopy of leaf area index 3 with random inclination and orientation of foliage, on a clear sky day (lat. 44°N , 120th day of year). The delay in recovery of photoprotection is predicted to decrease A_c' by 6.5–17% at 30°C and by 12.5–32% at 10°C . The lower value is for a chilling-tolerant species; the upper is for a chilling-susceptible species. Temperature is important because it decreases photosynthetic capacity and rate of recovery from the photoprotected state. On average, losses at typical temperatures for temperate crops would be of the order of ca. 15% (Zhu *et al.* 2004a). Much larger losses from photoprotection result when photosynthesis is decreased by stresses (Long *et al.* 1994).

Large gains in ϵ_c can be achieved if the lag in relaxation in photoprotection can be decreased or eliminated. Is this a possibility? Photoprotection fulfils a necessary function of decreasing the probability of oxidative damage to PSII, which in itself would lower photosynthetic efficiency and would require repair and replacement of the proteins before efficiency can be restored. In the longer term, a continued excess of excitation energy would lead to irreversible photooxidation (reviewed, Long *et al.* 1994). Can the loss found here be decreased without the risk of photoinhibition and photooxidation? Falkowski and Dubinsky (1981) identify algae associated with corals that can withstand $1.5 \times$ full sunlight without evidence of loss of maximum photosynthetic efficiency or photoinhibition, showing that the loss of efficiency is not an intrinsic requirement of the photosynthetic apparatus. In higher plants, Wang *et al.* (2002) show a close correlation between increased rate of recovery from the photoprotected state and increased biomass production in the 'super-high yield' rice cultivars. This increased rate of recovery is associated with an increase in concentrations of the intermediates of the xanthophyll cycle. Across plant species, higher rates of recovery have been associated with xanthophyll cycle capacity, including the epoxidation associated with recovery (Long *et al.* 1994). These findings suggest that up-regulation of capacity for recovery from photoprotection is feasible and may already have been achieved in rice.

Photorespiration

About 30% of the carbohydrate formed in C_3 photosynthesis is lost through photorespiration (Monteith 1977). The loss increases with temperature so that photorespiration is a particularly significant inefficiency for C_3 crops in tropical climates and during hot summer weather in temperate climates (Fig. 2). Photorespiration results from the apparently unavoidable oxygenation of RuBP by Rubisco (reviewed, Giordano, Beardall & Raven 2005). Beyond this point, the

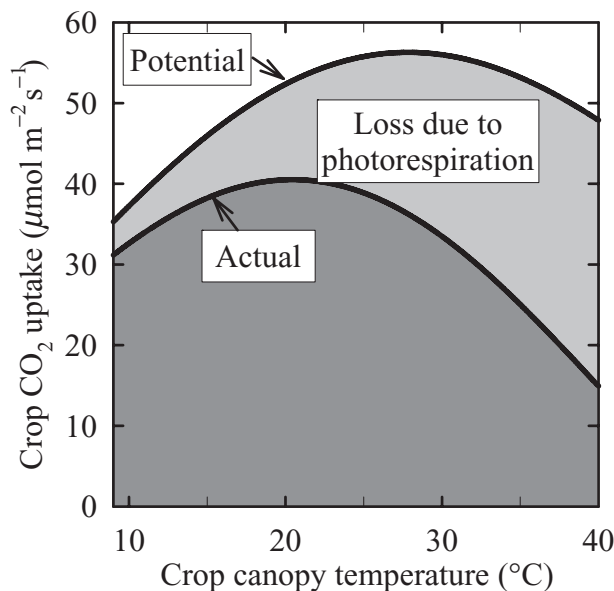


Figure 2. Calculated actual and potential rates of crop canopy photosynthesis versus temperature, where potential is defined as the rate in the absence of photorespiration. The difference represents the loss caused by photorespiration. Calculation assumes a crop with a leaf area index of 3 and a photon flux above the canopy of $1800 \mu\text{mol m}^{-2} \text{s}^{-1}$ (i.e. full sunlight). Parameters and method as detailed in Long (1991).

