Crop Productivity and Photoassimilate Partitioning

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Summary. The photosynthetic basis for increasing the yield of major field crops is examined in terms of improving the interception of seasonal solar radiation by crop foliage, the efficiency of conversion of intercepted light to photosynthetic assimilates, and the partitioning of photoassimilates to organs of economic interest. It is concluded that, in practice, genetic and chemical manipulation of light interception over the season and of partitioning offer the most potential for achieving further increases in yield. During the history of improvement of genetic yield potential of crops, increase in the partitioning of photoassimilates to harvested organs has been of primary importance.

The concept that crop yield is determined by a single limiting factor that, if made nonlimiting, would give way to the next most limiting factor has often been interpreted too literally. Even with respect to environmental variables, it is true only in the extreme. A crop is rarely limited in its yield (or even its daily growth rate) by a single environmental factor. Indeed, that a successful crop is adapted to its environment indicates that no one factor is limiting. The progressive increases in crop yields have been due to improvements in numerous co-limiting factors or phenomena, such as pest and disease control by chemical and genetic means; improved weed control by more effective and selective herbicides; increased supply of nutrients and water; better matching of the timing of crop life cycles to seasonal changes in the environment; closer matching of the crop's thermal requirement for development to the radiation regime; more timely and efficient operations enabled by greater mechanization; and increase in genetic yield potential.

Genetic yield potential has not been precisely defined. However, several genotypes can be operationally ranked for comparative genetic yield potential by growing them side by side in an experimental agricultural environment having near-optimal planting, water, and fertilizer regimes; effective control of pests and diseases; lack of lodging and weather damage; and a complete harvest. Although average farm yields are well below the best experimental yields, steady improvement in genetic yield potential has been found to contribute to increases in average yields where it has been examined. Seed yields of modern wheat and soybean cultivars are nearly 40 percent greater than those of earlier cultivars when tested under comparable favorable conditions (*1*). During the past 40 years genetic yield potential of corn hybrids increased about 50 percent (*2*) and peanuts nearly 100 percent (*3*) as modern genotypes, adapted to fertile environments and high planting densities, were developed.

Nevertheless, there remains potential for further improvement in genetic yield potential of crops grown in fertile environments. While record farm yields do not in themselves represent genetic yield potential, the fact that average yield of six major U.S. grain crops is only about 20 to 35 percent of record yields (*4*) is suggestive of a substantial improvement still to be achieved.

Since most of the dry weight of plants consists of carbon compounds, the increase in harvested yield is intimately linked to changes in the photosynthetic fixation of carbon dioxide per unit of land area and the subsequent partitioning of this photoassimilate between harvested and nonharvested portions of the crop. However, photosynthesis and photoassimilate partitioning have not traditionally been explicit selection targets for plant breeders. The photosynthetic basis for increasing harvested yield involves maximizing (i) the amount of light intercepted by foliage, (ii) the conversion efficiency of intercepted light to photosynthetic products, and (iii) the partitioning of photoassimilate to the harvested "economic sinks" (grains, tubers, sugar storage stem, and so forth).

A goal of crop physiologists is to understand these component phenomena sufficiently to identify attributes that are manipulable through management or by chemical (plant growth modifiers) or genetic means to increase crop yield.

Light Interception

Generally, seasonal dry weight growth of field crops is directly and linearly related to the absolute amount of light intercepted by green foliage (*5*, *6*). This relation usually holds whether the variation in intercepted light is achieved by variation in incident light flux (*7*) or in leaf cover of the ground due, for example, to variation in time of planting. Much of the impact of water supply and nitrogen fertilizer on growth is via leaf development and senescence and hence via light interception. Thus it is of primary importance to ensure that crop genotype and management are such that the radiation of the growing season is intercepted as fully as possible. This requires minimizing the period of incomplete ground cover at the beginning and end of the season. The growing season is usually circumscribed either by temperatures too low for plant development or by seasonal drought. During the period of full canopy cover, the leaf area per unit of ground area (leaf area index) ideally should not exceed that required for full interception (*8*) or the crop will have committed photoassimilate to making noneconomic leaves that might have been invested in economic yield components. It can be necessary, however, for peak leaf area index to exceed that needed for full interception at that time if this ensures larger interception near the beginning and end of the season. Much seasonal light is not intercepted by foliage in many crops (*5*, *6*); further improvement in this area could be rewarding.

For the season as a whole, increased production of total crop dry weight, whether it be achieved by greater light interception or by greater efficiency of light conversion due, say, to CO_2 enrich-

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ment (*9*), is generally manifest in improved economic yield (*10*). Analysis of this correspondence between net overall carbon fixation per unit of ground area and yield can be taken a step further by considering seasonally integrated daytime net CO_2 fixation by a field crop (night respiration excluded). This has been done with soybean crops planted at different densities and under different levels of shading (*11*) over 4 years. A broad linear relation was found between seed yield and seasonal daytime net photosynthesis per unit of land area. Many researchers have even sought to find relations between growth or yield and net photosynthesis rates determined at leaf, chloroplast, or enzymatic levels of organization, but they have not been successful.

