

Review

## NH<sub>4</sub><sup>+</sup> toxicity in higher plants: a critical review

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### Abstract

Ammonium (NH<sub>4</sub><sup>+</sup>) toxicity is an issue of global ecological and economic importance. In this review, we discuss the major themes of NH<sub>4</sub><sup>+</sup> toxicity, including the occurrence of NH<sub>4</sub><sup>+</sup> in the biosphere, response differences to NH<sub>4</sub><sup>+</sup> nutrition among wild and domesticated species, symptoms and proposed mechanisms underlying toxicity, and means by which it can be alleviated. Where possible, nitrate (NO<sub>3</sub><sup>-</sup>) nutrition is used as point of comparison. Particular emphasis is placed on issues of cellular pH, ionic balance, relationships with carbon biochemistry, and bioenergetics of primary NH<sub>4</sub><sup>+</sup> transport. Throughout, we attempt to identify areas that are controversial, and areas that are in need of further examination.

### I. Introduction

Ammonium (NH<sub>4</sub><sup>+</sup>) is a paradoxical nutrient ion in that, although it is a major nitrogen (N) source whose oxidation state eliminates the need for its reduction in the plant cell (Salsac et al. 1987), and although it is an intermediate in many metabolic reactions (Joy 1988), it can result in toxicity symptoms in many, if not all, plants when cultured on NH<sub>4</sub><sup>+</sup> as the exclusive N source (Vines and Wedding 1960, Givan 1979, van der Eerden 1982, Fangmeier et al. 1994, Gerendas et al. 1997). Observations of NH<sub>4</sub><sup>+</sup> toxicity to plants were made at least as early as 1882, when Charles Darwin described NH<sub>4</sub><sup>+</sup>-induced growth inhibition in *Euphorbia peplus* (cited in Schenk and Wehrmann 1979). Sensitivity to NH<sub>4</sub><sup>+</sup> may be a

universal biological phenomenon, as it has also been observed in many animal systems (Petit et al. 1990, Kosenko et al. 1991, 1995, Tremblay and Bradley 1992, Gardner et al. 1994), including humans, where it has been implicated in particular in neurological disorders (Marcaida et al. 1992, Mirabet et al. 1997, Butterworth 1998, Haghghat et al. 2000, Murthy et al. 2000), and also in insulin disorders (Sener and Malaisse 1980). Many research efforts have been directed toward unraveling the causes and mechanisms of NH<sub>4</sub><sup>+</sup> toxicity in plants, and while present knowledge is far from complete, a more comprehensive understanding of this phenomenon is beginning to emerge. This review will present key findings from this extensive body of work, with special focus on more recent developments in the field, and on nitrate (NO<sub>3</sub><sup>-</sup>) nutrition as a point of comparison. In addition, we offer clarification of central issues that have been clouded by speculation in the past, and identify several critical areas for further research.

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## II. Ecology of $\text{NH}_4^+$ toxicity

### 1. $\text{NH}_4^+$ in the biosphere

Nitrogen concentrations in soil solution can range over several orders of magnitude (Jackson and Caldwell 1993, Nerdoloy and Van Rees 1998). In many natural and agricultural ecosystems,  $\text{NH}_4^+$  is the predominant N source (Vitousek et al. 1982, Blew and Parkinson 1993, Pearson and Stewart 1993, van Cleve et al. 1993, Bijlsma et al. 2000), and is almost always present to some extent in the majority of ecosystems. For instance, a survey of boreal and temperate forest ecosystems shows forest-floor soil solution  $[\text{NH}_4^+]$  values ranging from approximately 0.4 to 4 mmol/L  $[\text{NH}_4^+]$ , with a mean value of 2 mmol/L (based on Vitousek et al. 1982, see also Bijlsma et al. 2000). In agricultural soils,  $[\text{NH}_4^+]$  can be even higher, often ranging from 2 to 20 mmol/L (Wolt 1994). The relative abundance of  $\text{NH}_4^+$  compared to  $\text{NO}_3^-$  in soil solution is determined by a number of factors, of which the accumulation of organic matter, soil pH, soil temperature, the presence of allelopathic chemicals, and soil oxygenation status are the most important (Rice and Panchoy 1972, Haynes and Goh 1978, Lodhi 1978, Dijk and Eck 1995). Typically, low pH, low temperature, accumulation of phenolic-based allelopathic compounds, and poor oxygen supply inhibit many nitrifying microorganisms (cf. Stark and Hart 1997), resulting in higher rates of net ammonification than net nitrification (Vitousek et al. 1982, Gosz and White 1986, Olf et al. 1993, Eviner and Chapin 1997). Soils exhibiting these conditions tend to be later-successional, while  $\text{NO}_3^-$ -rich soils tend to be early-successional (Smith et al. 1968, Rice and Panchoy 1972, Lodhi 1978, Klingensmith and van Cleve 1993).

