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Carbon and nitrogen assimilation in relation to yield: mechanisms are the key to understanding production systems

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Abstract

Improved understanding of crop production systems in relation to N-supply has come from a knowledge of basic plant biochemistry and physiology. Gene expression leads to protein synthesis and the formation of metabolic systems; the ensuing metabolism determines the capacity for growth, development and yield production. This constitutes the genetic potential. These processes set the requirements for the supply of resources. The interactions between carbon dioxide (CO_2) and nitrate (NO_3^-) assimilation and their dynamics are of key importance for crop production. In particular, an adequate supply of NO_3^-, its assimilation to amino acids (for which photosynthesized carbon compounds are required) and their availability for protein synthesis, are essential for metabolism. An adequate supply of NO_3^- stimulates leaf growth and photosynthesis, the former via cell growth and division, the latter by larger contents of components of the light reactions, and those of CO_2 assimilation and related processes. If the supply of resources exceeds the demand set by the genetic potential then production is maximal, but if it is less then potential is not reached; matching resources to potential is the aim of agriculture. However, the connection between metabolism and yield is poorly quantified. Biochemical characteristics and simulation models must be better used and combined to improve fertilizer-N application, efficiency of N-use, and yields. Increasing N-uptake at inadequate N-supply by increasing rooting volume and density is feasible, increasing affinity is less so. It would increase biomass and N/C ratio. With adequate N, at full genetic potential, more C-assimilation per unit N would increase biomass, but energy would be limiting at full canopy. Increasing C-assimilation per unit N would increase biomass but decrease N/C at both large and small N-supply. Increasing production of all biochemical components would increase biomass and demand for N, and maintain N/C ratio. Changing C- or N-assimilation requires modifications to many processes to effect improvements in the whole system; genetic engineering/molecular biological alterations to single steps in the central metabolism are unlikely to achieve this, because targets are unclear, and also because of the complex interactions between processes and environment. Achievement of the long-term objectives of improving crop N-use and yield with fewer inputs and less pollution, by agronomy, breeding or genetic engineering, requires a better understanding of the whole system, from genes via metabolism to yield.

Key words: Carbon assimilation, development, growth, metabolic systems, nitrogen assimilation, yield.

Introduction: the conceptual framework

During the autocatalytic cycle of growth and reproduction of higher plants, the embryo in the seed grows, under suitable conditions, to form a plant with leaves and roots. Leaves contain the complex biochemical photosynthetic machinery capable of capturing light and using the energy for the reductive assimilation of carbon dioxide (CO_2) and nitrate ions (NO_3^-) with the formation of carbohydrates and amino acids, respectively (Foyer *et al.*, 2001; Lawlor, 1994). Roots capture water and mineral nutrients which are required for metabolism; here the focus is on the supply of nitrogen. For simplicity only the supply and

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metabolism of nitrate is discussed: the roles of ammonia as NH_3 or as NH_4^+ (Macduff *et al.*, 1987; Ter Steege *et al.*, 2001), N_2-fixation from plant–rhizobium associations, and the uptake of organic-N are not considered. The C- and N-assimilates produced are exported to the growing regions of the plant where they are incorporated into new vegetative cells and tissues, giving rise to reproductive organs and seed, thus completing the growth cycle (Ourry *et al.*, 2001).

The plant functions as a complete system with a balance between the shoot and root in the capture of resources and the exchange of assimilates over the long term, but a quantitative understanding of these processes and how they are regulated is limited. Figure 1 summarizes the interactions. The processes and fluxes of N- and C-assimilates are dependent upon the supply of resources from, and conditions in, the environment. Discussion of the role of basic metabolism is often divorced from that of the determination of crop yield, because of the focus of the practitioners and the need to address specific points in the system for research purposes. However, because yield is a consequence of biochemistry, the two are closely linked (Lawlor *et al.*, 2001). From the viewpoint of agricultural production yield is the most important aspect, for the world's annual production of *c.* 1.6 billion tonnes of grain of wheat, maize and rice provides the bulk of energy and nutrients for human consumption. The production of large yields of high quality products with the minimal input of resources, particularly N-fertilizers, is the main aim.

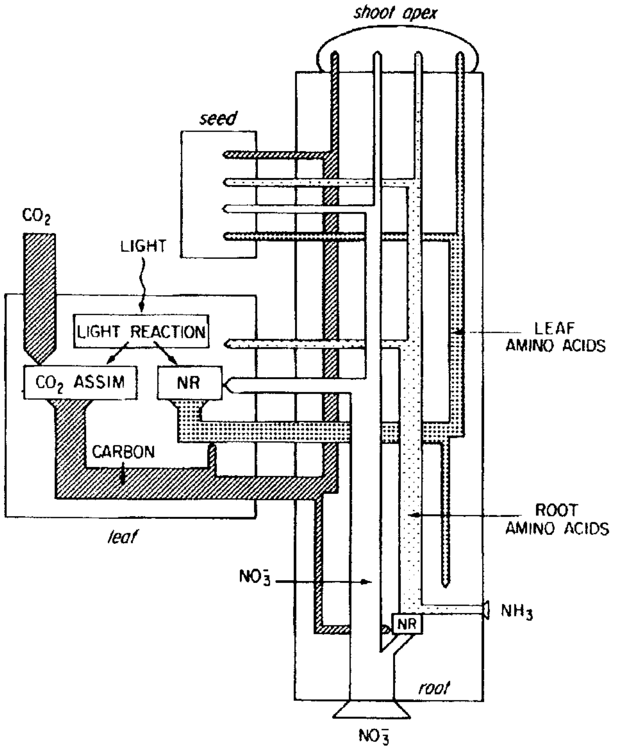

Fig. 1. Schematic diagram of the interactions between different processes and subsystems in the plant leading to the production of dry matter and yield (from Lawlor, 1994). Light provides the energy for photosynthetic assimilation of atmospheric CO_2 in the leaf, producing carbon assimilates (carbon) which are used in nitrate reduction (NR) in the leaf (and also root). The NO_3^- is obtained from the soil. Within the plant the carbon assimilates and amino acids produced are distributed to the growing organs where they provide the substrates for all aspects of metabolism and growth. If the supply of assimilates is less than demand, then metabolism, tissue composition and growth are impaired. If in excess, then amino acids and other metabolites, and nitrate, accumulate. To increase plant growth when N is limiting, more NO_3^- must be taken up. When the N-uptake is greater than the demand then growth is at the potential and additional capacity must be formed in the growing organs.

This review attempts to bring together different aspects of plant metabolism and physiology and the role of N in the processes leading to the production of yield. It emphasizes the importance of a mechanistic understanding of all parts of the system and, in particular, the integration of knowledge if the overall aims of greater crop production with minimal use of N without environmental pollution are to be achieved. It is argued that undue focus on particular parts of the system leads to poor assessment of the potentially limiting processes and neglect of important ones.

N-fertilizers and improving agriculture

The need for better understanding of the basic mechanisms relating N to crop production and for improvements in use of N is shown by considering the current situation of world agriculture and its dependence on industrially produced N-fertilizers (Bacon, 1995). Agriculture now exploits much of the most fertile land on earth, uses a large part of the available water and requires substantial inputs of fertilizers. Some of these (e.g. phosphates) are mined, but inorganic N is derived from the industrial conversion of N_2 to NH_3 to make urea, nitrates etc. Industrial N-fixation is close to the natural fixation of N_2 in the biosphere. There is a need for a greater production of food without an expansion of agricultural land (limited by other factors such as water supply) and with less fertilizer, the synthesis of which uses large amounts of energy from fossil fuels. The reduced use of less N-fertilizer will decrease costs and pollution, which is a consequence of methods of synthesis and of the inefficient application to and uptake by crops; achievement of these goals is urgently required (Ter Steege *et al.*, 2001). However, the reduced use of fertilizers conflicts with the need for a greater yield of crops. Yields of the major staples, for example, wheat, have not increased in the last decade (Evans, 1998). Thus, with the increasing demand for food from a rapidly increasing human population, the potential for food shortages worldwide must increase. To overcome the potentially dangerous social problems of food shortages and environmental degradation and pollution, agricultural yields per unit land area must be increased at the same time as dependence on applied fertilizers is decreased (Evans, 1998). A large part of global agriculture uses N-fertilizer very inefficiently (even in developed economies) often because other environmental conditions are limiting so improvement in the crop environment and in technology for N-application, as well as application of current best practice, could reduce losses and increase production (Bacon, 1995). Current knowledge of how plants metabolize N and of the behaviour of N in the environment is probably adequate for this, although improvements in understanding the system as a whole (see later discussion of modelling), are required. Improvements in the genetic potential of yield, which has not been improved by decades of selection breeding, in order to achieve better total production with less N and other resources would be desirable (see the discussions relating to increasing rice production in Sheehy *et al.*, 2000). However, even if this were possible it would require intensified selection breeding. In the case of genetic modification, much greater input of expertise and financial support, and further technological developments are probably required, despite some evidence of success (Ku *et al.*, 2000) for current technology is relatively untried (Matsuoka *et al.*, 2000) and the time required for effective application is probably long. The likelihood of success is difficult to assess; this also applies to selection breeding given the much slower, or non-existent, rates of increase in yields and yield potential. Genetic characteristics may be specifically altered to increase production and its efficiency, but the targets for modification must be clearly identified. Application of molecular techniques will aid in this and in breeding programmes, with major genes and quantitative trait loci controlling agronomic traits being identified and located in comprehensive genetic maps (Snape, 1996). However, the role of N in crops is so fundamental that these approaches must be combined with a detailed understanding of plant biochemistry and physiology.