Conversion of Intercepted Light to Photoassimilate

A typical rate of crop dry weight production per unit of seasonally intercepted radiation (*5, 12*) is about 3 grams per megajoule of light in the photosynthetically active wavelength band (400 to 700 nanometers). There has been a long history of efforts to improve photosynthetic efficiency by breeding for various subcomponents of crop photosynthetic CO_2 fixation. These efforts have included diffusionally, biochemically, and photochemically determined steps. Several reasons—both physiological and methodological—suggest why this approach has not been successful (*13*). Important among these reasons is the complexity of feedbacks in the crop photosynthetic system. The hierarchy of regulations operating over the full range of space and time from chloroplast membranes to field crop surface, and from less than nanoseconds for primary photochemical events to months for seasonal growth appear to ensure that no single plant attribute is rate limiting to CO_2 fixation for long, and that quantitative manipulation of one attribute is compensated for in time or space. So, although it is well established that total dry weight and economic crop yield can readily be increased by improving photosynthetic environment through CO_2 or light enrichment in the field (*9*), it has not been possible to improve yield by direct genetic or (nonsubstrate) chemical manipulation of the photosynthetic system at levels of organization below that of leaf canopy development. In fact, the progressive increases in yield potential for crops such as wheat, barley, oats, and soybeans have not been associated with increases in crop biomass. Similarly, increased yields in cultivars of wheat, corn, tomato, and rice have occurred without any increase in relative growth rate of young plants (*14*). Over the history of improvement in genetic yield potential of field crops, it has been the partitioning of photosynthetic product between economic yield and the rest of the plant ["harvest index" (HI) (*14*)] that has been of primary importance, even though selection was not directed specifically to that end. Harvest index, also termed coefficient of economic yield, can apply to the ratio of harvested dry weight to total aboveground dry weight (shoot HI) or to the ratio of harvested dry weight to above- plus belowground dry weight (plant HI). In the field, assessing root dry weight can be extremely difficult, so shoot HI is more commonly used in agronomic studies.

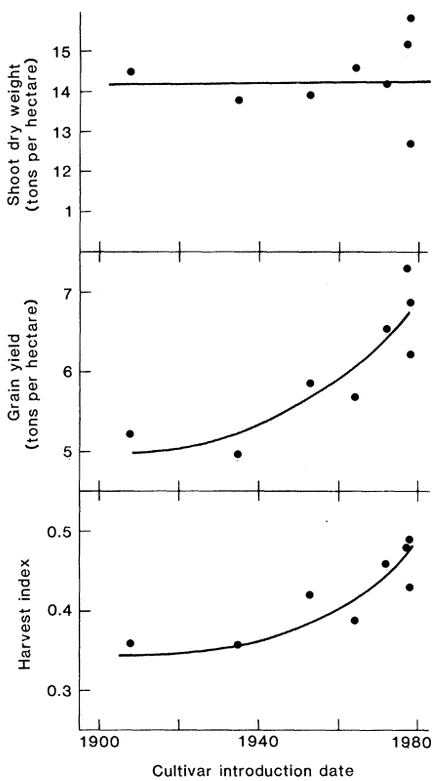

Fig. 1. Comparative yield potential and HI for eight British winter wheat cultivars, plotted against the year that each cultivar was introduced. The cultivars were grown in intensively managed experimental plots at a high-fertility site near Cambridge, England, in the 1977–1978 season (*16*).

Harvest Index

Improved HI was responsible for the grain yield potential increases among successively developed cultivars of the major cereal species over the past century (*1, 14, 15*). Eight major winter wheat cultivars, released in England during a period of 70 years (*16*), are compared in Fig. 1. There was no trend in total shoot dry weight yield when all cultivars were grown under the same field conditions, but grain yield had increased from 5 to 7 tons per hectare while HI rose from 0.35 to 0.5. Similarly, with a legume crop, peanuts (*3*), the doubling of pod yield was due primarily to increased HI rather than to increased total yield (Fig. 2), extra pods being produced at the expense of vegetative plant parts (Fig. 3) (*17*). Similar patterns have been found for barley and soybeans (*14*).

Such results concerning the importance of changes in dry weight partitioning between organs have focused attention on HI as a specific selection criterion. Although there must be an upper limit to HI because of the need for some investment in leaves and a robust stem, it does not yet appear to have been reached in most crops. A limit of 0.62 has been suggested for wheat (*16*). This compares with a value of about 0.5 for best modern cultivars and 0.2 to 0.35 for traditional varieties. Compared with most photosynthetic attributes, for which seasonally representative values

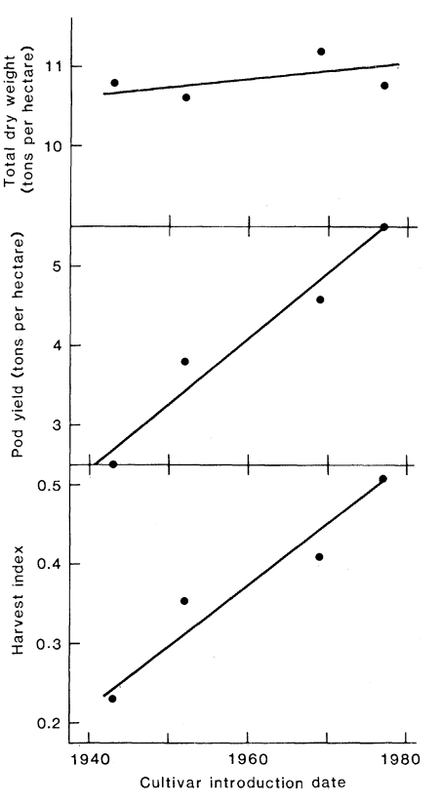

Fig. 2. Comparative yield potential and HI for four major peanut cultivars from the southern United States, plotted against the year that each cultivar was introduced. The cultivars were grown in intensively managed experimental plots in a favorable environment near Gainesville, Florida, in the 1976 season. [Adapted from W. G. Duncan *et al.* (*3*)]

