Biochemical aspects of inorganic nitrogen assimilation by woody plants

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Introduction

For the majority of woody plants, the mineralization of organic nitrogen in soil provides their source of nitrogen for growth. The relative activities of ammonifiers and nitrifiers, as influenced by a complex interaction of abiotic and biotic factors, will determine the availability of ammonium and nitrate ions, both spatially and temporarily. Nitrification rates show considerable variation in different forest soils and, for the most part, this is unrelated to total nitrogen, pH or carbon:nitrogen ratio (Robertson, 1982).

Not surprisingly, woody plant species exhibit differences in their capacities to utilize nitrate and ammonium ions (see *e.g.*, Chandler, 1981). These differences relate in part at least to the prevalent form of available nitrogen in their ecological niche. However, the assimilation of ammonium and nitrate ions carries different potential costs with respect to energy and water requirements, with ammonium ions being the more cost-effective nitrogen source (Raven, 1985). Differences in cost effectiveness of nitrate and ammonium ions

may be important with respect to the growth of understorey trees and shrubs in light- and water-limited environments, particularly as these species have high maintenance costs.

In this report, we will consider the characteristics of inorganic nitrogen assimilation in woody plants. The occurrence and localization of nitrate reductase and glutamine synthetase isoforms will be discussed.

Nitrate reduction

Nitrate assimilation in higher plants is catalyzed by 2 enzymes: pyridine nucleotide-linked nitrate reductase and ferredoxin-linked nitrite reductase. The capacity for nitrate reduction is widespread among woody plants (see, *e.g.*, Smirnoff *et al.*, 1984; Stewart *et al.*, 1988), although certain taxonomic groups exhibit a low capacity for leaf nitrate reduction. Rates of nitrate reduction in many gymnosperms and members of the Proteaceae and Ericaceae are at the low end of the range reported for higher plants (Smirnoff

et al., 1984). In a recent study of Australian rain forest plants, many understorey trees and shrubs were found to utilize nitrogen sources other than nitrate and to have a low capacity for nitrate reduction (Stewart et al., 1988).

Leaf nitrate reductase is a cytosolic enzyme which utilizes NADH as reductant. Trees of the genus Erythrina are quite atypical in having a nitrate reductase which can utilize both NADH and NADPH (Orebamjo et al., 1982). However, this enzyme has a high K_m for nitrate (10 mM), this being 20–100 times greater than K_m values reported for NADH nitrate reductases (Orebamjo et al., 1982). The activity of nitrate reductase in Erythrina species is very high, 20–50 pkat·g fw^{-1}. There are no other reports of woody plants having this unusual form of nitrate reductase and the physiological significance of a low affinity–high activity nitrate-reducing system is obscure. Although the properties of NADH–nitrate reductases are rather uniform, there are considerable differences between species as regards the sites of nitrate reduction. Measurements of the distribution of nitrate reductase activity between roots and shoots and of xylem sap nitrogenous compounds have led to the recognition of 3 groups of plants. One group consists of species in which the shoot is the major site of nitrate reduction and in these species little reduction occurs in their roots; a second group comprises species which exhibit the capacity for both root and shoot nitrate reduction and in which nitrate ions as well as reduced forms of nitrogen are present in the xylem sap; the third group includes species in which the root is the major site of nitrate reduction and little or no nitrate is present in xylem sap (see Pate, 1983). Andrews (1986) suggests that 3 generalizations can be made with respect to the partitioning of nitrate assimilation between roots and shoots: 1) temperate perennial species assimilate nitrate in their roots when the external concentration of nitrate is low <1 mol·m^{-3} and, as this is increased, shoot nitrate reduction becomes more important; 2) temperate annual species carry out most of their nitrate reduction in the shoot, irrespective of external nitrate reduction;

Table I. Root–shoot partitioning of nitrate reductase in woody plants.