Photosynthetic carbon and nitrogen assimilation and N-supply

The role of N in agricultural production is intimately connected with photosynthesis. In photosynthesis, the ‘physical energy’ of photons is converted into the ‘chemical energy’ of ATP and reduced metabolic intermediates, primarily NADPH, which are used in the synthesis of carbon and nitrogen assimilates of many different types, particularly carbohydrates and amino acids (Foyer *et al.*, 2001). These ‘fuel’ the synthesis of biochemical components of organs and ultimately provide the structure of the whole plant (Lawlor *et al.* 2001). The basic mechanisms of assimilate production are considered for they are central to the understanding of crop production.

CO_2 assimilation

CO_2 assimilation in relation to leaf composition and N-supply has been extensively reviewed (Evans, 1983; Sage *et al.*, 1987; Lawlor *et al.*, 2001) and may be summarized thus: N determines the synthesis of amino acids and therefore of proteins (see later) and, ultimately, of all cellular components. A rapid rate of CO_2 assimilation requires correspondingly large amounts of many components of the chloroplasts, particularly the light harvesting chlorophyll–protein complexes (LHCP), electron transport and $NADP^+$-reducing components of thylakoids, and the CO_2 assimilating enzyme ribulose bisphosphate carboxylase-oxygenase (Rubisco), plus other enzymes required for CO_2 assimilation in the stroma. The basic characteristics (molecular mass, structure) of proteins are genetically determined. However, many enzymes are regulated in very complex ways by environmental and plant factors (e.g. light and protein phosphorylation, respectively). Both the amounts and activities of proteins and other components in organs, as well as the size of organs determine the total capacity of metabolism. To achieve large rates of CO_2 assimilation per unit area of leaf, large numbers of molecules of components are necessary. Some proteins have high molecular mass in relation to their activity so that considerable amounts of protein are present in leaves (large mass per unit leaf area). To capture the energy used in photosynthesis efficiently, for example, a large amount of chlorophyll per unit area is needed and is ‘supported’ by proteins (light harvesting chlorophyll–protein complexes, LHCP, in the thylakoids), so thylakoids contain much N. The major protein of the chloroplast and leaf (particularly in C_3 plants; Evans, 1983; Sage *et al.*, 1987) is Rubisco, which catalyses the reaction between CO_2 and RuBP, giving rise to triose phosphate; this is exported from the chloroplast stroma to the cytosol, and then converted into sucrose. Rubisco has low catalytic rate per mass of protein, so the rates of CO_2 assimilation commonly determined in C_3 leaves requires a large amount of Rubisco. The amount of Rubisco may reach 8 g m^{-2} and constitute up to 30% of the N in wheat leaves and up to 50% of the soluble protein (Lawlor *et al.*, 1989). Actively metabolizing leaves contain correspondingly large N-contents. If N supply during leaf growth is below that required to sustain the potential rate of protein synthesis etc, the amount of components formed is inadequate for maximum CO_2 assimilation (Lawlor *et al.*, 1988, 1989). Of course, the situation is more complex than outlined, in that CO_2 assimilation depends not on the amounts of the components but on their activity, which depends on other factors. If Rubisco content, for example, is decreased then the activity increases, so tending to compensate for the change. At low temperature more Rubisco (and other proteins and components) is required to maintain a given rate of CO_2 assimilation as metabolic activity decreases due to the slower rates of enzyme reactions (Lawlor *et al.*, 1987*b*). Thus, there is considerable flexibility in development of the photosynthetic system and in metabolism, as well as complex interaction with the environment.

Nitrogen deficiency has great impact on chloroplasts, in size, composition and function. Compared with those from plants grown with ample N, they are smaller and flatter, with fewer thylakoid membranes which are poorly stacked and a smaller proportion of granal to intergranal thylakoids, and the proportion of stroma increases, for example, in rice (Laza *et al.*, 1993) and sugar beet (Kutik *et al.*, 1995). There is less LHCP, Rubisco and ATP synthase (Theobald *et al.*, 1998). However, Evans and Terashima showed that the composition of thylakoids is relatively unaffected by N-supply, with thylakoid components, electron transport activities and rates of O_2 evolution at CO_2 saturation expressed on a chlorophyll basis very similar in spinach grown with very different N-supply (Evans and Terashima, 1987). By contrast, the Rubisco and soluble protein contents per unit of chlorophyll was greater at large N-content, showing that with low N, the capacity for CO_2 assimilation was less than for electron transport. In wheat the ratio of Rubisco to chlorophyll is relatively constant over a range of N-treatments, although increasing at large N (Evans, 1983; Lawlor *et al.*, 1989). Other authors have shown that the mass ratios of Rubisco to total chlorophyll increased as N-supply increased more than did the ratio of ATP synthase to chlorophyll (Nakano *et al.*, 1997; Theobald *et al.*, 1998). The chlorophyll *a*/*b* ratio is largely unaffected by N-supply, despite the large decrease in chlorophyll, suggesting that N-supply does not differentially regulate the expression of LHCP (which contains chlorophyll *b*) relative to the other components of the light harvesting and reaction centre complexes. A generalization is that N-supply does differentially alter expression of the genetic information controlling chloroplast composition particularly expression of Rubisco and the ATP synthase, but not that of thylakoid membrane components (Fig. 2). The possibilities of altering the photosynthetic mechanisms to increase CO_2 assimilation are being actively considered (Paul and Lawlor, 2000). Some increases have been achieved by altering the composition of leaves, unexpectedly, by altering trehalose metabolism, probably through regulation of development (Paul *et al.*, 2001). Given the flexibility in composition and function of leaves in response to environmental conditions such as light, alterations to improve N-use might be possible. However, if these involve increasing the number but not the characteristics of enzymes etc, then the mass of protein will increase, and so will N content and demand for N. Increasing the content of Rubisco does not necessarily increase CO_2 assimilation per unit area in C_3 leaves, even in bright light, because of other constraints (e.g. CO_2 supply) (Lawlor *et al.*, 1987*a*, *b*; Millard, 1988; Millard and Catt, 1988) so increasing the amount of components may not always be effective.

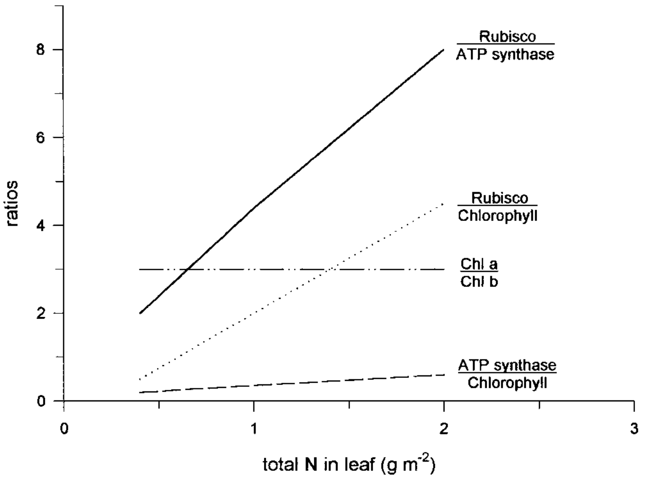

Fig. 2. Schematic diagram of the relative changes in biochemical components of the chloroplast in relation to the total N accumulated in the leaf of wheat. The increase in chloroplast stromal Rubisco compared to ATP synthase in the thylakoid membranes and light harvesting chlorophyll protein complex (judged from the ratio of chlorophyll *a* to *b*) is obvious (from data in Theobald *et al.*, 1998).