purpose of photorespiratory metabolism is to recover the carbon diverted into this pathway. Blocking photorespiratory metabolism downstream of Rubisco simply results in this carbon entering a dead-end metabolic pathway. Indeed, mutants that lack any one of the photorespiratory enzymes die unless they are grown at low oxygen or at very high CO_2 to inhibit oxygenation of RuBP. The only remaining prospect for decreasing photorespiration then, is decreased oxygenation. Would decreased oxygenation result in higher yields? Photorespiratory metabolism can dissipate excess excitation energy at high PPFD, involves the synthesis of serine and glutamate, and transfers reductive power from the chloroplast to the mitochondrion. This has led some to suggest that photorespiration is essential for normal plant function (e.g. Barber 1998; Evans 1998). However, xanthophylls provide a far more effective means of dissipating excess energy. Unlike photorespiration, this dissipation mechanism is not a significant drain on the ATP and NADPH produced by the light reactions. Further, dissipation of energy as heat through xanthophylls is induced by excess light and is reversed when light is no longer in excess. So unlike photorespiration, it does not continue to divert energy from photosynthesis when light is no longer in excess. In addition, the photosynthetic cell has pathways besides photorespiration for amino acid synthesis and transfer of reductive energy to the cytosol (reviewed, Long 1998), which suggests that the supposed 'beneficial' functions of photorespiration are redundant within the cell. Further, photorespiration can be eliminated without detri-

ment to the plant by growing plants in a very high concentration of CO₂, a competitive inhibitor of the oxygenase activity of Rubisco. For example, wheat can grow normally and can complete its life cycle under these unusual conditions (Wheeler *et al.* 1995). Commercial growers of some greenhouse crops increase [CO₂] to three or four times the normal atmospheric concentration (Chalabi *et al.* 2002). This inhibits the oxygenation reaction of Rubisco, increasing photosynthetic efficiency and final yield. At present, the global [CO₂] is rising and this, too, is diminishing photorespiration, but atmospheric change also includes many potentially negative effects for crops, including increased temperature, decreased soil moisture and an associated rise in phytotoxic tropospheric ozone (reviewed, Ort & Long 2003; Long *et al.* 2004, 2005). Healthy C₄ plants avoid photorespiration by concentrating CO₂ at the site of Rubisco. Despite earlier contradictory arguments, it is now clear that photorespiration is not an essential metabolic pathway in crops. Can it be eliminated? Two possibilities are conversion of C₃ crops to C₄ or improved specificity of Rubisco for CO₂.

C₄ photosynthesis a means to eliminate photorespiration?

Terrestrial C₄ plants differ from C₃ plants in containing two distinct layers of photosynthetic tissue, one external to the other, each containing morphologically and functionally distinct chloroplasts. This cellular differentiation within the photosynthetic tissue is termed 'Kranz' leaf anatomy. In this arrangement, the mesophyll surrounds the inner photosynthetic bundle sheath where Rubisco is localized. Only the mesophyll has intercellular air spaces and contact with the atmosphere. CO₂ is first assimilated into a C₄ dicarboxylate through phosphoenolpyruvate (PEP) carboxylase (c) in the mesophyll. The dicarboxylate is transferred to the bundle sheath where it is decarboxylated, releasing CO₂ at the site of Rubisco. The resulting pyruvate is transferred back to the mesophyll where it is phosphorylated to provide PEP, completing the C₄ cycle. The photosynthetic C₄ cycle is in effect a CO₂ pump that concentrates CO₂ around Rubisco to ca. 10 × current atmospheric concentrations (Hatch 1987; von Caemmerer 2003). It effectively eliminates photorespiration, but requires additional energy to operate the C₄ cycle (Table 1). C₄ photosynthesis in seed plants has evolved independently at least 45 times (Kellogg 1999; Sage 2003). The first clear evidence of C₄ plants in the fossil record coincides with the what appears to be the lowest atmospheric [CO₂] in Earth's history, a concentration that was maintained with only minor fluctuations until the Industrial Revolution (Cerling 1999). The repeated evolution of C₄ plants, despite the complexity of the process, is strong evidence that there may be no other adaptive variability to use among land plants for decreasing photorespiration. If there were forms of Rubisco with improved ability to discriminate against oxygenation, then it would surely have been a simpler route for evolution than selecting the complex syndrome of changes needed to provide functional

C₄ photosynthesis. Table 1 shows that from theory, C₄ plants will on average have a higher maximum ϵ_c than C₃. This difference increases with temperature because of the increase in photorespiration as a proportion of photosynthesis (Fig. 2), such that this advantage would be most pronounced in the tropics. Indeed the highest known productivity in natural vegetation is for a C₄ perennial grass in the central Amazon, which achieves a net production of 100 t (dry matter) ha⁻¹ year⁻¹ (Piedade *et al.* 1991; Long 1999; Morison *et al.* 2000). Of our major food crops, only maize and sorghum are C₄ (Long 1998). Is there a theoretical advantage in the C₄ process and can it be transferred to our major C₃ crops?