in the field are difficult to obtain, HI is simply measurable on a crop stand at the end of the season without destroying the next generation of seed. Furthermore, in spring wheat, HI for spaced plants appears to be representative of the parameter for a community of plants (*18*). Breeders require easily measured selection criteria because of the large populations to be screened and the relative difficulty of work in the field; they frequently need to use spaced plants in the early stages of new cultivar development.

Recognition of the importance of partitioning of fixed carbon between alternative sinks in past improvements in genetic yield potential has led to much research into the physiology and biochemistry involved. Figure 4B shows the main sinks for the gross quantity of carbon fixed in the leaves. It illustrates vividly what a small proportion of carbon fixed is finally harvested. Figure 4A shows the typical fate of annual solar radiation incident on crops. Carbon partitioning phenomena start at the very point of carbon fixation with partitioning between the photosynthetic carbon reduction (PCR) cycle and the photosynthetic carbon oxidation (PCO) cycle. They continue to be expressed in all aspects of carbon economy while harvestable yield is being generated. For example, carbon is partitioned between starch storage in the chloroplast (for later mobilization) and immediate export into the mesophyll cell cytoplasm; between sucrose retention in leaf mesophyll and loading into the phloem for export; between retention in the phloem and unloading into growing sinks; between vegetative growth and reproductive growth; and among competing alternative sinks, such as root and shoot, elongating wheat stem and ear growth, or grains within an ear. Little is known about the regulation of these processes. Given the numerous carbon partitioning steps that occur between primary fixation of CO_2 and final accumulation of a small fraction of the fixation product into harvested organs (Fig. 4B), only a few aspects can be dealt with here.

PCR Cycle Versus PCO Cycle

Concurrent with photosynthetic carbon fixation by the PCR cycle, the interlinked PCO cycle releases back to the atmosphere CO_2 that was recently fixed. In C_3 species the proportion of CO_2 fixed by the PCR cycle that is partitioned back to CO_2 by the PCO cycle is usually found to be 15 to 20 percent (*19*), but there remain methodological uncertainties, and figures based on other methods range as high as 50 percent (*20*). Since the function of this apparently wasteful partitioning is unknown, much research has been directed at its reduction or elimination by chemical or genetic means.

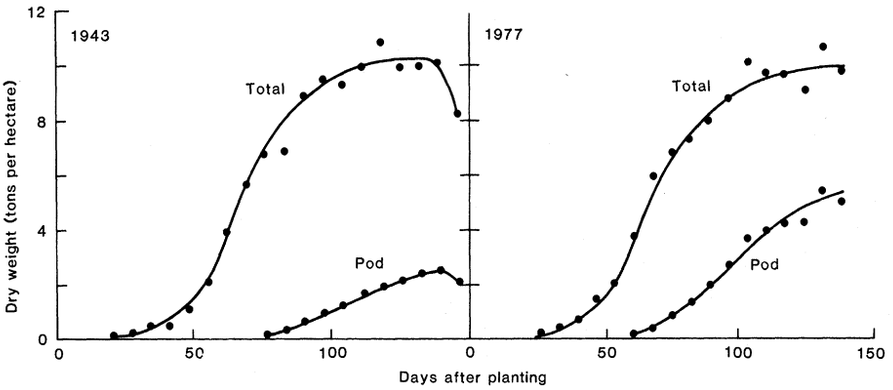

Fig. 3. Time course of total plant dry weight production ("total") and fruit growth ("pod") for the peanut cultivars grown in the study represented in Fig. 2. The cultivars are Dixie Runner (released about 1943) and Early Bunch (1977). [Adapted from W. G. Duncan *et al.* (*3*)]

The photorespiratory pathway arises from an oxygenase activity possessed by ribulosebisphosphate carboxylase (*21*), which catalyzes the primary CO_2 fixation of photosynthesis. The substrate for both oxygenase and carboxylase activity is the photosynthate intermediate ribulose-1,5-bisphosphate. This competition between O_2 and CO_2 for the active site in ribulosebisphosphate carboxylase reduces the gross rate of CO_2 fixation (Fig. 4B). A product of the oxygenase reaction is phosphoglycolate. It is the subsequent multistep conversion of phosphoglycolate to phosphoglycerate, an intermediate required in the PCR cycle, that involves release of photorespiratory CO_2. This photorespiratory pathway also accomplishes a partial recovery of carbon partitioned into phosphoglycolate. Attempts to chemically inhibit phosphoglycolate metabolism to prevent the CO_2 release have not been successful at increasing net CO_2 fixation: usually net fixation decreases instead (*13*). It seems that inhibition of any step in phosphoglycolate metabolism prevents a necessary recycling of carbon back into the PCR cycle, while the accumulated photorespiratory intermediates have no capability of feedback regulation of the partitioning between oxygenation and carboxylation of ribulosebisphosphate (*22*). This conclusion focused attention on the ratio of carboxylase to oxygenase activity of ribulosebiphosphate carboxylase. Evidence suggesting that the ratio is not immutable has encouraged a search in several laboratories for suitable manipulations of the protein structure by mutation or genetic engineering to suppress oxygenase activity while maintaining or enhancing carboxylase activity. This would have the double advantage of both reducing the competitive effect of O_2 at the active site and reducing the photorespiratory release of CO_2. However, no one has been able to refute the argument that the oxygenase reaction is a physiochemically inevitable consequence of the carboxylase mechanism (*23*). Nevertheless, recombinant DNA methodology provides a means for systematic manipulation of the amino acid composition of ribulosephosphate carboxylase. This, together with investigation of the exact mechanisms of carboxylation and oxygenation, offers hope of creating an improved carboxylase/oxygenase ratio for crop species or at least of determining why it is not possible to do so.