The supply of CO_2 for C_3 photosynthesis is inadequate in the current atmosphere (360 μmol mol^{-1}) and photosynthetic rates and crop growth and yields are increased by *c.* 30% under UK conditions if this is doubled. Thus, by this simple environmental change, the genetic potential (see later) is achieved. If the efficiency of CO_2 assimilation could be increased in C_3 plants by eliminating photorespiration then productivity per unit of N accumulated would increase. In the current CO_2 atmosphere, a large content of Rubisco, of low catalytic activity, in leaves grown with abundant N has frequently been remarked upon, and has led to the concept of decreasing the amount of Rubisco or altering its characteristics (reducing the amount of protein per active site, increasing the state of activation etc) to reduce N-requirements. However, there is no evidence that this is attainable (and there is evidence that such changes profoundly inhibit photosynthesis). Because of the large amount and low activity of Rubisco in leaves, especially with ample N-supply, it has been considered a storage protein: it is remobilized from older leaves and contributes a large part of the protein in growing leaves when N is limiting and for storage protein synthesis, for example, in cereal grain and potato tubers (Lawlor *et al.*, 1987*a*, 1989; Millard, 1988; Millard and Catt, 1988) supporting a storage function. It has been argued that synthesis of protein is an inefficient use of energy, but given the need for a large Rubisco content in C_3 plants for CO_2 assimilation, especially at high light, and the ionic and osmotic effects of storing NO_3^-, and amino acids, there may be advantages in the synthesis of Rubisco as a transient store of abundant N (Heilmeier and Monson, 1994). Such considerations should not be neglected when it is suggested that Rubisco content should be decreased to improve N-use efficiency, for example, under elevated CO_2 (Mitchell *et al.*, 2000). The complexity of the role of Rubisco, and its tight integration in the N-economy of wheat, is shown by the variable balance between the maintenance of CO_2-assimilating capacity in old leaves and the remobilization of Rubisco during senescence and grain filling. Changes in this balance have large effects on yield and grain composition. N-deficiency particularly influences these processes, decreasing total assimilation as leaves are shorter-lived, senescing and losing photosynthetic competence more quickly than when it is abundant, due to remobilization of N to growing organs (Brouqisse *et al.*, 2001); improving the effective life of leaves by application of N (Lawlor *et al.*, 1989) or breeding (Sheehy *et al.*, 2000) is a proven method of increasing yield. Although such interactions have been well described, there is a need for the improved quantitation of the relationship between N-supply on photosynthetic and related systems and to optimize N supply, Rubisco amounts and photosynthetic rate.

There are substantial differences between C_3 and C_4 plants in the content of photosynthetic components in leaves, as often discussed (Sage *et al.*, 1987; Evans and von Caemmerer, 2000; Lawlor *et al.*, 2001). Because C_4 photosynthesis provides a high CO_2 concentration in which Rubisco functions, less Rubisco is required for C_4 than C_3 CO_2 assimilation despite the greater rates of CO_2 assimilation in C_4 plants, even at large photon flux. Consequently, the N-content per unit leaf is smaller than in C_3 plants, and the N-requirement is less for greater production, so N-use efficiency is improved. To modify C_3 plants towards this happy state is a long-held ambition of plant biochemistry but requires production of a Rubisco with less, or no, oxygenase capacity, so minimizing or eliminating photorespiration (the cause of the relative inefficiency of C_3 plants). As mentioned above, the possibility of engineering Rubisco to eliminate photorespiration is remote. So altering the structural and metabolic characteristics of C_3 plants to achieve the high-CO_2 environment for Rubisco that occurs in C_4 plants has been suggested (Sheehy *et al.*, 2000). Emulating in C_3 plants the complexity of the C_4 syndrome will be difficult, because of its substantial enzymatic and anatomical complexity. It is unlikely that rapid advances will be made by altering C_3 to C_4 biochemistry and anatomy, despite apparent improvements in the C_3 photosynthesis of rice from the incorporation of enzymes likely to increase the CO_2 concentration in the vicinity of Rubisco (Matsuoka *et al.*, 2000) and some success in increasing CO_2 assimilation (Ku *et al.*, 2000), this desirable aim still seems a very distant prospect (Evans and von Caemmerer, 2000).

Nitrate uptake and assimilation

Uptake of NO_3^- by roots depends (Engels and Marschner, 1995) on the concentration of NO_3^- in the soil solution, on the volume of soil exploited by roots and rooting density, and on the efficiency of roots in absorbing NO_3^-. The latter is affected by metabolic demand and conditions such as temperature (Macduff *et al.*, 1987, 1993). The flux of NO_3^- to the root surface depends on the extent of contact with soil and water, on the rate of diffusion from the bulk soil in water, and particularly important for NO_3^-, transport in the mass flow of water. This is related to crop transpiration and soil water content. Root size is a function of growth and thus of N-supply, an example of the strong feed-forward effects in crop production. For large total N-uptake by a crop from low NO_3^- concentration, high affinity NO_3^- transporters are required, together with more transporters per unit root surface, and greater root length and surface area per volume of soil to minimize the diffusion limitation. The storage capacity for NO_3^- in cells should be large, particularly where metabolism is restricted, for example, at low temperatures when photosynthesis is slow. Intrinsic N-use efficiency of the whole plant (biomass production per unit N accumulated) is determined by the integrated biochemical processes in the plant. The single curve relating production to N over a wide range of N-uptake and environmental conditions shows (Fig. 3) that the efficiency of the processes is genetically determined. However, the capacity for biomass production and N-uptake may be very dependent on environmental conditions, including NO_3^- supply. Increasing total production requires sufficient N to reach the asymptote, but greatest production with least N requires optimization, which occurs at less than maximum production (Lawlor *et al.*, 2001).

To improve N-uptake from low NO_3^- concentrations in soil requires exploitation of a larger total soil volume, and greater root length and surface area per volume of soil to minimize the diffusion resistance which may be a major limitation. In addition, more transporters per unit of root surface will increase the transport characteristics of the root. Increasing the affinity of the transport system for NO_3^- would be a major advance; however, this is already large as NO_3^- can be removed from micromolar

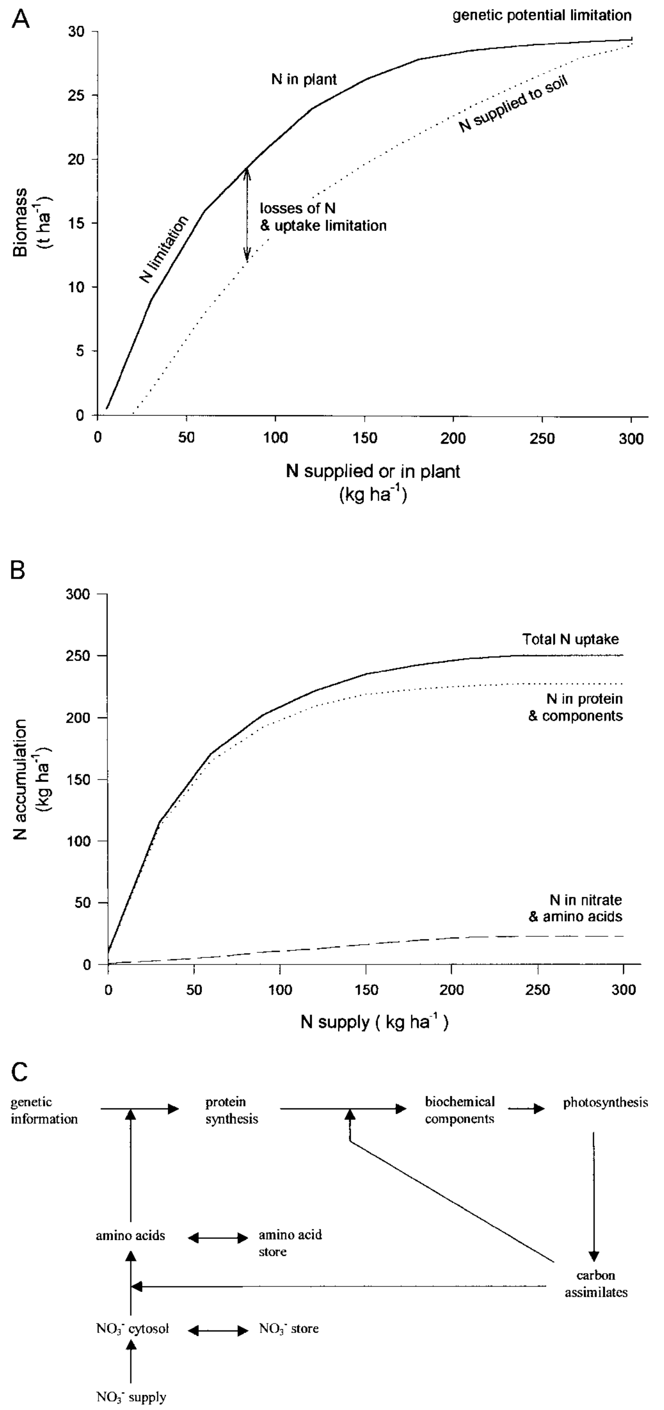

Fig. 3. Generalized response curves (A) relating the production of biomass to the uptake of nitrogen and to the supply of nitrogen. The difference between the curves is due to losses of N in the soil and limitations to uptake by the plant. In (B) the relationship between N in macromolecular biochemical components of the cell and nitrate and amino acids, which are the source of N for their synthesis is illustrated. The responses (which are to illustrate principles and are derived from experiments) can be interpreted as the consequence of limitation of the genetic potential, identified with protein synthesis, or limitation by N-supply as in the diagram (C).

concentration in well-stirred solutions (Engels and Marschner, 1995) and may be very difficult to achieve.