C₄ plants have the advantage of eliminating energy loss in photorespiration, but at the expense of additional energy, typically 2 ATPs per CO₂ assimilated. Because the specificity of Rubisco for CO₂ and the solubility of CO₂ relative to O₂ decline with increases in temperature, photorespiration as a proportion of photosynthesis increases with temperature. In dim light, when photosynthesis is linearly dependent on the radiative flux, the rate of CO₂ assimilation depends entirely on the energy requirements of carbon assimilation (Long, Postl & Bohlär-Nordenkampf 1993; Long 1999). The additional ATP required for assimilation of one CO₂ in C₄ photosynthesis, compared with C₃ photosynthesis, increases the energy requirement in C₄ plants (Hatch 1987). However, when the temperature of a C₃ leaf exceeds ca. 25 °C, the amount of light energy diverted into photorespiratory metabolism in C₃ photosynthesis exceeds the additional energy required for CO₂ assimilation in C₄ photosynthesis (Hatch 1992; Long 1999). This means that below ca. 25–28 °C, C₄ photosynthesis is less efficient than C₃ photosynthesis under light-limiting conditions [i.e. it has a lower quantum yield (Φ_{CO_2})]. This is demonstrated in Fig. 3a, in which values of Φ_{CO_2} were calculated from theory (Long 1999). This is very similar to actual measurements of the temperature response of Φ_{CO_2} in C₃ and C₄ species (Ehleringer & Björkman 1977; Ehleringer & Pearcy 1983).

Total photosynthesis by a crop canopy, however, reflects a combination of light-limited and light-saturated CO₂ assimilation. At light saturation, the efficiency of photosynthesis is independent of the maximum quantum yield of CO₂ uptake (Φ_{CO_2}) and depends on the maximum rate of photosynthesis (A_{sat}). Here, the C₄ plant has an advantage, even below 25 °C, because its maximum rate is greater than that of an equivalent C₃ leaf because of the absence of photorespiration, as shown in Fig. 2. Does a higher rate of light-saturated photosynthesis offset the lower rate of light-limited photosynthesis at the crop canopy level at temperatures below 25 °C? Note the dynamic nature of the balance between light-limited and light-saturated photosynthesis within a canopy over the course of a day. By combining established steady-state biochemical models of C₃ and C₄ leaf photosynthesis (Farquhar *et al.* 1980; Collatz, Ribascarbo & Berry 1992) with canopy radiation transfer models, the integrals of the diurnal course of photosynthesis can be calculated (Humphries & Long 1995). Using this approach, Fig. 3b shows that while the advantage of C₄

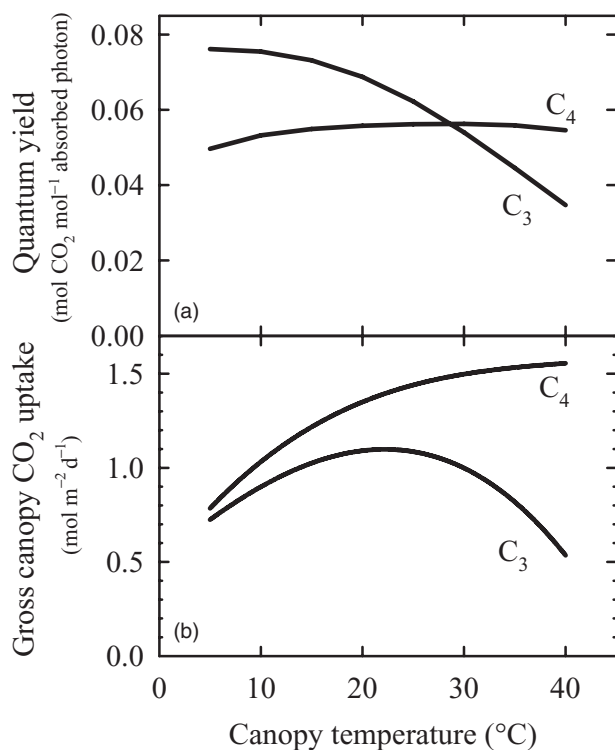


Figure 3. (a) The predicted maximum quantum yield of photosynthetic CO₂ uptake for leaves of C₃ and C₄ species under different temperatures following. (b) The predicted rates of gross canopy CO₂ uptake integrated over a diurnal course for a range of canopy temperatures. The simulation is for a leaf area index of 3 assuming a spherical distribution of foliar elements, on 30 June and with clear sky conditions (atmospheric transmittance = 0.75) at a latitude of 52 °N. Redrawn from Long (1999).

photosynthesis diminishes with temperature, there is still an advantage to the simulated daily integral of canopy CO₂ uptake even at 5 °C. Thus, even at the cool early growing season temperatures typical of temperate climates, some advantage can theoretically be gained from C₄ photosynthesis. That this can occur in practice is supported by the observation that the highest known dry matter productivity for the UK is for the cold-adapted C₄ perennial grass *Miscanthus × giganteus* that produces 29 t (dry matter) ha⁻¹ in southern England with a measured ϵ_c of 0.039 (Beale & Long 1995; Beale, Morison & Long 1999). At the least, this suggests that with continued improvement in cold tolerance, maize may outyield C₃ crops even in cool climates, such as NW Europe.

