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Edited by

H. W. WOOLHOUSE

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Department of Plant Sciences,
The University,
Leeds, England

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CONTRIBUTORS TO VOLUME 6

B. DELL, *School of Environment and Life Sciences, Murdoch University, Murdoch, Western Australia 6153, Australia*

K. JONES, *Jodrell Laboratory, Royal Botanical Gardens, Kew, Richmond, Surrey TW9 3DS, England*

J. A. LEE, *Department of Botany, University of Manchester, Manchester M13 9PL, England*

A. J. McCOMB, *Botany Department, University of Western Australia, Nedlands, Western Australia 6009, Australia*

A. J. E. SMITH, *School of Plant Biology, University College of North Wales, Bangor LLS7 2UW, Gwynedd, North Wales*

E. STEUDLE, *Institute of Biophysical Chemistry, Nuclear Research Centre, Jülich, West Germany*

G. R. STEWART, *Department of Botany, University of Manchester, Manchester M13 9PL, England*

U. ZIMMERMANN, *Institute of Biophysical Chemistry, Nuclear Research Centre, Jülich, West Germany*

PREFACE

Volume 6 of the *Advances in Botanical Research* continues our practice of critically appraising progress in subjects from across the whole spectrum of botanical studies.

Smith considers the evolution of the Bryophyta with particular reference to the evidence from cytological studies; the rapid progress in this field emphasizes the probable antiquity of the major groups which were delimited on other criteria. Jones draws on the evidence of his detailed work in the cytogenetics of the Commelinaceae to consider the evolution of the karyotype with particular reference to the role of fusion and fission processes.

Nitrogen is perhaps the plant nutrient which is most frequently in limiting supply in a wide range of habitats; Lee and Stewart explore the biochemical and physiological adaptations of the nitrogen nutrition of plants in a range of habitats. Dell and McComb are concerned with the borders between physiology, fine structure and ecology in their investigation of the structure, functioning and ecological significance of resin glands in plants.

Though many botanists are interested in plant water relations, the subject has lagged for want of new research techniques; in his paper Zimmerman points the way forward to important new work on the water relations of plant cells through the use of his micromethods for the direct measurement of the turgor potentials.

The Editor is indebted to Mrs Joan Long for work on the Subject Index and to Miss J. Denison for Secretarial Assistance.

H. W. Woolhouse

Department of Plant Sciences,
University of Leeds, 1978

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Ecological Aspects of Nitrogen Assimilation

J. A. LEE AND G. R. STEWART

Department of Botany, University of Manchester, Manchester, England

I. INTRODUCTION

Nitrogen is commonly the soil nutrient element required in largest quantities by higher plants. Plants of many species in a variety of habitats usually contain between 1 and 3% nitrogen on a dry weight basis. There are exceptions to this, e.g. ruderal species growing in nitrogen-enriched habitats may contain up to 6% nitrogen and, indeed, nitrogen may be so freely available in some of these habitats as to be toxic to some plants. However, plants rarely have less than 1% nitrogen, even in the extreme nitrogen deficient tundra habitats, and this is a reflection of the importance of nitrogen containing compounds in the structure and metabolism of cells.

Nitrogen assimilation has received considerable attention by plant physiologists in recent years, and this has resulted in a number of interesting and important discoveries. However, the majority of these investigations have been confined to crop plants, and relatively little regard has been paid to ecological considerations. This preponderance of work on fast growing annual species from habitats in which the supply of nitrogen and other nutrients is maintained at an artificially high level is understandable but perhaps it limits our knowledge of the diversity of assimilation processes. The growth of higher plants in many ecosystems is limited by nitrogen supply. Adaptation to low nitrogen availability presumably demands at least some modification of the assimilation processes shown by crop plants, if not different mechanisms of assimilation; and in many perennial species this will also be linked to the efficient conservation and recycling of nitrogen within the plant. Nitrogen is largely made available to plants through the activities of micro-organisms, and because of this soils may show marked seasonal variation in the supply and form of available nitrogen. Again this must demand specific adaptations by plants to utilize effectively the fluctuating supply. In this paper we bring together recent work on the nitrogen assimilation of crop plants with work in progress on ecological aspects of nitrogen metabolism. We review the mass of information on the availability of nitrogen in different ecosystems, and examine the variety of adaptations shown by plants to extremes of nitrogen supply.