Metabolism of nitrate, which can occur in leaves and roots depending on the species of plant, is well studied (see papers in Lea and Morot-Gaudry, 2001). Focusing only on leaf cells, NO_3^- is reduced to NH_3 by nitrate and nitrite reductases using electrons from photosynthetic electron transport. The NH_3 is converted to amino acids by the GS/GOGAT enzyme reaction, and the 'carbon-skeletons' are provided by organic acids derived from the tricarboxylic acid cycle in the mitochondria. Carbohydrates for organic acid synthesis come, ultimately, from photosynthetic CO_2 assimilation and ATP for the GS/GOGAT reaction is generated by photosynthesis and respiration. Thus, there is close interaction in the very earliest phases of N and C metabolism, both using the light energy, with some 10% of the electron flux in photosynthesizing leaves used in NO_3^- reduction (Foyer *et al.*, 2001). However, the ratio of carbon to N assimilated is not rigidly fixed, for if the supply of NO_3^- increases relative to CO_2 then more amino acids and proteins accumulate relative to the rate of CO_2 assimilation and to the carbohydrate content which may decrease as growth is stimulated (Lawlor *et al.*, 1987*a*, *b*, *c*). There is competition between the processes: in leaves, limited NO_3^- supply decreased the content of ribulose bisphosphate (RuBP) the substrate for CO_2 assimilation, but increased the ATP/ADP ratio, suggesting that there may be competition for reductant (Mächler *et al.*, 1998). Because of the greater potential CO_2 assimilation in leaves grown with large N-supply, the extent of the competition between CO_2 and NO_3^- assimilation may be hidden. The N/C ratio varies according to availability of resources, particularly N but also CO_2, decreasing when CO_2 supply rises (Theobald *et al.*, 1998), and is very dependent on temperature. For example, under cool conditions, organ growth slows more than N-assimilation and use, so N/C ratio increases compared to warm conditions. When ample NO_3^- is available a large proportion of N is 'stored' (i.e. metabolically inactive) as protein and, to a smaller extent, as amino acids and NO_3^- in short- or long-term pools until their capacity is saturated. Their capacity must be genetically determined, although how they are regulated is not well understood (Heilmeier and Monson, 1994).

The importance of protein synthesis and the genetic and environmental factors regulating the amounts and relative proportions of different proteins is central to any discussion of the effects of N on metabolism and growth. The rate of protein synthesis, when all other factors are not limiting, depends on temperature. It stops at very low temperatures, increasing to a maximum as temperature rises before decreasing with further increase. This affects all plant processes, and changes the demand for amino acids and hence for nitrate. Experimental evidence shows that as the requirement for protein synthesis decreases, so amino acids accumulate and the demand for NO_3^- falls, but not its uptake, so NO_3^- accumulates, the response to NO_3^- concentration thus changes and there is marked interaction between temperature and nitrate supply and growth (Lawlor *et al.*, 1987*a*, *c*, 1988; Miller *et al.*, 2001). With inadequate N, the amino acid content of leaves falls, less protein is synthesized and growth is decreased generally more than photosynthesis, so carbohydrates accumulate and the N/C ratio is low. In crops, details of these processes and interactions are scarce, although crucial to understanding the mechanisms of their growth and for N-requirements.

Modification of nitrogen metabolism is potentially possible as there is great natural variation in the processes and particularly in the capacity (Limami and de Vienne, 2001). Increasing the amount of nitrate reductase activity per unit leaf area is potentially a route for increasing the total amount of nitrate reduced; it is induced by NO_3^- and its regulation is complex, offering the possibility of altering the amount and therefore the rate of nitrate reduction. With ample NO_3^- it is clear (see earlier discussion) from nitrate accumulation (Lawlor *et al.*, 1987*a*) that there are limitations to the rate of reduction, depending on environmental conditions, especially temperature. Also, the accumulation of amino acids shows that protein synthesis is limiting. In considering how metabolism related to N might be modified, it is clear that there are very complex interactions throughout metabolism, and considerable impacts of environment, which combine to determine the growth of the plant and its composition.

Modification of the processes of protein synthesis would constitute a major alteration in basic metabolism. To increase the amount of a protein would require increasing the capacity for synthesis, for example by increasing the number of sites, because at a given temperature with ample amino acids, the existing capacity would be limiting. Indeed, this switch-on of capacity may be the situation in plants with a large protein content, and in the response to ample NO_3^-. However, such concepts have hardly been explored. To increase the amount of NO_3^- absorbed and used at subsaturating and saturating supply will require different approaches and, potentially, many changes to metabolism.

Leaf growth and composition

Leaf growth rate and composition are substantially affected by N during growth and are well documented and understood in terms of changes in structure, although the mechanisms are not so well understood (Nelson and Dengler, 1997). They are particularly responsive to increasing N-supply when that is limiting (see section on N-response curves). This was seen in experiments (Lawlor *et al.*, 1987*a*, *b*, *c*, 1988) examining the relative effects and interactions between temperature and N-supply on the wheat leaf. With cool (13/10 °C day/night temperature) compared to warm (23/18 °C) growth conditions and deficient (4.5 mM) compared to abundant (19.9 mM) NO_3^- supply, low N decreased the rate of leaf growth but did not decrease its duration, although it did decrease their final size (Table 1). Leaves contained fewer cells of smaller volume. Low N decreased the soluble (including Rubisco) protein content and the rate of protein synthesis per leaf, but increased the ratio of structural to non-structural protein. The supply of NO_3^- is crucial for leaf growth because of the role of proteins in the growth of cell walls and the cytoskeleton and hence in cell expansion (Lawlor *et al.*, 1988). Syntheses of structural protein appears to take precedence over other components as, at very low N availability, the residual (insoluble) protein N content increases relative to the soluble, suggesting that a certain amount (3 g m^{-2}) of protein is required for the synthesis of basic cell structures (Lawlor *et al.*, 1989; Theobald *et al.*, 1998) without which other structures cannot be formed. With abundant N-supply to metabolism, senescence of leaves etc is minimized and slowed, so extending their effective life (leaf area duration). What determines the relative sensitivity of synthesis of cellular components to NO_3^- is unclear. The relationship between, say, concentration of NO_3^- in particular cell compartments (Miller *et al.*, 2001) and amino acid concentrations, and how these are linked to rates of Rubisco or cell-wall protein synthesis, are not known. Such specificity in response is important if the mechanisms are to be understood, because cell growth is related differently to Rubisco accumulation and photosynthesis, depending on N-supply, thus affecting the relationships between cell size, assimilation rates and tissue composition. Lower temperatures decreased plant growth rate and final area, but increased the duration of leaf expansion. However, soluble and Rubisco protein per unit leaf area increased despite a decreased rate of protein synthesis per leaf. The interpretation is that lower temperature slowed protein synthesis less than it slowed leaf expansion so the protein content increased compared to the warm conditions (Lawlor *et al.*, 1988). This maintained the metabolic rate despite the lower temperature. The complex changes in the amounts of NO_3^-, amino acids and carbohydrates with N-supply and temperature, may be explained by the different process—protein synthesis, organ growth, photosynthetic rate—having different responses to the environmental conditions. Possible interactions are indicated in Fig. 4. Ultimately, the explanation of how crops grow, the impacts of N on the processes and the interactions with temperature etc will require much better understanding of what determines leaf growth and composition. Effecting changes to leaf development,

Table 1. *An illustration of the effects of N-supply and the interactions with temperature in wheat plants*

The measured growth rates, the area of the third leaf, together with the protein and amino acid contents and calculated rates of protein synthesis and N uptake are given. The plants were grown at 13/10 °C (cold) or 23/18 °C (warm) and with 4.5 mM NO_3^- (−N) or 19.9 mM NO_3^- (+N). Values in parentheses are the percentage of warm +N. (Data from Lawlor *et al.*, 1988.)