Figure 3b shows that for a tropical C₃ crop such as rice, substantial gains in ϵ_c may be made by engineering the addition of the photosynthetic C₄ cycle into the crop. Genes coding for the enzymes of the photosynthetic C₄ cycle have been isolated from maize and other C₄ plants, and have been used, both singly and in combination, to transform rice and other C₃ crop species (reviewed in detail by Raines 2006). While high activity of the introduced C₄ enzymes is achieved in many cases, there is little evidence that over-

expression of C₄ genes in C₃ species alters photosynthetic characteristics or increases yield (Häusler *et al.* 2002; Miyao 2003), with only a few exceptions (e.g. Sheriff *et al.* 1998; Ku *et al.* 2001). Furthermore, while it is now possible to transform C₃ plants to express the C₄ pathway enzymes in a single cell, C₄ plants differ not only in their use of the photosynthetic C₄ cycle, but also in spatial separation of PEPc and Rubisco. In C₄ plants, there is a semi-impermeable barrier between the mesophyll and bundle sheath cells, which limits the diffusion of CO₂ released in the bundle sheath back into the mesophyll. Any CO₂ that diffuses back must be reassimilated, increasing the requirement of ATP and energy requirement per CO₂ molecule assimilated. Figure 3b assumes a leakage rate of 10% (i.e. 1 in 10 CO₂ molecules diffuses back into the mesophyll). If the entire mechanism is engineered within a single cell as is being attempted in rice (i.e. PEPc in the cytoplasm and Rubisco in the chloroplast), then leakage of CO₂ would be very much higher. As such, the additional ATP required in recycling CO₂ would drive the maximum ϵ_c well below that of C₃ photosynthesis. von Caemmerer (2003) shows from theory that such a single cell system would be very inefficient because of the leakage of a large proportion of the CO₂ released at Rubisco. As such, a single-cell C₄ system would allow a plant to maintain a positive carbon balance under high light and drought conditions, but would be very inefficient at low light or in dense canopies. Two naturally occurring C₄ plants have been identified in which the process occurs within a single cell. However, these are elongated cells in which PEPc and Rubisco are spatially separated by distance (Voznesenskaya *et al.* 2001, 2002; Edwards, Franceschi & Voznesenskaya 2004). Both are slow-growing species of hot semiarid environments consistent with the theoretical analysis of von Caemmerer (2003). Although higher photosynthetic rates have been suggested to occur in rice transformed with pyruvate orthophosphate dikinase (PPDK) and PEPc, this appears a result of increased stomatal aperture rather than of increased capacity within the mesophyll (Ku *et al.* 2001). The analysis of von Caemmerer (2003) shows that simple expression of the C₄ enzymes in the mesophyll of C₃ crops is not adequate in obtaining the ϵ_c advantages of C₄ photosynthesis. This requires understanding of the integrated development of Kranz anatomy, localization of C₄ and C₃ enzymes, and necessary membrane transporters. Understanding of the development of C₄ photosynthesis is still too incomplete to determine the necessary transformations (Monson 1999), although an alternative route may involve the search for a simple 'genetic switch' that, when triggered, would induce the formation of Kranz anatomy (SurrIDGE 2002). At present, a more viable approach to concentrating CO₂ within a single cell may be to use some of the successful concentrating mechanisms found in algae (reviewed, Giordano *et al.* 2005). Equally, it should be noted that there are likely opportunities to improve the Y_p of C₄ crops in cool climates. Although maize and sorghum show a low Y_p north of ca. 50 °N, the related C₄ grass, *M. × giganteus* has been shown to be highly productive. Understanding how this is

achieved may be critical to increasing the Y_p of our existing C_4 crops (Beale & Long 1995; Naidu *et al.* 2003).

An alternative means of decreasing photorespiration is to decrease the oxygenation capacity of Rubisco, but as subsequently explained, this may come with the penalty of decreased carboxylation capacity.

Increasing the efficiency of Rubisco

In considering how to redesign plant canopies, it was noted that photosynthesis at the leaf level is saturated by a PPF well below full sunlight (Fig. 1c). Referring back to Fig. 1c, it can be seen that the solar radiation exceeds the PPF needed to saturate photosynthesis for much of a sunny day. Are there other approaches to using this excess energy? The response of photosynthesis to solar energy describes a non-rectangular hyperbola, rising rapidly with increasing solar radiation at low PPF, but saturating at about 25% of full sunlight. Why does this saturation occur?