Human intervention in the nitrogen cycle is presently adding more reduced nitrogen to the biosphere as the result of intensive agricultural activities, which can lead to runoff from fields and deposition via the atmosphere (Vitousek 1994, Vitousek et al. 1997, Bobbink 1998, Bobbink et al. 1998, Valiela et al. 2000). Deposition of ammonium that has been transported long distances can be significant, and N input has more than doubled since the 1950s in many areas, especially in Europe (Pearson and Stewart 1993, Falkengren-Grerup and Lakkenborg-Kristensen 1994, Bobbink 1998, Bobbink et al. 1998, Goulding et al. 1998). Moreover, it has been estimated that human-related N fixation has actually exceeded that from combined natural sources (Vitousek 1994). This additional N input has led to the N saturation of many natural ecosystems and has affected species composition; in at least one case, a local species extinction was documented as a consequence of increased  $\text{NH}_4^+$  deposition (de Graaf et al. 1998), while phenomena as important as large-scale forest decline have been linked to anthropogenic  $\text{NH}_4^+$  input and associated soil acidification (van Breemen et al. 1982, Nihlgard 1985, van Dam et al. 1986, van Dijk and Roelofs 1988, van Dijk et al. 1989, 1990). By contrast, it is interesting to note that, when the bulk of the nitrogen deposited is as  $\text{NO}_3^-$  rather than  $\text{NH}_4^+$ ,

forest expansion, rather than contraction, has been observed (Köchy and Wilson 2001). It is clear that  $\text{NH}_4^+$  toxicity is of increasing ecological importance, and deserves renewed attention.

### 2. Species response gradients

Ammonium toxicity may be universal, but the threshold at which symptoms of toxicity become manifested differs widely among plant species. Although varying experimental conditions used in different studies make a rigid classification of plants into tolerance groups difficult, some broad generalizations are possible. Domesticated plants most sensitive to  $\text{NH}_4^+$  toxicity (especially in terms of its effect on growth rates) include tomato (Claassen and Wilcox 1974, Magalhaes and Huber 1989, Feng and Barker 1992 a–d), potato (Cao and Tibbits 1998), barley (Lewis et al. 1986, Britto et al. 2001 b), pea (Claassen and Wilcox 1974, Bligny et al. 1997), bean (Chaillou et al. 1986, Zhu et al. 2000), castor bean (Allen and Smith 1986, van Beusichem et al. 1988), mustard (Mehrer and Mohr 1989, Vollbrecht et al. 1989), sugar beet (Harada et al. 1968, Breteler 1973), strawberry (Claassen and Lenz 1999), citrus species (Dou et al. 1999), marigold (Jeong and Lee 1992), and sage (Jeong and Lee 1992).  $\text{NH}_4^+$  becomes an increasingly predominant N source in the soils of many natural ecosystems as they go through the process of succession, and tree species which are  $\text{NH}_4^+$ -sensitive tend to be early-successional, including angiosperms such as poplars (Pearson and Stewart 1993), and gymnosperms such as Douglas-fir (Krajina et al. 1973, Gijsman 1990 a, b, Oltshoorn et al. 1991, de Visser and Keltjens 1993, Gorison et al. 1993, Min et al. 2000), Scots pine (Vollbrecht et al. 1989, Elmlinger and Mohr 1992), and western red cedar (Krajina et al. 1973). Wild herbaceous plants particularly sensitive to  $\text{NH}_4^+$  toxicity include *Arnica montana* and *Cirsium dissectum* (de Graaf et al. 1998), eelgrass (van Katwijk et al. 1997, Hauxwell 2001), and broomrape (Westwood and Foy 1999).