Treatment	cold −N	cold +N	warm −N	warm +N
Growth rate (g plant^{-1} d^{-1})	2.2 (34)	3.4 (53)	5.2 (80)	6.5 (100)
Area of leaf 3 (cm^2)	9.9 (58)	12.0 (70)	16.5 (96)	17.1 (100)
Protein content of leaf 3 (g m^{-2})	7 (88)	12 (156)	5 (63)	8 (100)
Rate of protein synthesis (g leaf^{-1} d^{-1})	0.58 (21)	1.20 (44)	1.65 (60)	2.74 (10)
N uptake rate (μmol N g^{-1} dry mass d^{-1})	25 (58)	85 (65)	50 (38)	130 (100)
Amino acid content (mM m^{-2})	1.2 (75)	5.0 (312.5)	0.8 (50)	1.6 (100)
Nitrate content (mM m^{-2})	0.5 (17)	2.6 (87)	1.2 (40)	3.0 (100)

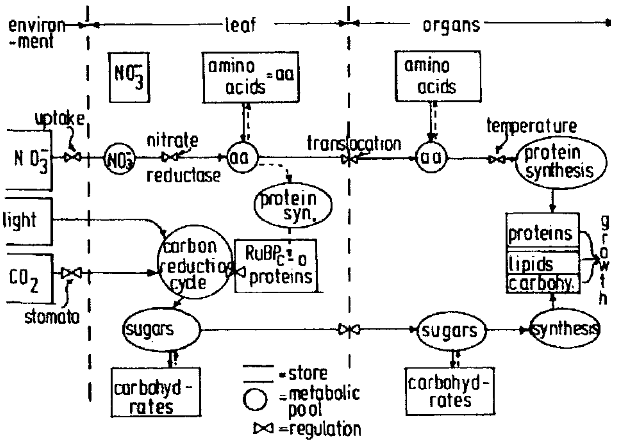

Fig. 4. Schematic diagram of processes involved in leaf growth and organelle, cell and tissue composition as affected by N-supply and temperature. The C and N fluxes in metabolic and storage pools of tissue components, leading from the environmental supply through the production of substrates in the leaf, to growth of organs is shown and sites of regulation indicated (after Lawlor *et al.*, 1988).

senescence and composition to increase production and efficiency of N use, will require modifications to the systems responsible for cell development, division and growth. The plasticity of growth and composition which occurs in response to N-supply and other environmental conditions suggests that there is potential for changing leaf growth etc, but as with metabolic processes the limiting processes are not clear and direct modification may be very difficult. It is not known how changes have occurred in the evolution of different species or of ecotypes adapted to different conditions, nor in the selection breeding of different varieties.

Crop growth, yield and N

Much has been written on this topic (Lawlor *et al.*, 2001) and so it is only very briefly considered here. There is essentially a linear relationship between the total dry matter produced by a crop and the radiation intercepted by it (Monteith, 1977); this is because light provides the energy for all metabolism. Radiation interception depends on the leaf area index (LAI; the ratio of leaf area to ground area) and thus on the size of leaves and their number, which is considerably affected by tillering in cereals and, as discussed, asymptotically dependent on N supply. However, the relationship between LAI and radiation interception is not linear: with LAI greater than about 3, PAR interception approaches *c.* 90%, and further increase in LAI does not increase light interception much. In addition to the effects of N on leaf area, there is the increase in photosynthetic rate at large N-supply, for reasons discussed. Consequent on these two related effects, there is a strong asymptotic relationship between biomass and N-supply (Fig. 2, see discussion on N-response curves). Biomass increases linearly as N-supply increases from small values (in this region considerable gains in production result from small increases in N) reaching a plateau at large supply when the crop grows at the genetically determined potential rate.

The N content/unit biomass (N% in dry matter) is frequently used as an indication of how adequate N supply is for crop growth (Lawlor *et al.*, 2001). However, N% is not a fixed value and decreases as the crop develops and its structure and biochemical composition change. Leaves, with a large content of proteins etc, are formed in early growth, and supporting stems, with more cellulose and lignin and little protein, later. So the N-content (N%) decreases with age and it also depends on environment. N deficiency reduces the N% as leaf : stem ratio decreases and protein and chlorophyll contents of leaves fall more than carbohydrates (see earlier). Use has been made of changes in N% to schedule N-application to crops (Lawlor *et al.*, 2001) and further developments of such techniques will undoubtedly be of practical economic value.

Yield is that part of a plant or crop used for a specific purpose, so will depend on the crop and human requirements. In the case of cereals, grain is the primary yield and total production depends on the number of plants per area, tillers per plant, number of ears per tiller, grains per ear and mass per grain. During early growth, the formation of tillers and leaves determines the later capacity for grain formation and assimilate production to fill them. Thus to achieve the genetic yield potential, all those factors affecting the growth of the many parts of the system, each regulated in complex ways, must be optimized over the whole life of the crop (Slafer *et al.*, 1996).

With adequate NO_3^- and CO_2 assimilation, the supply of assimilates to developing meristems is adequate to maintain their growth, so more tillers are produced and survive per plant and area, and similarly so with grains. Also, the capacity of grains to grow is increased, probably because more cells with greater enzyme capacity are made. Given adequate assimilates during grain filling, more grains are filled and they are larger. These factors together result in large yield. Under normal field conditions, the relative production of vegetative and reproductive organs varies between sites and years as a consequence of differences in environment, particularly N-supply. Hence, the ratio of grain yield to biomass (the harvest index, HI) is not constant, but varies with conditions. Where environmental variation is limited, HI is relatively constant, but if less assimilate is available during early growth, fewer tillers survive, resulting in fewer ears and grains per ear and so less yield. Later, limited assimilation may reduce grain filling. The N-supply is of considerable importance, affecting the availability of amino acids for the synthesis of protein during grain formation and later as a storage protein (Heilmeier and Monson, 1994).

Perhaps, it is too little appreciated that the different processes contributing to yield components are relatively independent with different factors influencing them, and that there is a substantial feed-forward effect from early growth. Also, the time at which environmental factors operate relative to the development of the plant and its individual organs, is of considerable importance. Because many factors are involved and they are dynamic, responses of crops cannot be easily predicted, yet agronomically they are of considerable importance. Many uses of crops are very sensitive to quality characteristics, for example, low protein N in grain is required in brewing barley so late N-supply should be minimal to decrease the protein content, but to obtain yield potential early N-applications are needed, leading to conflict which is complicated by the N-availability from the soil. It is important to understand the mechanisms of such processes better, in order to forecast plant requirements for N. Changes in yield and quality (including N content) by selection breeding have been remarkable and sustained. Greater yields have not resulted from increased biomass production in wheat, but largely from the increased number of grains and the size to which they can be filled. This has occurred at the expense of vegetative growth, so increasing HI (Evans, 1998). Clearly, supply of assimilates has kept pace with the genetic potential for grain production. However, the underlying changes in metabolism which have accompanied these changes are not understood, so offering little guidance for modifications directed at the level of metabolic processes.

Genetic potential

Genetic potential may be defined as the total capacity of the plant to form biomass or yield when environmental constraints are removed (Richards, 1996); breeders and agronomists frequently refer to yield potential, although total production is more relevant when considering how the potential growth of crops may be increased. The yield potential is a function of the genetic information which specifies the characteristics of proteins, so determining structure, growth and development, and size to which the system can grow. This maximum size over the growth cycle may be called the genetic potential. Also, the maximum rates of processes are genetically determined. Genetic potential cannot yet be defined quantitatively from basic principles, so an operational definition is required. Because plants (with wheat as a good example) often form large numbers of meristems capable of growth under favourable conditions, they respond greatly to the availability of resources, i.e. they are very 'plastic'. Hence, the genetic potential is defined under particular conditions (Richards, 1996). For agriculture, this is generally per unit area of ground surface, together with the normal incident solar radiation, temperature and atmospheric CO_2 for the site, which are relatively uniform and difficult to modify. As mentioned earlier, increasing current atmospheric CO_2 substantially increases biomass and yield, showing that the yield potential depends on conditions and how it is defined. Temperature is of particular significance, as it affects the rates of biochemical processes (see earlier discussion) and so determines the potential, with maximum potential occurring at an optimum temperature or within an optimum temperature range. However, those environmental factors which can be changed in agriculture, such as nutrition, water supply and pests and diseases, are modified so that production is not affected, i.e. they do not limit the genetic potential. Because of the many processes in agriculture which are affected differently by particular conditions, it may be necessary to optimize conditions rather than maximize them. Thus, the measurement of genetic potential may vary with environment (the genotype × environment interaction or G × E; Hollamby and Bayraktar, 1996).

About half of the substantial increase in yield over the last century was obtained from improved genetic characteristics of crops, the rest from improved husbandry, particularly increased fertilization (Austin *et al.*, 1993).

Indeed, the 'green revolution' was based on improving both the genetic potential and in supplying the correct conditions, especially nutrients, in the correct amount and at the appropriate time (Evans, 1998). Plant breeding has not increased the genetic potential for biomass production of a crop such as wheat, as old and new varieties produce similar biomass given the correct conditions. However, it has increased the grain yield potential and thereby the ratio of grain mass to total above-ground biomass or harvest index. Grain quality characteristics such as protein to carbohydrate ratios (e.g. in barley for animal feed or brewing) or the proportion of different proteins (e.g. for bread making) have also been modified: they are also affected by environment, such as the N-supply.