Several analyses suggest a colimitation by Rubisco and by capacity for regeneration of RuBP, the primary substrate for CO_2 assimilation in C_3 leaves. So why not just increase the amount of Rubisco per unit of leaf area? Rubisco is already the most abundant protein in crop leaves, constituting about 50% of the soluble protein of the leaf. Calculations of volumes suggest there may not be physical capacity to add more (Pyke & Leech 1987).

Rubisco appears to carry a double penalty. Firstly, it catalyses oxygenation of RuBP leading to photorespiration. Secondly, the maximum catalytic rate of Rubisco (k_c^c) is remarkably slow compared with most plant enzymes, such that large amounts of the protein are required to achieve the photosynthetic rates necessary to support high productivities in C_3 crops. This inefficiency explains why Rubisco is so much more abundant than any other protein in leaves.

It has long been recognized that genetic modification of Rubisco to increase the specificity for CO_2 relative to O_2 (τ) would decrease photorespiration and would potentially increase C_3 crop productivity. However, when the kinetic properties of Rubisco forms from different photosynthetic organisms are compared, it appears that forms with high τ have low maximum catalytic rates of carboxylation per active site (k_c^c) (Bainbridge *et al.* 1995). Theoretical considerations also suggest that increased τ may only be achieved at the expense of k_c^c . If a fixed inverse relationship between k_c^c and τ implied from measurements is assumed, and if increased concentration of Rubisco per unit leaf area is not an option, will increased τ result in increased leaf and canopy photosynthesis?

Zhu, Portis & Whitmarsh (2004b) use a mathematical model to explore these questions. From values of τ and k_c^c reported for Rubisco across diverse photosynthetic organisms, an inverse relationship between k_c^c on τ was defined. Following the steady-state biochemical model of leaf photosynthesis of Farquhar *et al.* (1980), the C_3 photosynthetic CO_2 uptake rate (A) is either limited by the maximum Rubisco activity ($V_{c,max}$) or by the rate of regeneration of RuBP, which, in turn, is determined by the rate of whole

chain electron transport (J). If J is limiting, increase in τ would increase net CO_2 uptake because products of the electron transport chain would be partitioned away from photorespiration into photosynthesis. The effect of an increase in τ on Rubisco-limited photosynthesis depends on both k_c^c and $[CO_2]$. As in the case of C_4 photosynthesis, there are conflicting consequences at the level of the canopy. Increased τ would increase light-limited photosynthesis, while the associated decrease in k_c^c would lower the light-saturated rate of photosynthesis. Zhu *et al.* (2004b) simulated the consequences of variation in τ assuming an inverse change in k_c^c for carbon gain by crop canopies. An increase in τ results in an increase in leaf CO_2 uptake at low light, but it decreases CO_2 uptake in high light. Over the course of a day, total crop canopy CO_2 uptake (A_c') results from significant amounts of both light-limited and light-saturated photosynthesis. Simulation of A_c' suggests that the present average τ found in C_3 terrestrial plants is supraoptimal for the present atmospheric $[CO_2]$ of $370 \mu mol mol^{-1}$, but would be optimal for $200 \mu mol mol^{-1}$, a value remarkably close to the average of the last 400 000 years. This suggests that Rubisco in higher terrestrial plants has adapted to the past atmospheric $[CO_2]$, but that further adaptation has been slow and has failed to change in response to the relatively rapid rise in $[CO_2]$ that has occurred since the start of the Industrial Revolution.

The thesis that increased $[CO_2]$ favours the selection of forms of Rubisco with increased k_c^c and decreased τ is consistent with the observation that Rubisco from C_4 plants, in which the enzyme functions in a high $[CO_2]$, typically has a higher k_c^c and lower τ than in C_3 land plants (Seemann, Badger & Berry 1984; Sage 2002;). Similarly, Galmes *et al.* (2005) suggest that lower $[CO_2]$ is found in the mesophyll of plants from saline and arid habitats because of their persistently lower g_s , and they provide evidence that this has led to the selection of higher τ forms of Rubisco in some C_3 species. Zhu *et al.* (2004b) show that if Rubisco from the non-green algae *Griffithsia monilis* can be expressed in place of the present C_3 crop Rubisco, then canopy carbon gain can be increased by 27%. These simulations suggest that very substantial increases in crop carbon gain may result if exotic forms of Rubisco can be successfully expressed in C_3 plants. Much evidence and theory points towards a strong negative relationship between specificity and catalytic rate of carboxylation in Rubisco. In this case, an indirect result of engineering higher specificity would be lower crop canopy photosynthesis because the detrimental effect of lowered catalytic rate would outweigh the beneficial effect of increased specificity (Zhu *et al.* 2004b). Ideally, a crop would express a high k_c^c Rubisco in the upper canopy leaves exposed to full sunlight and a high τ Rubisco in the shaded lower canopy leaves.