Starch Versus Sucrose Synthesis in Leaves

Early products of the PCR cycle in the chloroplast are the triose phosphates, phosphoglycerate, and dihydroxyacetone phosphate. These sugar phosphates are biochemically and spatially partitioned between two major biosynthetic pathways, one leading to the synthesis and retention of starch in the chloroplast and the other to sucrose synthesis in the cytoplasm. Up to 50 percent of the photosynthetically fixed carbon can be allocated either to starch or to sucrose depending on several factors, including plant species, environment, nutritional status, and developmental stage of the plant (*24, 25*). This partitioning is important to plant growth because the formation of sucrose (the principal phloem transport sugar in most crops) is a prime determinant of carbon export from photosynthesizing leaves and because leaf starch is a major carbohydrate reserve

that is mobilized to sucrose when current photosynthesis is low relative to sink demand for photoassimilate, as occurs in low light or darkness (*26*) or, in the longer term, when the leaves are senescing (*27*).

Dynamic and balanced partitioning of the PCR-derived triose phosphates between starch synthesis in the chloroplast and sucrose synthesis in the cytoplasm appears to be mediated by the levels of certain key metabolites and by the so-called phosphate translocator protein in the chloroplast membrane. Export of triose phosphates across the chloroplast envelope to the cytoplasm is stoichiometrically and obligatorily coupled in a one-to-one counterexchange with inorganic phosphate (P_i) through this specific translocator protein (*28*). In the cytoplasm, sucrose is synthesized from triose phosphates. Key enzymes involved are fructose-bisphosphatase (FBPase) and sucrose-phosphate synthase (SPS) (*29*). Sucrose synthesis is a phosphate-liberating process (net reaction, 4 triose phosphates + 3 $H_2O \rightarrow$ 1 sucrose + 4 P_i). The liberation of inorganic phosphate in the cytoplasm during sucrose synthesis favors continued triose phosphate export from the chloroplast by counterexchange through the phosphate translocator. Thus, under conditions that favor sucrose synthesis, triose phosphates are partitioned away from the starch biosynthetic pathway that resides in the chloroplast. If sucrose synthesis in the cytoplasm is reduced, triose phosphates remain within the chloroplast for starch synthesis. The resulting increase in phosphoglycerate within the chloroplast stroma (high phosphoglycerate to P_i ratio) also favors starch synthesis by allosterically activating the starch-synthesizing enzyme adenosine diphosphate–glucose pyrophosphorylase (*30*).