Improvement of crop potential requires identification of specific parts of the metabolism which must be altered to achieve given outputs. When N-supply is such that the existing yield potential is reached, the only way of increasing production is to improve the efficiency with which N is used in metabolism. Metabolism must be altered either to achieve more C-assimilation per unit of N, or to increase the capacity for N-use. The former will increase biomass but decrease the C/N ratio and provide no more protein. The latter may not be attainable because light would be a major limitation, as with large N, the LAI is already so large that more than 95% of available radiation is used (Sheehy *et al.*, 2000). To overcome this limitation would require the most extensive changes to photosynthetic metabolism and would present a formidable challenge (Evans and von Caemmerer, 2000). When N is limiting, it is the capacity to increase N-uptake from dilute solution or to increase the volume of soil exploited that is required, i.e. increased absorption efficiency or root growth. The efficiency of C-assimilation per unit of N in the plant would remain the same but biomass production would increase as well as the N/C ratio, as with current crops supplied with ample N. Increasing the C-assimilation per unit of N would potentially increase biomass (if light energy were available), but decrease N/C. If the N-supply to the root varies so that it does not match the growth rate in the short-term, then providing large stores of N in the plant would be a way of providing sufficient N to meet the biochemical requirements (Macduff *et al.*, 1993). Such an approach might be more easily achieved than modifying basic metabolism because increasing capacity for protein storage, an 'end product', would not involve extensive regulation. However, to be effective as a temporary N-store the remobilization machinery would be needed, with systems to detect N-deficiency and trigger protein breakdown. Of course, if the rate of supply from storage plus soil drops below the requirement in the long-term then growth will be restricted. Here, an understanding of the basic processes of carbon and nitrogen assimilation and how they relate to crop yield is of importance. It could guide agronomic practice, breeding and genetic engineering.

N-response curves

To determine the relationship between growth and N-supply, with limiting N, and the genetic potential with non-limiting N, crops are grown in a standard (or at least the same) environment of light, temperature and CO_2—which may not be optimal for greatest production—with all other conditions not limiting except the N-supply (Angus, 1995). N-fertilizer is then applied to different areas of the crop in different amounts (from zero to exceeding the requirement). The biomass or yield growth of the crop is then determined as a function of the amount of applied N. The relationship between crop growth and the amount of N applied has been determined in innumerable experiments and has been frequently discussed (Ter Steege *et al.*, 2001). A generalized response (Fig. 2) shows that with very deficient N, production is very small, increasing linearly with the supply of N until eventually the asymptote is reached, i.e. further increase in N does not increase production, which is the genetic potential. If growth is related to N accumulated by the crop, the initial slope, which is the true or intrinsic efficiency of N-use by the plant, is steeper (more biomass per unit of N) than if related to N-applied. The difference is a measure of the efficiency with which N is used, as is the difference between the point at which the plateau for biomass production is reached when expressed per unit of absorbed N and applied N is reached. The slope of the curve relating production to applied N is the efficiency of applied N use. With low N supply efficiency is large and decreases as N supply rises in both cases. The difference between N applied and accumulated provides valuable information about sources and losses of N (Bacon, 1995; Ter Steege *et al.*, 2001). N may come from rainfall and soil reserves as well as from applied fertilizers, so greatly affecting the apparent response derived from applied N. Similarly, losses of N due to leaching, bacterial metabolism and emissions from soils and plants etc affect interpretation.

Use of N-response curves have provided much quantitative information about the needs for N, timing etc (Angus, 1995; Lawlor *et al.*, 2001). Early studies showed the essentially asymptotic nature of plant responses to nutrient, including N supply, as discussed for N-response curves. Blackman's 'law of limiting factors' summarized the concept. Mitscherlich, for example, developed an equation ($Y = Y_{\max}(1-e^{-cN})$) relating yield, Y, to the amount of nitrogen applied N, and to the maximum yield, $Y_{\max}$, given by the asymptote of the response curve of Y versus N-supply; c is a curvature factor. Y_{max} (which is a measure of genetic potential) and c vary between seasons and also sites and cannot be easily applied to different conditions, but the approach allows the N-requirement to be estimated (see Angus, 1995, for discussion). To achieve Y_{max} the correct amount of N (N_{max}) must be supplied, for example, if a crop with 10 t ha^{-1} grain at N_{max} of 2% N (total 200 kg N) and 10 t ha^{-1} straw at N_{max} 0.5% N (total 50 kg N) then 250 kg ha^{-1} is required for the whole crop. This also represents the crop demand for N and is identifiable with the genetic potential for N uptake and yield production. If the difference between the N in the plant (N_{min}) is less than N_{max}, then the N required (the demand, D) is given by $D=(N_{max}-N_{min})\times$crop mass. This provides a simple assessment of the N requirement, but must be based on averages of long-term repeated measurements.

Indicators of N-status and metabolism in agriculture

Given the need to adjust the amount and timing of N applications to crops to increase production and efficiency and to decrease pollution, it logical to measure N in the plant to indicate N requirements (Neeteson, 1995). Measurement of N in the soil is less direct and may not reflect the dynamics of crop processes and the impact of environment on the crop. Measurement of N must be rapid, easy and effective, as well as inexpensive. As leaves contain a large proportion of the crop N, measurement of total N, or of components closely related to N-supply which respond quickly to changing N-supply and its relation to demand, are usually measured. Total N analysis is slow, requiring sampling and preparation of tissues and specialist analytical equipment and so is generally not used to measure N dynamics. Rapid colorimetric tests for NO_3^- concentration (which depends on supply and demand for NO_3^-) in small samples of expressed sap can be done in the field, are rapid and inexpensive and have been considered a useful indicator of N status, but they have not been extensively used as a guide for N application (Neeteson, 1995). Chlorophyll content, which is strongly related to N supply, can be measured in the field with a photometer, such as the Minolta SPAD-502 chlorophyll meter (Minolta Camera Company, Ramsey, NJ, USA), which is rapid and repeatable. The SPAD readings are calibrated to obtain the chlorophyll content of the leaves, or correlated directly with plant performance (Peltonen *et al.*, 1995), providing a practical method of assessing N status and N requirements, although one less dynamic than tests for NO_3^- content. Such approaches, based on biochemical and physiological understanding, should be encouraged.

Modelling nitrogen in the environment and plant

The use of N-response curves has been of great value in assessing N-requirements of crops (Angus, 1995). However, such methods are generalizations, which cannot easily account for the differences between crops at different sites and in different years as a consequence of varying N supply and weather which affects crop demand, N losses etc. Dynamic approaches are necessary, to allow rapid responses and to 'fine-tune' supply to demand. Models of these processes are well developed and widely used to recommend N-fertilizer applications (e.g. in decision support systems), increasing N-use efficiency and reducing pollution (Addiscott *et al.*, 1995). Improvements are required in the models, specifically addressing crop processes (e.g. growth rates which can be measured or modelled on the basis of temperature) over the short-term, to assess N requirements at specific times or crop growth stages. Relatively little use has been made of biochemical information in simulation models to advance understanding of the mechanisms and also to improve application of N (timing and amount).

Major constraints on the type of simulation modelling employed are: inadequate quantitative understanding of the individual parts of the mechanisms (enzyme characteristics, pool-sizes of intermediates), poor knowledge of connection between different mechanisms (link between C and N assimilation, transport within the plant) and difficulty of relating short-term measures of basic metabolism to the overall crop responses. The complexity of the system precludes the development of truly 'mechanistic' simulation models and raises the question of how complex can a model be to be understandable, stable and testable? Complex metabolic models are valuable to explore and quantify mechanisms and indicate what information is required (Evans and von Caemmerer, 2000). Experience suggests that very complex models are of limited value in practice. Models usually contain empirical relationships, which if well tested may provide acceptable accuracy in application. To improve the efficiency of N-use, more effort is required to develop and improve simulation models based on empirical relationships, and incorporating biochemical information.

Future possibilities

It is a truism that the processes involved in crop production are very complex and multi-layered, ranging from the molecular to the whole organism, and environmental factors affect all levels of organization. The essential points are that when N-supply is less than required for the genetic potential to be reached, it is N uptake that must be increased to get greater biomass. This may be done either by increasing the volume of soil exploited and reducing diffusion limitations with a greater density of rooting, or by increasing the affinity of the root for N. All are probably required. Alternatively, the C-assimilation per unit of accumulated N could be increased, so that a greater biomass would result from the smaller N accumulation. Increasing genetic potential for biomass could, in theory, be achieved by increasing the C-assimilation per unit N accumulated, at the cost of increasing the C/N ratio. This assumes that adequate light energy would be available, which is unlikely as nearly all energy is absorbed now with a full canopy. If more N was assimilated, but without changing the balance with C-assimilation, this would theoretically increase biomass and maintain the current C/N ratio. However, then light limitation would be likely. Alternatively, biomass could be increased by extending the period over which light is absorbed (longer growing season) and used effectively ('stay green' genotypes). The former is one of the main reasons for the larger biomass and yield of winter wheat, compared to spring wheat. The latter is also being exploited.