Most C_3 annual crop canopies form leaves at progressively higher levels so that leaves start life at the top of the canopy and then become progressively shaded as new leaves form above. Shading is sensed in plant leaves by the balance of red/far-red light via the phytochrome system (Gilbert, Jarvis & Smith 2001). One possibility would be for

Species	A_c' (mmol m ⁻² d ⁻¹)	A_c' (%)	A_{sat} (μ mol m ⁻² s ⁻¹)
Current average C ₃ crop ($k_c^c = 2.5$, $\tau = 92.5$)	1040	100	14.9
<i>Griffithsia monilis</i> ($k_c^c = 2.6$, $\tau = 167$)	1430	127%	21.5
<i>Amaranthus edulis</i> ($k_c^c = 7.3$, $\tau = 82$)	1250	117%	28.3
<i>A. edulis</i> /current ($k_c^c = 2.5$, $\tau = 92.5$)	1360	131%	28.3

Table 2. Estimates of the daily canopy carbon gain (A_c') after Zhu *et al.* (2004b) and assuming the hypothetical replacement of the average form of Rubisco from C₃ crop species with Rubiscos from other species

Reported values for k_c^c and τ of these species (Jordan & Ogren 1984; Seemann *et al.* 1984; Whitney *et al.* 2001) are listed. The final row extends to the results of Zhu *et al.* (2004b) to simulate the gain that can be achieved if a form of Rubisco with a high k_c^c (*A. edulis*) can be expressed in the sunlit leaves and if a form with high τ (current C₃ average) can be expressed in the shade leaves.

k_c^c , maximum catalytic rate of Rubisco; τ , specificity of Rubisco for CO₂ relative to O₂; A_{sat} , maximum rate of photosynthesis; Rubisco, ribulose 15-biphosphate carboxylase/oxygenase.

plants to trigger the replacement of a high k_c^c Rubisco with a high τ form as the leaf acclimates to shade. Table 2 shows that such a system can increase ϵ_c by 31%, in comparison with an equivalent crop canopy with the current typical Rubisco of C₃ crops. This would increase ϵ_c both by decreasing photorespiratory losses in the lower canopy and by increasing the light-saturated rate of photosynthesis in the upper canopy.

Regeneration of RuBP

As noted previously, light-saturated photosynthesis in crop leaves is typically colimited by $V_{c,max}$ and by capacity for regeneration of RuBP, termed J_{max} in the context of the model of Farquhar *et al.* (1980). This has become the standard framework for analysing limitations to C₃ leaf photosynthesis. If the rate of carboxylation at Rubisco is increased, then J_{max} should also be increased to gain maximum benefit. By 2050, atmospheric [CO₂] will be about 50% higher than today. This change, without any modification of the protein, will increase the efficiency of Rubisco by partially inhibiting oxygenation. From kinetic data, it may be calculated that as a result, $J_{max}/V_{c,max}$ would need to increase by 30% to maintain an optimal distribution of resources (Long *et al.* 2004). Interestingly, acclimation of soybean to growth under elevated [CO₂] in the field involves a significant increase in $J_{max}/V_{c,max}$, but the increase is 7%, less than the theoretical change needed to maximize response (Bernacchi *et al.* 2005). Increases in J_{max} will therefore be necessary, simply to adapt plants to rising [CO₂].

Unlike $V_{c,max}$, regeneration of RuBP does not depend on the amount or the properties of any single protein, but on the complete photosynthetic electron transport chain and on all the enzymes of the Calvin cycle except Rubisco. Transgenic plants with small decreases in the quantities of specific proteins produced by antisense technology in tobacco suggest that two points in this chain limit J_{max} : the cytochrome *b₆/f* complex in the electron transport chain