Plants that are the most highly adapted to  $\text{NH}_4^+$  as a nitrogen source include such domesticated species as rice (Harada et al. 1968, Sasakawa and Yamamoto 1978, Wang et al. 1993 a, b), blueberry and cranberry (Greadanu et al. 1972, Ingestad 1973, Peterson et al. 1988, Troelstra et al. 1995, Claassen and Lenz 1999), and onion and leek (Gerendas et al. 1997, cf. Abbes et al. 1995 for onion). Wild plants in this category include the heather *Calluna vulgaris* (de Graaf et al. 1998), the sedge *Carex* (Lee and Stewart 1978, Falkengren-Grerup 1995), many proteaceous plants (Smirnoff et al. 1984), some temperate angiosperm trees (e.g. oak, beech, hornbeam – Clough et al. 1989, Pearson and Stewart 1993, Truax et al. 1994, Rennenberg 1998, Rennenberg et al. 1998, Bijlsma et al. 2000; eucalypts – Garnett and Smethurst 1999, Warren et al. 2000, Garnett et al. 2001) and late-successional conifers (spruce species – Marschner et al. 1991, Kronzucker et al. 1997; hemlock – Krajina et al. 1973, Smirnoff et al. 1984).

Even species whose tolerance to  $\text{NH}_4^+$  nutrition is pronounced can suffer toxicity symptoms, given a high enough application of ammonium. For instance, rice plants can undergo leaf orangeing (Liao et al. 1994) and growth suppression (our unpublished results) under excessive  $\text{NH}_4^+$  regimes, particularly at low  $\text{K}^+$ , and their growth potential is not fully realized unless nitrate is co-provided with ammonium (see section IV). High  $\text{NH}_4^+$  deposition has also been implicated in the decline of some forests of red spruce, although this tree is considered to be highly adapted to  $\text{NH}_4^+$  as an N source (Holldampf and Barker 1993). Substantial variations in  $\text{NH}_4^+$  tolerance can also be seen amongst closely-related species (Monselise and Kost 1993), within species (Feng and Barker 1992a, Magalhaes et al. 1995, Schortemeyer et al. 1997), and at different developmental stages (Vollbrecht et al. 1989). Such differences, as well as differences in experimental systems (for instance,  $\text{NH}_4^+$  concentrations, pH regimes, supply of other nutrients, light intensity, temperature, and standards of comparison in terms of growth on other N sources and choice of contrasting species), have led to some apparent contradictions in the literature (compare, for instance, van den Driessche 1971 and Krajina et al. 1973, for conifers). While there is no perfect resolution of this question, some studies have managed to compare a large number of species within a consistent framework. Smirnov et al. (1984) used constitutive levels and inducibility of nitrate reductase as an indicator of N-source adaptation, identifying certain families as extreme nitrate specialists (Chenopodiaceae, Rosaceae, Urticaceae) and ammonium specialists (Ericaceae, Pinaceae, Proteaceae). Falkengren-Grerup (1995) classified 23 plant species into three tolerance groups, while in an approach using 276 parameter combinations ("species"), Bijlsma et al. (2000) identified five response categories based upon species' relative responses to  $\text{NO}_3^-$  and  $\text{NH}_4^+$ . From this and other studies, it emerges that certain plant families tend to be more tolerant or sensitive to  $\text{NH}_4^+$ ; these families are compiled tentatively, albeit not exhaustively, in Table 1. Notably

**Table 1.** Tentative assignment of plant families according to their tendency towards tolerance or sensitivity to  $\text{NH}_4^+$  toxicity. For details, see text.