Understanding how processes respond to N is the key to improving production and N-use efficiency, and is essential if genetic potential is to be increased (Sheehy *et al.*, 2000). The response of the whole plant, as the central element in agricultural systems, to environmental conditions can only be understood if the organization of the system is appreciated. In addition, the environmental factors governing supply of N must be evaluated. However, it is impossible to analyse all aspects together under a range of conditions in a single research programme, or to combine them for practical application, hence the need for modelling. This has led to different concepts of how to deal with the 'problem' of understanding the need for N, and how to use N to modify and improve the quality and quantity of agricultural products. At the extremes are the reductionist and agronomic approaches. Reductionism considers that knowledge of genome structure will provide sufficient information to allow the characteristics of plants to be modified in selected directions to improve the response to N and to increase productivity, efficiency and yield potential of agricultural systems. Such a view appears accepted amongst molecular biologists and genetic engineers (Matsuoka *et al.*, 2000). Currently, much effort and enthusiasm is directed to understanding the genomes of plants and hopes of increasing food production are focused on genetic modification to increase genetic potential. Given the complexity of the subsystem nature of plants, and the considerable interaction with the environment. I consider this view to be ill-based and untenable even in its less extreme form. The role of biochemistry was once similarly viewed (e.g. the regulation of crop production by nitrate reductase activity), but, in practice, relatively few specific modifications and agronomic improvements resulted. Biochemical ideas and information have been essential to understanding the plant–environment system. Current concepts are of interactive and dynamic biochemical systems, with many feed-back and feed-forward regulatory processes which provide stability and flexibility in metabolism, growth etc, but may be more difficult to alter in specific ways than is assumed. The agronomic approach is essentially empirical, based on experimental determination of input requirements for maximum (or optimum) production: this time-honoured method is successful, but limited in space and time and, therefore, specific, and so transferable only in general terms. This is not what is required for fine-tuning of resource with production in agriculture, and has resulted in failure to exploit more mechanistic biochemical understanding (perhaps linked to simulation modelling) to improve efficiency and reduce pollution etc.

The increase in yield potential over the last 50 years has arisen from empirical selection breeding, with biochemical and physiological input leading to better understanding of processes (such as those regulating protein quality of grain, disease resistance etc) and selection criteria, and to substantially improved analytical methods. Selection breeding largely recombines genetic information and selects for 'end-products' such a grain amount and quality. This procedure results in the selection of biochemical processes, and their combinations, which optimize the subsystem activities, but essentially treats the system as a 'black box'. Whilst this approach will probably continue to provide most of the improvements in crop characteristics for many years, the potential offered by molecular biology for altering the genome and thereby the basic biochemical processes must be exploited. However, it requires integration with understanding of biochemical processes, and of their interaction with environment, if it is to succeed (Snape, 1996). Genetic engineering alters the system at specific points identified as key, or limiting, steps in metabolism. However, there is not a gene for N-use efficiency or for grain yield or quality, but many genes coding for proteins which determine the structures and behaviours of different biochemical and physiological subsystems. So it is likely (and now frequently demonstrated) that changes to the genome will not result in major alterations to basic metabolism of the type discussed here (Paul and Lawlor, 2000). Also, the response to environmental conditions may be complex, involving the G×C interactions mentioned, requiring a shift in concept away from the genome to the genome in an environment (Sheehy *et al.*, 2000). Unless this is rapidly appreciated, the current loss of plant biochemical and physiological expertise in many countries in favour of molecular biology, will distort the knowledge base. It will substantially weaken the more classical methods of attack on the fundamental problem of how to improve production by slowing the application of current knowledge of fertilizer technology. It will also inhibit further improvement of approaches more likely to have major impact on the provision of food in the next half-century when demand will grow fastest. The combination of molecular, biochemical and physiological information is required to assess the possibilities for crop improvement effectively.

In conclusion: crop production is totally dependent upon the provision of N in suitable amounts at the correct time for growth. This is the consequence of metabolic events, based on proteins, with light energy used in the reduction of CO_2 and NO_3^- and the synthesis of assimilates which are used in vegetative and reproductive growth and yield formation. However, the relationships between the many processes leading to yield are complex and although genetically determined are subject to considerable influence from the environment, particularly N supply. Understanding of the mechanisms determining crop production, yield and efficiency is advanced, but insufficiently exploited in agriculture, although there are ways of applying the information. Changes to crops required to increase biomass are at low N supply: (a) increase total N accumulation by increasing volume of soil exploited, rooting density and affinity of the root surface for NO_3^-. This will maintain the current C/N ratio of the crop; (b) increase C-assimilation per unit of N, which will increase biomass and C/N ratio. At low N-uptake and LAI less than 3, light energy would not be limiting in (a) or (b); both approaches could be used. At ample N-supply and uptake, the possibilities are: (c) to increase the C-assimilation per unit N so increasing biomass and C/N ratio or (d) to increase the N accumulated in active components, but maintain the same C-assimilation, so increasing biomass and maintaining the C/N ratio. Both assume that sufficient energy will be available, which at large LAI is unlikely, and that CO_2 will not be limiting. Currently it is, and the rate of increase in atmospheric CO_2 is unlikely to be fast or large enough to increase production to the genetic potential obtained in elevated CO_2. Hence, increasing genetic potential for crop growth and yield will be needed when the N-supply meets demand, but ways of doing this are unclear. To increase photosynthetic efficiency per unit N or light is not a trivial task. The changes occurring in basic metabolism during improvements achieved by plant breeding are unknown and cannot show what limitations have been overcome to improve genetic yield potential. They have largely involved increasing the capacity for grain growth whilst decreasing vegetative growth and maintaining the ability of C- and N-metabolism to supply the grain, and extending the growing season. Improvements in basic C- and N-metabolism are not documented. Analysis of the metabolic and physiological aspects of plant responses to NO_3^- supply and temperature show the interaction of many processes determining the response of plants to N. Such information is important for guiding the development of methods to improve N-application to crops, in breeding and for assessing the potential of genetic manipulation to improve N-use efficiency and genetic yield potential. Appreciation of genotype–environment interactions is essential. The analysis suggests that increasing N-uptake will require changes in root systems and in the affinity of NO_3^- transporters. The increased efficiency of N-use in photosynthesis would require substantial alterations in photosynthetic metabolism. Improving genetic yield potential will be a major, long-term task, unlikely to be achieved by current genetic engineering technology. A combination of selection breeding and molecular methods may provide ways of improving N-use efficiency and genetic potential.

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References

Addiscott TM, Whitmore AP, Powlson DS. 1991. *Farming, fertilizers and the nitrate problem*. Wallingford: CAB International.

Angus JF. 1995. Modeling N fertilization requirements for crops and pastures. In: Bacon PE, ed. *Nitrogen fertilization in the environment*. New York: Marcel Dekker, Inc., 109–127.

Austin RB, Ford MA, Morgan CL, Yeoman D. 1993. Old and modern wheat cultivars compared on the Broadbalk wheat experiment. *European Journal of Egronomy* **2,** 141–147.

Bacon PE. (ed.) 1995. *Nitrogen fertilization in the environment*. New York: Marcel Dekker, Inc.

Brouqisse R, Masclaux C, Feller U, Raymond P. 2001. Protein hydrolysis and nitrogen remobilization in plant life and senescence. In: Lea PJ, Morot-Gaudry J-F, eds. *Plant nitrogen*. Berlin: Springer-Verlag, 275–293.

Engels C, Marschner H. 1995. Plant uptake and utilization of nitrogen. In: Bacon PE, ed. *Nitrogen fertilization in the environment*. New York: Marcel Dekker, Inc., 41–81.

Evans JR. 1983. Nitrogen and photosynthesis in the flag leaf of wheat (*Triticum aestivum* L.). *Plant Physiology* **70,** 1605–1608.

Evans JR, Terashima I. 1987. Effects of nitrogen nutrition on electron transport components and photosynthesis in spinach. *Australian Journal of Plant Physiology* **14,** 59–68.

Evans JR, von Caemmerer S. 2000. Would C_4 rice produce more biomass than C_3 rice? In: Sheehy JE, Mitchell PL, Hardy B, eds. *Redesigning rice photosynthesis to increase yield*. Amsterdam: Elsevier Science bv, 53–71.

Evans LT. 1998. *Feeding the ten billion*. Cambridge: Cambridge University Press.

Foyer C, Ferrario-Mery S, Noctor G. 2001. Interactions between carbon and nitrogen metabolism. In: Lea PJ, Morot-Gaudry J-F, eds. *Plant nitrogen*. Berlin: Springer-Verlag, 237–254.

Heilmeier H, Monson RK. 1994. Carbon and nitrogen storage in herbaceous plants. In: Roy J, Garnier E, eds. *A whole plant perspective on carbon-nitrogen interactions*. The Hague, The Netherlands: SPB Academic Publishing bv, 149–171.