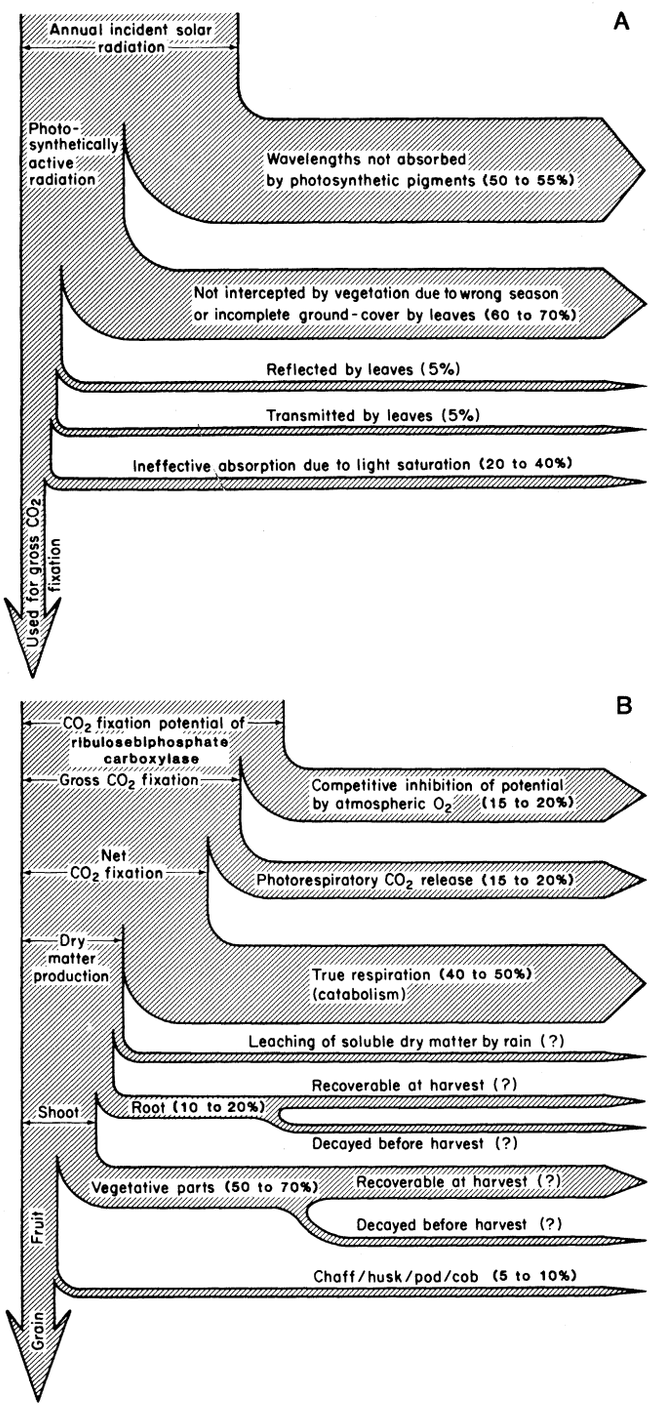

Fig. 4. (A) Fate of solar radiation incident over 1 year on a field used for a typical grain crop in a temperate agricultural environment. (B) Partitioning of gross photosynthetic CO_2 fixation by a generalized temperate grain crop. The values in parentheses and relative width of the bars are intended as guides to the proportion of the previous fractional flow remaining that is diverted into each successive sink. Foraging by insects has not been included because of its extreme variability.

Studies on source-sink manipulations of whole plants show that photosynthetically fixed carbon can be preferentially partitioned into sucrose available for export during periods of high sink demand or retained by starch when sink demand is low (*31*). However, in other plants, export from photosynthesizing leaves continues undiminished in response to a rapid change in sink demand, at least in the short term; the exported carbon accumulates in alternative sinks (*32*).

Starch synthesis and accumulation in leaves may be controlled indirectly by the rate of sucrose synthesis. A growing body of evidence (*29, 33*) suggests that the FBPase and SPS are key regulating enzymes in sucrose synthesis. As such, the activities of these enzymes, when acting in coordination with the phosphate translocator, may represent an important link between sink demand and rates of carbon partitioning into starch and sucrose. For example, the level of SPS in plants appears to be negatively correlated with the total starch content of leaves and with biochemical partitioning of photosynthetic carbon into starch. Plants that form little starch, such as wheat, barley, and spinach, have high SPS activity compared to plants that form large amounts of starch, such as tobacco, peanuts, and soybeans. Intraspecific differences in SPS activity and carbon partitioning have been noted in wheat (*25*). Recent studies of soybeans showed that altering the source-to-sink ratio by partial defoliation, pod removal, or changes in photosynthetic irradiance caused changes within hours in extractable SPS activity and in starch and sucrose synthesis (*24, 34*). These results support the hypothesis that SPS activity is important to starch-sucrose partitioning in leaves.

Regulation of FBPase is receiving increased attention with the recent discov-

ery of fructose-2,6-bisphosphate (FBP) in plants. FBP plays an important regulatory role in glycolysis and gluconeogenesis in animal liver (*35*). In plants the level of FBP responds to changes in light, specific metabolites, sugars, and CO_2. That FBP is a potent inhibitor of cytoplasmic FBPase and sensitizes FBPase to the effects of FBP and P_i suggests that it plays a key regulatory role in sucrose biosynthesis (*36*).

Catabolism Versus Anabolism

Energy for growth and maintenance of plant structure and for ion uptake is derived largely from respiratory catabolism of photosynthetically derived sugars to CO_2 and water (*37*). Up to 50 percent of the net CO_2 fixation by leaves of an annual plant may ultimately be lost to the plant by subsequent respiration. Crop scientists have long questioned whether the partitioning of photosynthetically fixed carbon between biosynthesis (anabolism) and respiration (catabolism) could be shifted in favor of more biosynthesis. Can respiratory efficiency be improved, and if so, would it improve crop yield?

Two possible routes to greater efficiency in respiratory utilization of fixed carbon can be envisioned. Either the efficiency of conversion of stored carbohydrate to adenosine triphosphate might be increased or utilization of adenosine triphosphate for processes not strictly required for plant growth in the crop environment might be reduced.

While bioenergeticists traditionally have viewed respiration as an optimized process under tight control, in plants there is an alternative terminal oxidase that transfers electrons from the cytochrome oxidase chain to oxygen at ubiquinone. This cyanide-insensitive alternative oxidase is not coupled to adenosine triphosphate synthesis (*38*) and as such appears to be wasteful of energy. The pathway is found in many species, in both shoots and roots (*39, 40*), but does not operate until the capacity of the cytochrome oxidase system has been exceeded or blocked after ubiquinone. Considering the degree of engagement of the pathway in roots, it was calculated that in wheat the carbon loss to that pathway was at least 6 percent of the carbon in final grain yield (*39*). If a loss of such magnitude occurs and can be shown not to be associated with an essential function, its elimination could translate into a significant increase in the partitioning of carbon to economic yield.