and sedoheptulose-1:7-bisphosphatase (SbPase) in the Calvin cycle have been shown to strongly control the rate of RuBP synthesis (Price *et al.* 1998; Harrison *et al.* 2001; Raines 2003). Of course, decreased photosynthesis as a result of a decrease in a specific protein, even in the absence of pleiotropic effects, is not proof of limitation in the wild-type plant because if several proteins are present in just sufficient amount to support observed *in vivo* rates of photosynthesis, antisense reduction in any one would cause a decrease in rate. Transgenic tobacco plants over-expressing an *Arabidopsis* SbPase, however, have now been produced and show a significantly greater light-saturated photosynthetic rate and greater daily carbon gain in young leaves than the wild-type plants from which they are derived. The growth rate of these plants is accelerated and leaf area and leaf biomass are increased up to 30% (Lefebvre *et al.* 2005; reviewed, Raines 2006). Transgenic tobacco plants over-expressing a dual-function cyanobacterial fructose-1,6-bisphosphatase/SbPase targeted to chloroplasts also show enhanced photosynthetic efficiency and growth. Compared with wild-type tobacco, final dry matter and photosynthetic CO₂ fixation of the transgenic plants are said to be 24–50% higher, respectively (Miyagawa, Tamoi & Shigeoka 2001). The next and critical challenges are to see if these large gains can be extended into food crop plants and also if the increases in photosynthesis and yield can be validated in field conditions.

OVERVIEW OF OPPORTUNITIES AND BARRIERS

Theoretical considerations suggest that further increases in Y_p of the major crops will depend largely on increasing crop photosynthesis. Although other analyses have suggested a lack of correlation between increased photosynthesis and crop yields, the overwhelming weight of evidence from elevated [CO₂] research now shows a very close link between increased photosynthesis and yield, when the increase in

Table 3. Summary of possible increases in solar radiation conversion efficiency (ϵ_c) that may be achieved and the speculated time horizon for provision of material that can be introduced into plant breeding programs (Adopted from Long *et al.* 2005b)

Change	% Increase in ϵ_c relative to current realized value	Speculated time horizon (years)
Rubisco with decreased oxygenase activity but without decreased catalytic rate	30% (5–60%)	???
Efficient C ₄ photosynthesis engineered into C ₃ crops	18% (2–35%)	10–20
Improved canopy architecture	10% (0–40%)	0–10
Increased rate of recovery from photoprotection of photosynthesis	15% (6–40%)	5–10
Introduction of higher catalytic rate foreign forms of Rubisco (Table 2)	22% (17–30%)	5–15
Increased capacity for regeneration of RuBP via overexpression of SbPase	10% (0–20%)	0–5

The time to realizing sufficient seed for commercial cultivation would be longer.

The value under the heading ‘% Increase ...’ is the suggested mean, followed by the range of possible change, calculated by substituting the changed properties into the simulation model of Humphries & Long (1995). See text for references.

Rubisco, ribulose 15-biphosphate carboxylase/oxygenase; RuBP, ribulose biphosphate.

photosynthesis is achieved without otherwise altering the genetics of the plant. Achieving similar increases in photosynthesis to those achieved by artificial elevation of [CO₂] will most likely be realized by genetic engineering.

Sinclair *et al.* (2004) argue that transformation of a few genes is unlikely to result in major yield increases. In this respect, they note the lack of success, great difficulty and complexity of translating biochemical and physiological research into improvements in crop yield by conventional plant breeding. They also note the several generations, and therefore several years, needed to introduce such changes. However, this ignores two major benefits of molecular transformation (Gepts 2002). Firstly, if a single gene transformation can, for example, produce a targeted change without pleiotropic effects (e.g. decreased photorespiration), then the phenotype is otherwise preserved. This is exemplified by the single transformations that provide roundup-ready cultivars, which otherwise preserve the properties of the untransformed cultivar (e.g. Hu *et al.* 2003). Secondly, it avoids the several generations of backcrossing needed when transferring a single gene or several genes by conventional breeding into the desired background. Time is still required to bulk up the population of the transformed genotype, but several years are still gained (reviewed by Gepts 2002). The issue of whether a single or few gene transformations can increase photosynthesis and yield, however, is critical. As outlined in the previous section, an increase in the expression of a single photosynthetic protein increases the dry matter production of tobacco. However, the elevated [CO₂] literature perhaps provides the best evidence that a single manipulation can increase yield. Because the direct effect of elevated [CO₂] is to suppress oxygenation and photorespiration at the level of Rubisco leading to higher photosynthesis and yield, then similar increases can reasonably be expected if Rubisco can be transformed to decrease oxygenation.

What transformations are likely to increase photosynthesis and yield, and on what timescale might they be realized? Table 3 provides a speculative view of the possible increases and time horizons over which changes may be

achieved. The maximum theoretical ϵ_c are ca. 0.051 and 0.060 for C₃ and C₄ crops, respectively. The C₃ maximum can be raised by decreasing photorespiration, either by identifying Rubisco with an increased specificity for CO₂ or by engineering C₄ photosynthesis into C₃ crops. At present, higher specificity forms of Rubisco found in other photosynthetic organisms carry the penalty of lower catalytic rates. It is reasoned that replacement of existing Rubisco with these forms would result in lower, not higher, rates of canopy carbon uptake. At the present atmospheric [CO₂], introduction of Rubisco forms with higher catalytic rates would increase ϵ_c .