$\text{NH}_4^+$ -tolerant	$\text{NH}_4^+$ -sensitive
Alliaceae	Solanaceae
Ericaceae	Cucurbitaceae
Pinaceae	Asteraceae
Fagaceae	Fabaceae
Cyperaceae	Chenopodiaceae
Proteaceae	Brassicaceae
Taxaceae	Salicaceae
Myrtaceae	Rosaceae
	Euphorbiaceae
	Urticaceae

members are highly variable in their N-source adaptation (Harada et al. 1968, Gigon and Rorison 1972, Sasakawa and Yamamoto 1978, Findenegg 1987, Magalhaes and Huber 1989, Adriaanse and Human 1993, Cramer and Lewis 1993, Falkengren-Grerup and Lakkenborg-Kristensen 1994, Falkengren-Grerup 1995, Gerendas and Sattelmacher 1995). Moreover, we hypothesize that a species' adaptation to the successional stage of an ecosystem, and thus N-speciation dominance in the native soil habitat (Vitousek et al. 1982), might be more important than family affiliation (see Kronzucker et al. 1997, Bijlsma et al. 2000).

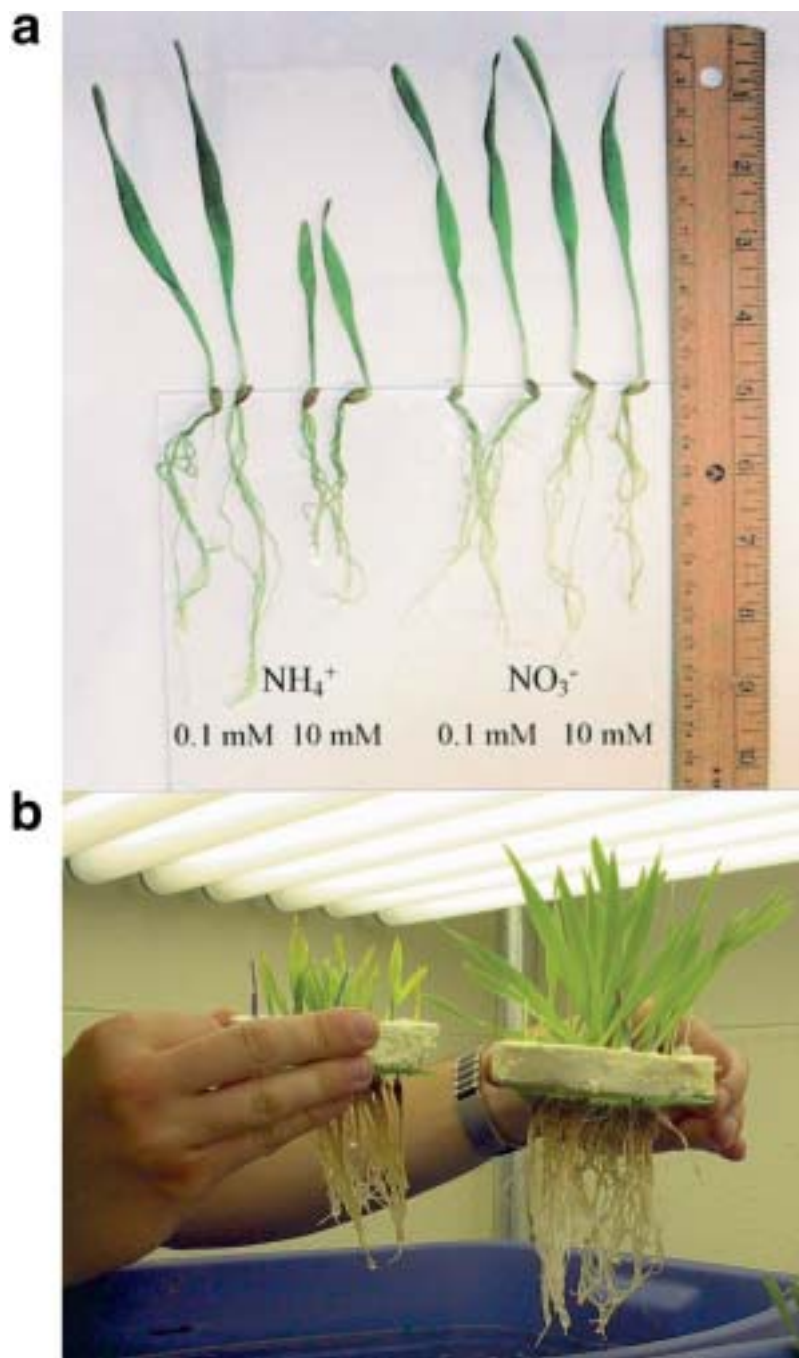
### III. Symptoms and proposed mechanisms of $\text{NH}_4^+$ toxicity