II. NITROGEN UPTAKE AND ASSIMILATION

A. THE UPTAKE OF NITRATE AND AMMONIA

In contrast to many micro-organisms, higher plants often exhibit an accumulation of nitrate ions in both the root and shoot system. This accumulation of nitrate, in common with that of other anions, occurs against the electrochemical gradient, suggesting the active inward transport of these ions (Higinbotham *et al.*, 1967). Nitrate accumulation is regarded as being the net result of two independent processes, an influx and an efflux process, operating across the plasma lemma (Morgan *et al.*, 1973; Jackson *et al.*, 1976). Studies of nitrate uptake are complicated by its subsequent assimilation and are limited by the lack of a suitable radioactive isotope of nitrogen, consequently the precise mechanism underlying the active influx process remains largely unknown.

One characteristic of nitrate uptake which has been studied in several species is the apparent induction of uptake. Following transfer of urea-grown tobacco cells to a nitrate medium, a lag period of 1–2 h was observed before nitrate accumulation reached its maximum rate (Heimer and Filner, 1970). Similar observations have been made on nitrogen-deficient seedlings—these exhibit a low initial rate of nitrate uptake over the first 3–4 h following their transfer to a nitrate-containing medium and this is followed by a period in which the rate of nitrate uptake increases to its maximum value (see e.g. Ashley *et al.*, 1975). The increase in the rate of nitrate uptake (Rao and Rains, 1976) or accumulation (Heimer and Filner, 1970), as well as the increase in nitrate reductase level is inhibited by inhibitors of protein synthesis such as cycloheximide. The lag phase in nitrate uptake and the inhibition of the subsequent accelerated rate of uptake have been interpreted as being indicative of the synthesis of a specific nitrate transport system or permease (Heimer and Filner, 1970; Jackson *et al.*, 1973). In general there is a close relationship observed between the development of nitrate uptake and the appearance of nitrate reductase activity (see e.g. Jackson *et al.*, 1973). Recently Butz and Jackson (1977) have proposed a mechanism for nitrate transport in which a tetrahedron-shaped, transmembrane nitrate reductase tetramer is also the nitrate carrier protein. While this speculative model fits many of the observations regarding the relationship between nitrate reductase levels and the rate of nitrate uptake it is unlikely to be of general application. Species such as *Xanthium pennsylvanicum* exhibit no nitrate reduction in the root system, the xylem sap containing 95–99% of its nitrogen as free nitrate (Pate, 1972). Unless one assumes such species possess root nitrate reductases having only the capacity to function as a carrier protein or the absence of any active nitrate uptake it is difficult to see how the model can be applied to them.

In contrast to its active influx, the efflux of nitrate is a passive leakage phenomenon. The rate of efflux does not, however, appear to be simply related to the nitrate content of root tissue (Jackson *et al.*, 1976). Thus seedlings of *Triticum vulgare* grown on 15 mmol NO_3 exhibited a much greater efflux rate when transferred to 1 mmol NO_3 than seedlings grown on 1 mmol NO_3, even though their roots had similar nitrate contents. This is suggested to result from a differential permeability of the plasmalemma or differential compartmentation of accumulated nitrate.

Regrettably, most studies of nitrate absorption have not distinguished between effects on the influx and efflux processes. However, a number of factors of at least potential ecological importance have been observed to influence net nitrate influx. Several workers have shown external pH exerts a marked influence on nitrate uptake. At pH values above 6–7 nitrate uptake decreases (Van den Honert and Hooymans, 1955; Lycklama, 1963). In contrast Arnon *et al.* (1942) found little influence of pH on nitrate uptake by Bermuda grass over the range 4–9.

High tissue chloride levels are reported to inhibit nitrate uptake (Cram, 1973; Smith, 1973) although no competition between external nitrate and chloride occurs (Smith, 1973). Presumably the situation may be somewhat different in some halophytic plants which accumulate very high chloride levels and contain appreciable levels of nitrate (Stewart *et al.*, 1974).