Theoretical analysis now shows that introduction of C₄ photosynthesis into a single cell is energetically highly inefficient. This appears confirmed in rice transformed to express the C₄ photosynthetic pathway (Raines 2006). Engineering C₄ photosynthesis into C₃ crops would therefore require not only the introduction of the C₄ photosynthetic cycle, but also the Kranz leaf anatomy and associated differential expression of photosynthetic proteins. While the basis of development of this differential expression is not as yet fully understood, it is possible that a genetic trigger that induces Kranz anatomy may yet be found (SurrIDGE 2002). Still, such a complex transformation would probably not be possible for decades (SurrIDGE 2002; Table 3). In the shorter term, extending the environmental range of existing C₄ crops is likely to be a more successful route to higher total productivities.

The theoretical ϵ_c is not achieved even by the most productive systems under optimum conditions. The major cause is likely the fact that in direct sunlight, significant parts of the crop canopy are light saturated (i.e. absorbed light simply exceeds the capacity of the leaf to use light). Here, there are four areas that can allow significant increases in ϵ_c : (1) alteration of crop canopy architecture to improve the distribution of radiation and to minimize the period over which any leaf is light saturated. Although this is an approach that has fallen from fashion, clearly, for many crops, canopy architecture remains far from optimal. This can increase ϵ_c by as much as 40% at midday in full

sunlight. Increased computational capacity now allows the use of complex reverse-ray tracing algorithms to identify optimal architecture for different environments and to identify selection criteria. This is also one area of improvement that can be approached by conventional plant breeding. (2) Increased recovery from photoprotection to increase efficiency of photosynthesis of leaves in the shade can increase daily ϵ_c by ca. 15% and by more at lower temperatures. Again there is variability among photosynthetic organisms that can be used. The molecular mechanism of photoprotection and its relaxation is understood and may be improved by relatively simple transformations. (3) The amount of Rubisco is possibly already close to the capacity of photosynthetic cells and represents the largest single investment of nitrogen within most growing crops. Despite its high concentration, it is a limitation to light-saturated photosynthesis. Simulations show that replacement of current C₃ crop Rubisco with forms with higher catalytic rates from other photosynthetic organisms can increase daily ϵ_c by up to 31%. Because Rubisco is formed from two types of subunit, one coded for in the nucleus and one in the plastid genome, transformation is particularly challenging. However, such transformations have already been achieved with tobacco (reviewed, Maliga 2004; Raines 2006). Given the progress in transformation technology over the past 10 years, such changes appear achievable on a 10-year time horizon. Even larger increases in ϵ_c would be possible if leaves can be engineered to express a high catalytic rate form of Rubisco initially and then if this can be replaced with a high specificity form during shade acclimation. (4) To gain full advantage of an engineered increase in Rubisco efficiency or the increase in Rubisco efficiency that will simply result from rising [CO₂], an increase in capacity for RuBP regeneration is necessary. Antisense transformations suggest that the cytochrome b6/f complex and the SbPase are the major limitations to RuBP regeneration. Preliminary evidence suggests that engineered over-expression of SbPase can increase ϵ_c by 10%. Thus, this transformation is already realizable as a means to increase ϵ_c .

In conclusion, while opportunities to increase the theoretical maximum ϵ_c of C₃ or C₄ crops do not appear realizable on a 10–20 years time horizon, there are a number of opportunities to improve the ability of crops to approach the current theoretical maxima. Some increases may be achieved by conventional breeding, although most will require introduction of foreign genetic material. Despite the apparent complexity of yield as a genetic trait, the modification of single genes has, indeed, resulted in increased plant yield (Gepts 2002; van Camp 2005). Consequently, such genetic modifications appear achievable within a 10–20 years time horizon (Table 3). Finally, both environmental stress and respiration are beyond the scope of this analysis. Clearly, there is a wide range of opportunities for improving the tolerance of ϵ_c to stress, although most are specific to the individual stress (Ort & Long 2003; Raines 2006). Respiration, as a factor that may be decreased to increase ϵ_c , has received very little attention,

in part because knowledge of the limitations and the full role of the respiratory process is far less complete than for photosynthesis; in particular, the role of cyanide-insensitive respiration appears to impose a variable inefficiency on net carbohydrate accumulation. In the one example in which respiration was used as a selection criterion, substantial yield increases were achieved by selecting for decreased respiration rates (Wilson & Jones 1982).

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