Hollamby G, Bayraktar A. 1996. Breeding objectives, philosophies and methods in South Australia. In: Reynolds MP, Rajaram S, McNab A, eds. *Increasing yield potential in wheat: breaking the barriers*. Mexico: CIMMYT, 52–65.

Ku MSB, Cho D, Ranada U, Hsu T-P, Li X, Jiao D-M, Ehleringer J, Miyao M, Matsuoka M. 2000. Photosynthetic performance of transgenic rice plants over-expressing maize C_4 photosynthesis enzymes. In: Sheehy JE, Mitchell PL, Hardy B, eds. *Redesigning rice photosynthesis to increase yield*. Amsterdam: Elsevier Science bv, 193–204.

Kutík J, Nátr L, Demmers-Derks HH, Lawlor DW. 1995. Chloroplast ultrastructure of sugar beet (*Beta vulgaris* L.) cultivated in normal and elevated CO_2 concentrations with two contrasted nitrogen supplies. *Journal of Experimental Botany* **46,** 1797–1802.

Lawlor DW. 1994. Relation between carbon and nitrogen assimilation, tissue composition and whole plant function. In: Roy J, Garnier E, eds. *A whole plant perspective on carbon-nitrogen interactions*. The Hague, The Netherlands: SPB Academic Publishing bv, 47–60.

Lawlor DW, Boyle FA, Kendall AC, Keys AJ. 1987*a*. Nitrate nutrition and temperature effects on wheat: enzyme composition, nitrate and total amino acid content of leaves. *Journal of Experimental Botany* **38,** 378–392.

Lawlor DW, Boyle FA, Young AT, Keys AJ, Kendall AC. 1987*b*. Nitrate nutrition and temperature effects on wheat: photosynthesis and photorespiration of leaves. *Journal of Experimental Botany* **38,** 393–408.

Lawlor DW, Boyle FA, Young AT, Kendall AC, Keys AJ. 1987*c*. Nitrate nutrition and temperature effects on wheat: soluble components of leaves and carbon fluxes to amino acids and sucrose. *Journal of Experimental Botany* **38,** 1091–1103.

Lawlor DW, Boyle FA, Keys AJ, Kendall AC, Young AT. 1988. Nitrate nutrition and temperature effects on wheat: a synthesis of plant growth and nitrogen uptake in relation to metabolic and physiological processes. *Journal of Experimental Botany* **39,** 329–343.

Lawlor DW, Kontturi M, Young AT. 1989. Photosynthesis by flag leaves of wheat in relation to protein, ribulose bisphosphate carboxylase activity and nitrogen supply. *Journal of Experimental Botany* **40,** 43–52.

Lawlor DW, Lemaire G, Gastal F. 2001. Nitrogen, plant growth and crop yield. In: Lea PJ, Morot-Gaudry J-F, eds. *Plant nitrogen*. Berlin: Springer-Verlag, 343–367.

Laza RC, Bergman B, Vergara BS. 1993. Cultivar differences in growth and chloroplast ultrastructure in rice as affected by nitrogen. *Journal Experimental Botany* **44,** 1643–1648.

Lea PJ, Morot-Gaudry J-F. (eds) 2001. *Plant nitrogen*. Berlin: Springer-Verlag.

Limami A, de Vienne D. 2001. Natural genetic variability in nitrogen metabolism. In: Lea PJ, Morot-Gaudry J-F, eds. *Plant nitrogen*. Berlin: Springer-Verlag, 370–378.

Mächler F, Oberson A, Grub A, Nösberger J. 1998. Regulation of photosynthesis in nitrogen-deficient wheat seedlings. *Plant Physiology* **87,** 46–49.

Macduff JH, Hopper MJ, Wild A, Trim FE. 1987. Comparison of the effects of root temperature on nitrate and ammonium nutrition of oilseed rape (*Brassica napus* L.) in flowing solution culture. I. Growth and uptake of nitrogen. *Journal of Experimental Botany* **38,** 1104–1120.

Macduff JH, Jarvis SC, Larsson C-M, Oscarson P. 1993. Plant growth in relation to the supply and uptake of NO_3^-: a comparison between relative addition rate and external concentration as driving variables. *Journal of Experimental Botany* **44,** 1475–1484.

Matsuoka M, Fukayama H, Tsuchida H, Nomura M, Agarie S, Ku MSB, Miyao M. 2000. How to express some C_4 photosynthesis genes at high levels in rice. In: Sheehy JE, Mitchell PL, Hardy B, eds. *Redesigning rice photosynthesis to increase yield*. Amsterdam: Elsevier Science bv, 167–175.

Millard P. 1988. The accumulation and storage of nitrogen by herbaceous plants. *Plant, Cell and Environment* **11,** 1–8.

Millard P, Catt JW. 1988. The influence of nitrogen supply on the use of nitrate and ribulose 1,5-bisphosphate carboxylase/oxygenase as leaf nitrogen stores for growth of potato tubers (*Solanum tuberosum* L.). *Journal of Experimental Botany* **39,** 1–11.

Miller AJ, Cookson SJ, Smith SJ, Wells DM. 2001. The use of microelectrodes to investigate compartmentation and the transport of metabolized inorganic ions in plants. *Journal of Experimental Botany* **52,** 541–549.

Mitchell RAC, Theobald JC, Parry MAJ, Lawlor DW. 2000. Is there scope for improving balance between RuBP-regeneration and carboxylation capacities in wheat at elevated CO_2? *Journal of Experimental Botany* **51,** 391–397.

Monteith JL. 1977. Climate and the efficiency of crop production in Britain. *Philosophical Transactions of the Royal Society, London* **B281,** 277–294.

Nakano H, Makino A, Mae T. 1997. The effects of elevated partial pressures of CO_2 on the relationship between photosynthetic capacity and N content in rice leaves. *Plant Physiology* **115,** 191–198.

Neeteson JJ. 1995. Nitrogen management for intensively grown arable crops and field vegetables. In: Bacon PE, ed. *Nitrogen fertilization in the environment*. New York: Marcel Dekker, Inc., 295–325.

Nelson T, Dengler N. 1997. Leaf vascular pattern formation. *The Plant Cell* **9,** 1121–1125.

Ourry A, Macduff JH, Volenec JJ, Gaudillere JP. 2001. Nitrogen traffic during plant growth and development. In: Lea PJ, Morot-Gaudry J-F, eds. *Plant nitrogen*. Berlin: Springer-Verlag, 255–273.

Paul MJ, Lawlor DW. (eds) 2000. Genetic manipulation of photosynthesis. *Journal of Experimental Botany* **51,** Special Issue, 319–496.

Paul M, Pellny T, Goddijn O. 2001. Enhancing photosynthesis with sugar signals. *Trends in Plant Science* **6,** 197–200.

Peltonen J, Virtanen A, Haggrèn E. 1995. Using a chlorophyll meter to optimize nitrogen fertilizer application for intensively-managed small-grain cereals. *Journal of Agronomy and Crop Science* **174,** 309–318.

Richards RA. 1996. Increasing the yield potential of wheat: manipulating sources and sinks. In: Reynolds MP, Rajaram S, McNab A, eds. *Increasing yield potential in wheat: breaking the barriers*. Mexico: CIMMYT, 134–149.

Sage RF, Pearcy RW, Seemann JR. 1987. The nitrogen use efficiency of C_3 and C_4 plants. I. Leaf nitrogen, growth, and biomass partitioning in *Chenopodium album* (L.) and *Amaranthus retroflexus* (L.). *Plant Physiology* **84,** 954–958.

Sheehy JE, Mitchell PL, Hardy B. (eds) 2000. *Redesigning rice photosynthesis to increase yield*. Amsterdam: Elsevier Science bv.

Slafer GA, Calderini DF, Miralles DJ. 1996. Yield components and composition in wheat: opportunities for further increasing yield potential. In: Reynolds MP, Rajaram S, McNab A, eds. *Increasing yield potential in wheat: breaking the barriers*. Mexico: CIMMYT, 101–133.

Snape JW. 1996. The contribution of new biotechnologies to wheat breeding. In: Reynolds MP, Rajaram S, McNab A, eds. *Increasing yield potential in wheat: breaking the barriers*. Mexico: CIMMYT, 167–181.

Theobald JC, Mitchell RAC, Parry MAF, Lawlor DW. 1998. Estimating the excess investment in ribulose-1,5-bisphosphate carboxylase/oxygenase in leaves of spring wheat grown under elevated CO_2. *Plant Physiology* **118,** 945–955.

Ter Steege MW, Stulen I, Mary B. 2001. Nitrogen in the environment. In: Lea PJ, Morot-Gaudry J-F, eds. *Plant nitrogen*. Berlin: Springer-Verlag, 379–397.