A close correlation has been observed between net nitrate and net potassium uptake. In *Triticum vulgare* nitrate uptake was observed to occur without the concomitant uptake of potassium, whereas uptake of the latter was largely dependent on nitrate uptake (Jackson *et al.*, 1976). If this phenomenon occurs generally then it has important ecological consequences as regards the mineral nutrition of species which grow in soils where nitrate is not the major source of available nitrogen.

Another factor which can influence nitrate uptake is ammonium. In some species nitrate uptake is inhibited by ammonium ions (see e.g. Lycklama, 1963). It is interesting to note, however, that this ammonium inhibition is a species specific characteristic in that no inhibition was observed with *Lemna minor* (Orebamjo and Stewart, 1975).

The absorption of ammonium ions seems to be a passive process (Higinbotham, 1973) and generally occurs at rates considerably greater than those observed for nitrate uptake. In studies of plants grown on ammonium nitrate it has been observed that in many species there is a preferential absorption of ammonium ions (see e.g. Fried *et al.*, 1965; Minotti *et al.*, 1969). Thus while the absorption of nitrate may be a rate limiting step in nitrogen assimilation it is unlikely that this is the case for ammonium absorption.

B. NITROGEN FIXATION

Nitrogen fixation in free-living and symbiotic micro-organisms occurs through the activity of an ATP-dependent nitrogenase. Six electrons are required in this reaction which can be described by the following equation:

$$N_2 + 6e^+ + 6H^+ + nATP \rightarrow 2NH_3 + nADP + nPi.$$

It is generally accepted that the reaction requires at least six molecules of ATP.

Nitrogenase preparations can be resolved into two component proteins, neither of which has the capacity by itself to reduce nitrogen. Component I protein has a molecular weight of 210 000–280 000 daltons, with four identical subunits (Kleiner and Chen, 1974). It is a molybdenum-iron protein, containing 1–2 atoms of molybdenum and 18–40 atoms of iron. The Component II protein has a molecular weight of 56 000–64 000 daltons, with two subunits and contains four atoms of iron per molecule. The precise roles of these two proteins in the fixation process are still not fully elucidated. It is suggested that nitrogen is bound to, and reduced by, the molybdenum-iron protein. The iron protein is thought to be involved in the reduction of the Component I protein. Reduced ferredoxin acts as electron donor reducing Component II protein which can bind Mg-ATP. This binding of Mg-ATP lowers the potential (from −294 mV to −400 mV) of the Component II protein thus allowing it to reduce the Component I protein. The reduced Component I protein then effects the reduction of molecular nitrogen (Winter and Burris, 1976).

Studies of symbiotic nitrogen fixation have seen great advances in the past 2 years. The elaboration of a chemically defined medium on which free-living Rhizobia can reduce nitrogen or acetylene (Keister, 1975; Kurz and La Rue, 1975; McComb *et al.*, 1975; Pagan *et al.*, 1975; Tjepkema and Evans, 1975) provides the basis for detailed studies into the biochemistry of fixation in the asymbiotic state. It is interesting that one of the requirements for nitrogen fixation of asymbiotic Rhizobia cultures is a source of combined nitrogen (ammonia, nitrate and glutamine being effective). Earlier studies which led to the development of free-living nitrogen fixing Rhizobia cultures included the demonstration that callus cultures of several legume species infected with Rhizobium could develop nitrogenase activity (see e.g. Scowcroft and Gibson, 1975). It is significant, however, that other non-leguminous higher plant cell cultures infected with Rhizobium (see e.g. Child, 1975) are also able to develop the capacity to utilize molecular nitrogen.

The initial observation of Dilworth (1966), that the ATP-driven nitrogenase reaction would also catalyse the reduction of acetylene to ethylene resulted in the development of a simple and sensitive technique for assessing nitrogen fixation *in situ* (Stewart *et al.*, 1967). The "acetylene reduction test" is now widely used in determining the rates of nitrogen fixation in natural environments and has provided invaluable information regarding the contribution of nitrogen fixation to the nitrogen economy of plant communities. Much of this work has been brought together in Stewart (1975) and Nutman (1976) and readers are referred to these accounts for a detailed consideration of this area.