Shoot–root NRA <1.0	Shoot–root NRA 1–5	Shoot–root NRA >5.0
Aegiceras corniculatum	Aspidospermum australe	Astronium lecointeri
Aegialitis annulata	Anadenanthera pergrina	Cedrella fissilis
Avicennia nitida	Casearia sylvestris	Chorisia speciosa
Bauhinia rufescens	Cordia eucalypta	Croton urucurana
Calophyllum brasiliense	Dalbergia brasiliense	Ficus exasperata
Centrolobium tomentosum	Enterolobium contortisilicum	Genipa americana
Dimorphandra mollis	Hymenea coubaril	Guierra senegalensis
Kielmeyera coriacea	Lonchocarpus muellbergiana	Hybanthus atropurpurea
Pithecellobium dulce	Machaerium stipitatum	Nectramdra rigida
Rhizophora mangle	Metrodonea nigra	Solanum prianthum
Styrax sp.	Platypodium elegans	Urera barrifra
	Prunus sellowii	Tabebuia chrysotricha
	Pterocarpus violacea	
	Rapanea ferruginea	
	Rauvolfia vomitoria	
	Schinus terebentifolium	

See Stewart et al. (1988) for assay details.

3) tropical and subtropical species both annual and perennial carry out nitrate assimilation predominantly in their shoots, external nitrate concentration having little effect on the localization of assimilation. However, studies of the distribution of nitrate reductase activity (NRA) between the roots and shoots of tropical and subtropical trees indicate that about 30% of the species exhibit shoot to root nitrate reductase ratios of less than 1, for 40% of the species, the ratio was between 1 and 5 and, for the remaining 30%, the ratio was greater than 5 (see Table I).

Moreover, most of the species examined showed marked changes in shoot to root nitrate reduction when the nitrate supply was increased. Typically, species with high shoot nitrate reductase activities are those characteristic of forest margins and gaps and early stages of forest succession. Previous studies of herbaceous and woody species (Stewart et al., 1987) and Australian rain forest species (Stewart et al., 1988) also support this idea that shoot nitrate reduction is a characteristic of pioneer species both temperate and tropical.

Many of the woody species, which in pot experiments exhibit a predominance of root nitrate reduction, are species which can either utilize dinitrogen through symbiotic association with a prokaryotic nitro-gen fixer or are species which normally grow in habitats where ammonium ions are likely to be the available nitrogen source. The assimilation of ammonium ions derived from dinitrogen or directly absorbed from the soil solution occurs exclusively in the root system. Consequently, these species have the necessary biochemical components for nitrogen assimilation in their root cells, are active in the transport of nitrogenous compounds between root and shoot and their leaves are biochemically competent in the catabolism and re-assimilation of translocated nitrogenous compounds. A predisposition to root nitrate reduction may simply be a consequence of an obligatory root nitrogen assimilation imposed by adaptation to dinitrogen or ammonium ion utilization.

Pathways of ammonium ion assimilation

Various approaches, including ^{15}N-tracer studies, the use of enzyme-specific inhibitors and studies with mutants lacking one or more of the assimilatory enzymes, give results consistent with the view that ammonium assimilation occurs through the combined action of glutamine synthetase

Table II. Ammonium assimilating enzymes in the roots and shoots of woody plants.

Species	Enzyme activity (nkat·g fw^{-1})					
	root			shoot		
	GS	GOGAT	GDH	GS	GOGAT	GDH
Avicennia nitida	150	60	758	200	312	207
Dalbergia ecastophyllum	200	70	400	515	300	295
Kielmeyera coriacea	189	16	280	291	256	558
Peltophorum dubium	116	6	107	381	122	907
Pseudobombax marginatum	130	48	1014	391	160	545
Trema guineensis	100	20	800	450	310	287

See Stewart et al. (1988) for assay details.

and glutamate synthase and that glutamate dehydrogenase (GDH) makes, at the best, a minor contribution (see Wallsgrove, 1987). There have been few studies of the ammonia assimilatory enzymes in woody plants. Glutamine synthetase has been demonstrated in leaves of several tree species (Chandler, 1981; NcNally *et al.,* 1983; Stewart *et al.,* 1988).

The results in Table II show the presence of glutamine synthetase (GS) and glutamate synthase (GOGAT) in shoots and roots of woody plants representative of a range of forest types. In common with many herbaceous species, substantial activities of glutamate dehydrogenase are present, particularly in roots. *Avicennia nitida* roots exhibit high activities of NADH glutamate dehydrogenase which are nearly 5 times greater than those of glutamine synthetase. However, when such roots are treated with methionine sulphoximine, an inhibitor of glutamine synthetase, not only is glutamine synthesis inhibited but there is also an accumulation of ammonium ions and a decline in the concentrations of amino acids. These results suggest that, even in tissues where the activity of glutamate dehydrogenase is high, the preferred pathway of ammonium assimilation is the glutamate synthase cycle.