#### 1. Visual symptoms

The reported symptoms of  $\text{NH}_4^+$  toxicity range widely, and generally appear with external  $\text{NH}_4^+$  concentrations above 0.1 to 0.5 mmol/L (Schenk and Wehrmann 1979, Peckol and Rivers 1995, van Katwijk et al. 1997). Figure 1 shows, in the sensitive species barley, two of the most dramatic of these symptoms: the chlorosis of leaves, and the overall suppression of growth (Kirkby and Mengel 1967, Kirkby 1968, Gigon and Rorison 1972, Breteler 1973, Holldampf and Barker 1993, Gerendas et al. 1997). Yield depressions among sensitive species can range from 15 to 60% (Woolhouse and Hardwick 1966, Chaillou et al. 1986), and even death can result (Gigon and Rorison 1972, Magalhaes and Wilcox 1983a, b, 1984a, b, Pearson and Stewart 1993, de Graaf et al. 1998). Other visual symptoms often include a lowering of root:shoot ratios (Haynes and Goh 1978, Atkinson 1985, Blacquièrre et al. 1987, Boxman et al. 1991, Wang and Below 1996, Bauer and Berntson 1999), although the reverse effect has been observed for some species (Gigon and Rorison 1972, Troelstra et al. 1985). A decrease in the fine:coarse root ratio is also part of the syndrome (Haynes and Goh 1978, Boxman et al. 1991), but this can be accompanied by stimulation in root branching (Ganmore-Neumann and Kafkafi 1983). Symptoms not so readily visible, but equally important, can include a decline in mycorrhizal associations (Boxman et al. 1991, Lambert and Weidensaul 1991, van Breemen and van Dijk 1998, van der Eerden 1998, Boukcim et al. 2001, Hawkins and George 2001). Finally, seed germination and seedling establishment can be inhibited by  $\text{NH}_4^+$  toxicity (Cooke 1962, Hunter and Rosenau 1966, Megie et al. 1967, Barker et al. 1970, Westwood and Foy 1999), a feature of high ecological significance.

#### 2. Ionic balance and biochemical responses

Chemical changes in the plant induced by  $\text{NH}_4^+$  exposure include the well-documented total tissue depression, com-



**Figure 1. a**, 8-day-old seedlings of barley (*Hordeum vulgare* L. cv. «Klondike»), hydroponically cultured in ammonium (two pairs at left) or in nitrate (two pairs at right). Nitrogen concentrations in solution were as indicated.  $[K^+]$  in all solutions was 0.023 mmol/L. **b**, Barley seedlings cultured as in Figure 1, but only with ammonium, at a concentration of 10 mmol/L (left, held in researcher's right hand) or 0.1 mmol/L (right, held in researcher's left hand)  $NH_4^+$ . Note the leaf chlorosis and severe growth suppression in roots, and, especially, shoots at high ammonium concentrations.

pared to  $NO_3^-$ -fed plants, of essential cations such as potassium, calcium, and magnesium (Kirkby 1968, Salsac et al. 1987, van Beusichem et al. 1988, Boxman et al. 1991, Holl-dampf and Barker 1993, Troelstra et al. 1995, Gloser and Gloser 2000). This decline in cations other than  $NH_4^+$  is accompanied by an increase in tissue levels of inorganic anions such as chloride, sulfate and phosphate (Kirkby 1968, Cox and Reisenauer 1973, van Beusichem et al. 1988). In addition, tissue levels of non-amino dicarboxylic acids, such as malic acid, often decline in  $NH_4^+$ -grown plants, compared to plants