C. NITRATE REDUCTION

Nitrate reduction can be carried out by two distinct biochemical mechanisms. The first is the process of nitrate assimilation in which nitrate is reduced, via nitrite, to ammonia and subsequently enters into organic nitrogenous compounds. The second process, dissimilatory nitrate reduction, is one in which nitrate acts as an alternative electron acceptor to oxygen and is reduced to nitrite. In this process ammonia formation is uncommon, the normal products being nitrite or molecular nitrogen. Dissimilatory nitrate reduction is common in obligately or facultatively anaerobic bacteria. Fungi such as Neurospora and Aspergillus exhibit dissimilatory nitrate reduction when grown under conditions of limited oxygen supply, it is uncertain, however, to what extent this reflects the operation of a pathway different from the assimilatory one (see Pateman and Kinghorn, 1976). Some higher plants exhibit high rates of nitrate reduction when subjected to flooding (see Garcia-Novo and Crawford, 1973) and a role for nitrate as an alternative electron acceptor to oxygen has been proposed. Dissimilatory nitrate reduction may also be of importance as a component of the nitrogen cycle in soils of low oxygen tension.

Assimilatory nitrate reduction is catalysed by two enzymes, nitrate reductase which reduces nitrate to nitrite, and nitrite reductase which reduces nitrite to ammonia. The overall reduction of nitrate to ammonia requires eight electrons. Nitrate reductase is a complex protein exhibiting three functions: (1) NAD(P)H nitrate reduction; (2) NAD(P)H dehydrogenase in which cytochrome *c*, DCPIP, ferricyanide and tetrozolium can function as acceptors; (3) $FMNH_2$, $FADH_2$, reduced benzyl and methyl viologen nitrate reduction. Like Component I protein of nitrogenase it is a molybdenum-containing protein but may also contain heme-iron (see Amy and Garret, 1974). Detailed structural studies of the enzyme from higher plants are lacking at present, most of the detailed information coming from the characterization of fungal or algal nitrate reductase and readers are referred to Hewitt (1975) for such information.

The only form of nitrite reductase so far characterized from higher plants uses reduced ferredoxin as an electron donor. While this enzyme has been found in both chlorophyllous and non-green tissue the physiological reductant appears to be restricted to chlorophyllous tissue (see Beevers and Hageman, 1969). In the absence of any alternative electron donor the role of the ferredoxin-dependent nitrite reductase in root nitrate assimilation is uncertain. There is no evidence that higher plants have a pyridine nucleotide-dependent nitrite reductase of the kind found in fungi.

Ferredoxin-dependent nitrite reductase contains two atoms of iron per molecule (see e.g. Huckelsby *et al.*, 1974) and has a molecular weight between 60 000 and 70 000 daltons (Cardenas *et al.*, 1972). Highly purified preparations can catalyse the stochiometric reduction of nitrite to ammonia and can also reduce hydroxylamine but not hyponitrite or nitric oxide (see Hewitt, 1975).

D. AMMONIA ASSIMILATION

The product of both nitrate assimilation and molecular nitrogen fixation is ammonium. Thus the assimilation of ammonia, whether it is derived from more highly oxidized forms of nitrogen or is absorbed directly from the soil solution is of key importance in plant nitrogen metabolism. Until quite recently it was generally assumed that the principal route of ammonia assimilation in higher plants was via the reaction catalysed by the enzyme glutamate dehydrogenase:

$$\text{2-oxoglutarate} + NH_3 + \text{NAD(P)H} + H^+ \rightleftharpoons \text{L-Glutamate} + \text{NAD(P)} + H_2O.$$

The discovery in higher plant tissues of glutamate synthase activity (Dougall, 1974; Fowler *et al.*, 1974; Lea and Miflin, 1974) has, however, led to the suggestion that an alternative route, via the combined action of glutamine synthetase and glutamate synthase, is the major pathway of ammonia assimilation in higher plants. The reactions catalysed by glutamine synthetase and glutamate synthase are:

$$\text{L-Glutamate} + NH_3 + \text{ATP} \xrightarrow{Me^{2+}} \text{L-Glutamine} + \text{ADP} + \text{Pi}$$

$$\text{L-Glutamine} + \text{2-oxoglutarate} + \text{NAD(P)H} + H^+ \rightarrow \text{2-L-Glutamate} + \text{NAD(P)} + H_2O$$

or

$$\text{L-Glutamine} + \text{2-oxoglutarate} + \text{FdH} \rightarrow 2\ \text{L-Glutamate} + \text{Fd}.$$

The coupling of glutamine synthetase and glutamate synthase results in the net synthesis of one glutamate molecule. Thus the net result is the same as the reaction catalysed by glutamate dehydrogenase but the route involving glutamine requires energy expenditure in the form of ATP. The discovery of glutamate synthase and the evidence for its participation in higher plant ammonia assimilation has been fully discussed in a recent review (Miflin and Lea, 1976).