Utilization of the high-energy intermediates from respiration may be operationally divided into that required for the processing of stored carbohydrate to new growth and that required for maintaining existing cells in a viable state (*41*). While it has been argued that the biosynthetic pathways that convert glucose to the various lipids and nitrogenous monomers required for growth are efficient (*42*), energy required for maintenance of existing cells may be as much as 50 percent of the overall respiration rate in whole plants, and the utilization of this energy is poorly understood. If the continuous breakdown and resynthesis of existing cellular compounds (turnover) could be slowed, or if the energetic cost of maintaining inter- and intracellular chemical and electrochemical gradients across membranes could be reduced, more carbon might be available for economic yield.

While improvement of respiratory efficiency may not have the potential for very large yield increases, there is evidence of increased plant growth rate and yield in association with decreased respiration. In corn and tall fescue genotypes possessing high growth rates, low rates of leaf respiration were found (*43*). More directly, selection for intravarietal variation in respiration rate of mature leaf segments of perennial ryegrass readily gave lines with high or low respiration rates. Measurement of forage yield under simulated cropping conditions in both greenhouse and field studies (*44*) showed that the lines with low respiration rates had a consistent 6 to 13 percent greater annual dry matter yield than the parent population from which they were derived. This increase came primarily from later summer cuttings in a continuous cropping regime, when growth temperature was relatively high and the amount of root and stem tissue was large. As such, it may indicate that some portion of the maintenance respiration requirement was decreased in these lines.

The metabolic basis for this reduction in respiration is not known, nor is there any indication that yield increases associated with it would necessarily follow if genotypes with such reduced respiration were established in annual grain crops. About half of the yield increase was associated with an improved ability of the lines with low respiration rates to establish new leaf cover after defoliation (*45*). So the yield enhancement from a reduction in respiration rate might not be significant in annual grain crops, which are not required to undergo multiple regrowth.

Vegetative Versus Reproductive or Storage Growth

The observed increase in HI for several improved crop species reflects a shift from excessive vegetative development in traditional varieties to greater partitioning into fruits or other sinks for photoassimilate, such as potato tubers or swollen storage roots, in modern cultivars. The basis for this change can be examined from two perspectives. The first concerns the timing and establishment of large numbers of fertile flowers or other storage organ initials. The second concerns regulation of transport and partitioning of current photoassimilate and prior reserves into these developing harvestable organs rather than into further branching, rooting, or ineffective flowering.

For traditional varieties of species from which seeds are harvested, such as cereals and grain legumes, the number of flowers formed and fertilized far exceeds the number that fill to mature seeds. Despite having less reproductive redundancy, modern high-yielding cultivars still have much fruitless flowering. In terms of yield components, however, the improvement during the era of systematic breeding has mostly involved number of fruits harvested per hectare rather than fruit size.

A key to high yield is the establishment of a large number of seeds before their rapid filling commences. Barring extraordinary weather or other calamity, once seed number has been established mature yield has largely been predetermined. The average mature weight per seed is a more conservative property of a cultivar than is seed number per hectare. These generalizations can be illustrated with wheat. Two weeks before pollination starts, a modern dwarf wheat has several times more partly developed flowers than will set and fill grain (*46*). Each spikelet (that is, inflorescence branchlet) in the central part of the spike can have nine to ten primordial florets. However, about two of these florets have ill-formed anthers that render them incompetent to set grain. During the 2 weeks before pollination there is a collapse in floret competency, such that typically only two or three florets will actually set grain. This 60 to 70 percent loss of competency to set grain appears to be closely related to the supply of photosynthetic assimilates at the time. Consequently, in the 10- to 20-day period before pollination final seed number is particularly sensitive to irradiance. Low light at that time can cause an irretriev-

able loss of yield because fewer seeds are established (*47*). As a consequence of this sensitivity of floret abortion to photoassimilate supply in temperate cereals, CO_2 enrichment to boost photosynthesis of field crops before anthesis can raise final yield by increasing grain number per unit of ground area (*48*). By contrast, CO_2 enrichment after anthesis has a lesser effect on yield.

Although the main component of variation in yield of wheat (and other species) is the seed number per unit of ground area, mean seed weight can nevertheless vary somewhat by improved photoassimilate supply (*48, 49*) and certainly differs among genotypes. Just as kernel number is established before rapid grain filling gets under way, so is the average potential size of the seed. This is closely related to the mean number of cells per seed in other species as well as cereals (*50*). Cell division in the starchy endosperm of wheat is virtually complete within 2 weeks after pollination (*51*). Once kernel number per unit of ground area and cell number per kernel are established, the filling of the grain usually proceeds rapidly (in about 4 to 6 weeks) to a substantially predetermined mean kernel size. From that perspective, by 2 weeks after grain set the partitioning of photoassimilate appears to be largely regulated by the filling sinks themselves. The physiology behind such regulation has attracted much research: regulation of CO_2 fixation rate per unit of leaf area by sink demand; control over the ordered senescence of the leaf canopy by storage sinks; and regulation of the day-to-day partitioning of current photoassimilate between competing alternative sinks. The last of these topics has been studied phenomonologically by establishing principles of competition between sinks of different sizes and distances from the source (*52*). It is also being studied by tracing the mechanisms and potential points of regulation of carbon compound flow between chloroplasts in the leaves to uptake in cells of the developing sink.