A combination of ^{15}N-labelling and enzymic specific inhibitors has been used to determine the pathway of ammonium assimilation in isolated beech mycorrhizal roots and the results again suggest the operation of the glutamate synthase cycle (Martin *et al.,* 1986). Glutamate dehydrogenase was found to play little if any part in mycorrhizal ammonium assimilation, even though studies of the mycorrhizal fungus suggest it assimilates ammonium by the glutamate dehydrogenase route (Genetet *et al.,* 1984). However, our recent studies with another mycorrhizal fungus, *Pisolithus tinctorius,* suggest it may utilize the glutamate synthase cycle

rather than glutamate dehydrogenase for ammonium assimilation.

Glutamine synthetase isoforms

Although the first enzyme of the glutamate synthase cycle is ubiquitous in plant tissues, it occurs as tissue/organ-specific isoforms, whose activities differ between species. There is a root-specific isoform and in legumes there is a nodule isoform (Cullimore *et al.,* 1983). The leaves of many species have 2 isoforms, one located in the chloroplasts and the other in the cytosol (Mann *et al.,* 1979; McNally *et al.,* 1983).

Among woody plants, there are considerable differences in the relative proportions of chloroplastic and cytosolic isoforms (see (McNally *et al.,* 1983; Stewart *et al.,* 1987). Recently, it has been shown that the leaves of woody pioneer species exhibit predominantly the chloroplastic isoform, while, in the leaves of species typical of closed, climax forest, the cytosolic isoform predominates (Stewart *et al.,* 1988). Moreover, several of these trees of climax forest appear to lack the chloroplastic isoform (Stewart *et al.,* 1987; 1988). Other higher plants in which the chloroplastic isoform is absent are achlorophyllous parasitic species (McNally *et al.,* 1983). Woody species which exhibit low levels or completely lack chloroplastic glutamine synthetase also have a low capacity for leaf nitrate reduction (Stewart *et al.,* 1988). Low levels of chloroplastic glutamine synthetase imply re-assimilation of photorespiratory ammonium by cytosolic glutamine synthetase. Curiously, the original model for the photorespiratory nitrogen cycle did, in fact, propose that the ammonium released was re-assimilated by cytosolic glutamine synthetase (Keys *et al.,* 1978). However, the absence of this

isoform in many C_3 species (McNally *et al.,* 1983) and the rapid accumulation of ammonium under photorespiratory conditions in mutants lacking chloroplastic glutamine synthetase (Wallsgrove, 1987) led to an acceptance of the idea that photorespiratory ammonium is re-assimilated by the chloroplastic isoform. Our observations with woody plants suggest species differ in the extent to which cytosolic and chloroplastic isoforms participate in the photorespiratory nitrogen cycle and that no simple generalization can be made.

Conclusions

The results presented here indicate that inorganic nitrogen assimilation in woody plants resembles, in general, that of herbaceous species. The differences observed between species relate to the site(s) of nitrate assimilation at the whole plant level and the site of glutamine synthesis at the cellular level.

Woody pioneer species exhibit a high capacity for nitrate reduction and are in general leaf assimilators of nitrate. In most, chloroplastic glutamine synthetase accounts for most of the total leaf activity. In contrast, many under- and overstorey-species generally utilize sources of nitrogen other than nitrate but, if nitrate ions are available, they are assimilated in the root system. Many tree species with a low capacity for leaf nitrate reduction exhibit low levels of chloroplastic glutamine synthetase and assimilate photorespiratory ammonium in the cytosol of leaf cells.

These differences in sites of nitrate and ammonium ion assimilation at the whole plant and cellular levels may relate to the influence of light on nitrogen metabolism. In leaf cells, the reductant and ATP for nitrate reduction and the subsequent assimilation of ammonium can be generated directly by the light reactions of photosynthesis. If photosynthesis is light-saturated, as is likely for pioneer species, then there will be sufficient reductant and ATP to support these reactions and those of CO_2 assimilation. If, however, light is limiting, as will be the case for understorey plants and for overstorey species early in their growth, nitrate and ammonium ions will compete with CO_2 for photochemical energy. Thus the spatial separation of both nitrate reduction, in the roots, and ammonium re-assimilation, in the cytosol, from CO_2 assimilation in the chloroplast provides a mechanism which allows control over the use of limited light between the assimilatory reactions of carbon and nitrogen metabolisms.

Acknowledgments

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