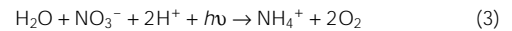
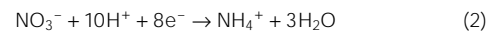
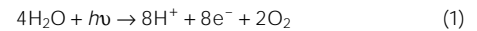
grown on  $NO_3^-$  (Kirkby 1968, Haynes and Goh 1978, Allen and Smith 1986, Allen and Raven 1987, van Beusichem et al. 1988, Goodchild and Givan 1990, Lepout et al. 1996), while amino acid concentrations increase (Margolis 1960, Harada et al. 1968, Kirkby 1968, Magalhaes and Wilcox 1984 a, b, Rosnitschek-Schimmel 1985, Chaillou et al. 1986, 1991, Allen and Raven 1987, Blacquièrre et al. 1988, Majerowicz et al. 2000). It is important to point out that almost no information is available on the intracellular localization of these changes in ion concentration (see Speer et al. 1994, Speer and Kaiser

1994), and much more work is necessary to resolve whether what is concluded from total tissue analyses also pertains to, in particular, the cytosolic compartment. Even large changes in total tissue contents, given the enormous capacity of the vacuole to sequester metabolites, including malate, and waste products (Martinoia et al. 1981, Martin 1987, Kaiser et al. 1989, Siebke et al. 1992, Heber et al. 1994, Yin et al. 1996a, Dietz et al. 1998, Oja et al. 1999, Blumwald 2000, Andreev 2001), may not have direct bearing on growth, fitness, and mortality. Until these questions are resolved, a causative role of such changes in the NH<sub>4</sub><sup>+</sup> toxicity syndrome will be difficult, if not impossible, to determine.

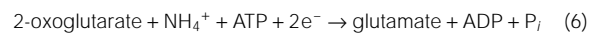
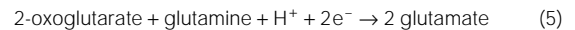
Although the uptake of many inorganic cations is reduced under NH<sub>4</sub><sup>+</sup> nutrition, the uptake of NH<sub>4</sub><sup>+</sup> itself is so high that NH<sub>4</sub><sup>+</sup>-fed plants generally take up an excess of cations relative to anions (Kirkby 1968, Clark 1982, van Beusichem et al. 1988). At the same time, NH<sub>4</sub><sup>+</sup>-fed plants normally acidify the external medium (Mevius and Engel 1931, Runge 1983, Findenegg 1987, Goodchild and Givan 1990, Schubert and Yan 1997), suggesting that proton efflux from the plant is one means of compensating for the charge imbalance. By contrast, NO<sub>3</sub><sup>-</sup>-fed plants cause a net alkalization of the medium (Dijkshoorn 1962, Runge 1983, Goodchild and Givan 1990, Schubert and Yan 1997), probably in response to the excess uptake, in this case, of anions relative to cations (however, for both N sources, differences in proton uptake and extrusion along the longitudinal root axis, and between the rhizoplane and bulk solution, demonstrate that the actual situation is considerably more complicated – see Henriksen et al. 1992, Taylor and Bloom 1998). Indeed, van Beusichem et al. (1988) showed that the cumulative number of protons excreted by *Ricinus communis* plants grown on NH<sub>4</sub><sup>+</sup> over 40 days closely approximated the excess cation uptake, while the “hydroxyl” ions excreted (not distinguishable from protons taken up; see below) under NO<sub>3</sub><sup>-</sup> provision approximated the excess anion uptake. The ammonium response, and the resulting acidification of the rhizosphere under both field and laboratory conditions, is often considered to be one fundamental cause of NH<sub>4</sub><sup>+</sup> toxicity, particularly since relief from toxicity symptoms has often been observed when growth solutions are pH-buffered (Gigon and Rorison 1972, Findenegg 1987, Vollbrecht and Kasemir 1992, Dijk and Eck 1995, Dijk and Grootjans 1998). However, in some cases the relief is only partial (Gigon and Rorison 1972, Breteler 1973), and in many other instances is absent (Kirkby 1968, Cox and Reisenauer 1973, Pill and Lambeth 1977, Blacquièrre et al. 1987, 1988, van Beusichem et al. 1988), so it is more likely that plants that benefit from pH-buffering are not suffering from NH<sub>4</sub><sup>+</sup>-toxicity *per se*, but rather from externally acidic conditions as a superimposed, but essentially separate, stress (see Goodchild and Givan 1990). Nevertheless, it appears that a prerequisite of NH<sub>4</sub><sup>+</sup>-tolerance is acid-tolerance, and therefore it is no coincidence that most, if not all, of the NH<sub>4</sub><sup>+</sup>-tolerant plants listed above are also acid-tolerant (see, for instance, Yan et al. 1992). This is not surprising,