Carbon Transport from Source to Sink

Complementary to biochemical partitioning between sucrose and starch is physical compartmentation of these products in the leaf. Histochemical and kinetic studies show at least five major types of photoassimilate compartmentation in leaves: (i) differential starch storage among the different leaf cell types; (ii) different sucrose storage pools among mesophyll cells; (iii) intracellular compartmentation between cytoplasm and vacuole; (iv) compartmentation between the symplast (or intracellular volume) and apoplast (or extracellular "free space" in cell walls and intracellular spaces); and (v) accumulation of solutes by the sieve element–companion cell complex of the vascular tissue. The size, distribution, and composition of these various pools varies with species and with translocation status of the plant. In soybean leaves, for example, there is a preferential net accumulation of starch in the second palisade mesophyll layer during the stage from flowering to early seed filling. This starch does not turn over diurnally. It is a longer term reserve, being mobilized during the middle to late stages of seed filling, when leaf photosynthesis has markedly declined (*27*).

A portion of the sucrose synthesized through FBPase and SPS in the cytoplasm is stored in the vacuoles. Kinetic studies (*53*) show that the cytoplasmic (or transport) sucrose pool has a much faster turnover than the larger vacuolar pool, which is less available for immediate export through the mesophyll toward the veins. The vacuolar sucrose pool, exchangeable with the cytoplasmic pool, is the first source for export during the night. Starch appears to be mobilized to sucrose at night only when the vacuole pool is substantially depleted.

The sieve element–companion cell complex of the phloem contains a much higher concentration of sucrose than the surrounding tissues. The partitioning step leading to that high concentration is called phloem loading. The following sketch, based on detailed reviews (*54*), is our currently favored view of how phloem loading occurs.

The intricately reticulated network of veins in leaves ensures that no more than two or three cells need be traversed between photosynthetic mesophyll cells and sugar-accumulating phloem cells. Photoassimilates, mostly sucrose, travel symplastically down their concentration gradients to the mesophyll cells close to the companion or phloem parenchyma cells, where they are released without hydrolysis by a facilitated efflux mechanism into an apoplastic solution of relatively low concentration. An energy-requiring active process then loads sucrose into the phloem, creating a high sucrose concentration in the sieve tube symplast. This energetically "uphill" loading of sucrose appears to occur, at least in part, by a plasmalemma-bound, sucrose-specific carrier involving a sucrose-proton cotransport mechanism. Regulation may occur at the carrier level or by a cell turgor–dependent process in which information about the rate of unloading in sinks is relayed instantly to the source as a hydrostatic pressure change in the whole transport network or by changes in the rate of water entry into the sieve elements (*54*).

The sieve elements form a continuous transport network throughout the plant, with numerous branches and anastomoses. There is no evidence of one-way valves, so in principle any source leaf can supply solutes to any sink without leaving the sieve tube network. Experiments with radioactively labeled solutes show that in some species there are preferred routes from certain source leaves to certain sinks but that selective leaf or sink removal rapidly alters the pattern of movement in most species (*15, 55*). It seems unlikely that source leaves have mechanisms to determine where their solutes are destined (*56*). Generally, the preferred channels of transport seem to be associated more with the least resistive vascular route between source and sink (*15*).

Although the mechanism of long-distance transport has not been unequivocally proven, the consensus is that there is a bulk flow of solution along sieve tubes under the influence of a hydrostatic pressure gradient. This gradient is maintained osmotically by regulated loading of solutes into the sieve element–companion cell symplast in source regions and regulated unloading in sink regions. Measurement of sieve element sucrose concentrations and gradients in sugarbeets, soybeans, and other species showed them to be sufficient to generate the necessary hydrostatic pressure gradient (*57*). Long-distance transport seems unlikely to impose appreciable limitation on the rate of transport from sources to sinks even when partial incisions or restrictions are made in the dominant transport routes (*15, 58*)—alternative paths appear to take over.

The discussion so far suggests that photosynthesis and the mechanism of phloem loading determines the amount of photosynthetic assimilate made available for translocation, whereas the mechanism and kinetics of unloading into, and associated uptake by, competing sinks, in association with relative distances between sources and sinks, determines the partitioning of loaded material in the short term.

Less is known of the regulation and coordination of phloem unloading (solute efflux from the phloem and interconnected tissues of the vascular bundles) than

of the subsequent uptake and assimilation of solutes for growth and storage product formation in sink tissues. The diversity of sink types suggests that there are several mechanisms of unloading.

Unloading from the vascular bundles can be through symplastic plasmodesmatal connections to growing cells, as in pea and corn roots (*59*) and in young leaves (*60*), or unloading may be across a cell membrane into the apoplast of the sink cells, as in sugarbeet tap roots (*61*) and in the storage stem of sugarcane (*62*). In crop seeds solutes may first pass symplastically from the phloem through one or more maternal seed coat tissues before entering the apoplast of the embryonic sink. Furthermore, solutes (principally sucrose and amino acids) may remain unaltered during unloading or, alternatively, be partially metabolized after (or during) unloading (*63*).

The seed coat of the developing legume seed is proving to be a convenient system for studying unloading (*64*). In that system, the phloem reticulates to varying degrees, depending on species, throughout the seed coat, supplying assimilates to the embryo across the apoplast separating the two generations. Unloading from the seed coat appears to involve an energy-dependent, carrier-mediated process (*65*), rather than just the passive leakage postulated earlier (*15*), despite a large downhill concentration gradient from sieve tube symplast to embryo apoplast. This suggests that unloading may serve as a potential control point in source-to-sink transfer of photosynthate and in intraplant competition for photoassimilate.