Glutamine synthetase is widely distributed in plant tissue. The enzyme from some sources, e.g. pea leaf tissue and *Lemna minor*, has a high affinity for ammonia, the K_m values being in the range 1 to 2×10^{-5}M (O'Neal and Joy, 1974; Stewart and Rhodes, 1977a). The enzyme has a requirement for a divalent metal cation and while Mn^{2+}, Co^{2+}, Ni^{2+} can satisfy this, Mg^{2+} is regarded as being the most important physiological co-factor. In addition to catalysing the synthesis of glutamine, the enzyme can catalyse the formation of glutamyl-hydroxamate when hydroxylamine is substituted for ammonia. The enzyme will also catalyse the so-called transferase reaction:

$$\text{L-Glutamine} + NH_2OH \xrightarrow[\text{ADP; } AsO_4]{Me^{2+}} 2 + \text{L-}\gamma\ \text{glutamyl hydroxamate} + NH_3.$$

The transferase reaction has no known physiological function but is generally 6–20 times more active than the synthetase assay and is consequently widely used to determine "glutamine synthetase" activity.

Two forms of glutamate synthase have been reported in higher plant tissues. One of these is pyridine-nucleotide-linked, exhibiting activity with both NADH and NADPH (Dougall, 1974; Fowler *et al.*, 1974). This form of the enzyme appears to be restricted to non-green tissue (Miflin and Lea, 1975). The other form of the enzyme utilizes reduced ferredoxin as the physiological electron donor and has been found in both chlorophyllous and non-green tissue (Lea and Miflin, 1974; Miflin and Lea, 1975). There was initially some doubt as to the substrate specificity of NAD(P)-linked glutamate synthase, crude cell preparations exhibiting apparent activity with asparagine (Dougall, 1974; Fowler *et al.*, 1974). Subsequent work has shown this lack of substrate specificity resulted in part from the use of impure asparagine (Miflin and Lea, 1976).

Glutamate dehydrogenase is widely distributed in higher plants; in general the enzyme exhibits activity with both NADH and NADPH as electron donors. Two forms appear to be present in some plants—one which is equally active with both NADH and NADPH and the other which shows greater activity with NADH than with NADPH (Lea and Thurman, 1972). In general, plant glutamate dehydrogenases have a low affinity for ammonium ions, K_m values in excess of 1×10^{-2}M being reported (see e.g. Pahlich and Joy, 1971; Teixiera and Davies, 1974). This kinetic characteristic is of course one of the major reasons why a role for this enzyme in ammonia assimilation is doubtful.

E. SITES OF NITROGEN ASSIMILATION

Evidence regarding the sites of nitrate assimilation can be found by two approaches: analyses of the enzymic complement of root and shoot tissues and analyses of the nitrogenous compounds exported by the root. Both approaches, largely experimental in nature, have a number of limitations, but when used in combination can yield useful information relating to the sites of nitrate assimilation (see Pate, 1972). Species exhibit marked differences as regards the site(s) of nitrate assimilation and two groups can be recognized. There are those in which nitrate assimilation is restricted to shoot tissue—plants such as *Borago* spp. and *Xanthium pennsylvanicum* are characteristic of this group. In these the xylem sap contains 95–99% of its nitrogen in the form of nitrate ions and no nitrate reductase is measurable in the root system (Pate, 1972). The other group consists of species in which both the root and shoot contribute in the assimilation of nitrate. At the extreme end of this group are several woody species where little nitrate is present in the xylem sap and the bulk of their nitrate reductase activity is located in the root system. The majority of species examined exhibit a pattern intermediate between these two extremes. In these both root and shoot tissues have appreciable levels of nitrate reductase and the xylem sap contains both free nitrate and organic nitrogen (Pate, 1971, 1972). The actual