Assessment and Conclusions

In terms of carbon economy, the route to increased commercial yield can be from either the carbon source (photosynthesis) or the carbon sink (commercial product), but preferably both simultaneously. High-yield cultivars and good management strategies are those that are successful at maximizing the amount of solar radiation intercepted seasonally by foliage without excessive investment of plant matter into vegetative rather than reproductive parts. In a given environment, breeding has not so far been successful at increasing yield through increasing net photosynthesis rate per unit of photosynthetic tissue, but has been very successful at increasing the interorgan partitioning of dry matter from unnecessary vegetative parts to the commercially useful sinks. In grain crops improving crop photosynthesis rate by environmental amelioration is most effective at increasing yield (by increasing the number of grains competent to fill) if effected before grain filling starts. Increasing sink size (grain number per hectare) by chemical or breeding methods is likely to be an effective route to higher yields of seed crops; this will involve prevention of abscission or abortion of flowers and young seeds while retaining the ability to fill these extra sinks.

The rate of growth of established, developing seeds seems to be determined primarily by phenomena operating either at the stages of unloading of photoassimilate into the extracellular solution surrounding seed cells or accumulation and conversion to storage product of this unloaded photoassimilate. However, if HI is further increased, the ability of the reduced photosynthetic surface per sink organ to supply the necessary photoassimilate will be reduced, leading to a greater prospect for improvement in net photosynthesis rate per unit of leaf area being expressed in higher grain yield.

In leaves, partitioning of fixed carbon between its retention in the plant and its photorespiratory release is readily maintained by environmental change, but has so far defied attempts at genetic alteration. The potential agronomic rewards for success are so high, however, that the remaining avenues in that endeavor must be followed. Conversely, the apparent scope for improving partitioning of fixed carbon between carbon skeletons for plant synthesis on the one hand, and substrate for respiration on the other, is relatively small but appears to have been effectively exploited in perennial ryegrass. It is yet to be seen whether some detrimental consequences follow or whether similar experience can be had with annual crop species.

The apparent success at improving ryegrass yield by selecting against mature leaf respiration is surprising: the more general experience in attempts to use physiological and biochemical knowledge to suggest selection criteria or chemical targets for improvement in genetic yield potential has been one of disappointment born of system complexity. This complexity results largely because yield is governed by numerous polygenic, organismic, and community traits that have enormous plasticity as a crop develops with multiple colimiting factors—both metabolic and environmental. Although some claim that the art of breeding is on the brink of substantial developments through genetic engineering, and although yield-enhancing chemicals hold promise, the complexity of yield and our lack of understanding of plant physiology and development are such that the rational design of such approaches for yield improvement is only in its infancy.

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RESEARCH ARTICLE

Evolutionary Relatedness of *Plasmodium* Species as Determined by the Structure of DNA

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Malaria parasites are classified in the genus *Plasmodium*. Historically, the species were grouped according to the hosts that they infected and then were subdivided according to morphological and biological characters (*1, 2*). Thus *Plasmodium* species are classified into primate, rodent, avian, and lizard malarias. The implication is that the parasites have evolved with their hosts and that there is greater relatedness among parasites in related hosts than those in hosts greatly separated in evolution. In the present article we examine this hypothesis using analysis of base composition and organization of DNA from various species of *Plasmodium*. We find a major exception to the classically derived organization of the malarial evolutionary tree. Surprisingly, *P. falciparum*, the malaria of man that causes the most morbidity and mortality, appears more closely related to rodent and avian malarias than to other primate malarias. Further, our data allow us to suggest which common characteristics of different *Plasmodium* species are the result of direct inheritance and which may be the result of independently occurring but convergent events.

Analysis of Base Composition

The deoxyguanosine · deoxycytidine (dG · dC) content of DNA from *P. falciparum* (*3*) and *P. berghei* (*4*) (18 and 24 percent, respectively) differs greatly from that of the mammalian host (~37 percent). We purified DNA samples from *P. falciparum, P. knowlesi*, and *P. berghei*, tested them for host DNA contamination, and then analyzed them for total dG · dC content by a DNA duplex melt procedure. Purification of parasite DNA is critical for these experiments since one nucleated host cell per 100 parasites would result in approximately a 50 percent contamination due to differences in total genome size. Each preparation of parasite DNA was tested for purity as described (see legend to Fig. 1). *Plasmodium falciparum* DNA that had been analyzed previously (*3*) was used as a control. *Plasmodium falciparum* parasites in continuous culture in human erythrocytes in vitro (*5*) were nearly free of nucleated host cells. The *P. falciparum* DNA was radiolabeled by nick translation with either deoxyadenosine triphosphate or deoxycytidine triphosphate and the dG · dC content was determined. The melting temperature (T_m) of both deoxyadenosine- and deoxycytidine-labeled DNA indicated a dG · dC content of 18 percent, consistent with the published figure (*3*). *Plasmodium berghei* DNA was extracted from infected mouse erythrocytes (*6*) and was further purified by $Hg^{2+}CsSO_4$ or Hoechst dye CsCl gradients as shown below. The dG · dC content of the *P. berghei* DNA was found to be 18 percent. It had previously been reported to be 24 percent (*4*). The difference probably can be attributed to host DNA contamination in the previous study. Determination of the dG · dC content of *P. knowlesi*, a malaria

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