given that NH<sub>4</sub><sup>+</sup>-rich soils are typically low in pH (Vitousek et al. 1982).

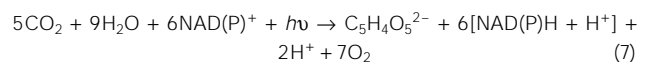
*Intracellular* pH disturbance has also been proposed to be a mechanism of NH<sub>4</sub><sup>+</sup> toxicity (Kosegarten et al. 1997; see also McQueen and Bailey 1991), but this possibility has been largely dismissed by studies using NMR and fluorescent dyes (Bligny et al. 1997, Kosegarten et al. 1997, Wilson et al. 1998, Gerendas and Ratcliffe 2000). However, because cellular nitrogen-pH relations in plants have long been clouded by incorrect and piecemeal speculation, this subject deserves a more detailed treatment. It has become a textbook argument (Salisbury and Ross 1992, Marschner 1995) that cytosolic pH must increase with nitrate feeding and decrease with ammonium feeding, unless buffered by a cellular pH-stat mechanism. In support of this argument, the two-step reduction of NO<sub>3</sub><sup>-</sup> to NH<sub>4</sub><sup>+</sup> (via nitrate and nitrite reductases) is usually cited, as it involves a transfer of 10 protons and 8 electrons. Because of this imbalance, nitrate reduction is a proton-consuming process overall. Starting with water, the ultimate source of both H<sup>+</sup> and e<sup>-</sup> (in the Hill reaction of photosynthesis – note that this applies to roots as well as shoots, in the long run), the two partial reactions for this redox transfer, and their sum, are as follows:



NH<sub>4</sub><sup>+</sup> assimilation, on the other hand, involves the release of protons (Kirkby 1968, Raven and Smith 1976, Smith and Raven 1979), although this release results neither from NH<sub>4</sub><sup>+</sup> acting as a weak acid (NH<sub>4</sub><sup>+</sup> → NH<sub>3</sub> + H<sup>+</sup>), nor from the primary assimilatory reaction sequence catalyzed by GS (4) and GOGAT (5) themselves, as can be seen when the partial reactions are summed:



While proton-neutral, however, this reaction sequence consumes two electrons (in reaction 5), which leads, again, to an imbalance between proton and electron consumption. Interestingly, however, in this case the proton/electron imbalance is the mirror image of that noted for reactions 1–3 in the reduction of NO<sub>3</sub><sup>-</sup> to NH<sub>4</sub><sup>+</sup>. Therefore, because NO<sub>3</sub><sup>-</sup> reduction is almost always coupled to NH<sub>4</sub><sup>+</sup> assimilation, NO<sub>3</sub><sup>-</sup> assimilation as outlined above is, overall, a pH-neutral process. This important conclusion is not usually drawn (cf. Gerendas and Ratcliffe 2000); nor is it usually considered that the production of each dicarboxylic carbon skeleton (2-oxoglutarate) for N assimilation involves the generation of two protons, as summarized in the following equation:



When C metabolism is included in the analysis, then, equations 3, 6, and 7 show that  $\text{NH}_4^+$  assimilation generates 4  $\text{H}^+$ , whereas  $\text{NO}_3^-$  assimilation generates 2  $\text{H}^+$ , and thus both processes impose a net acid load on the plant cell. Furthermore, it is crucial to this issue, but rarely considered, that in addition to purely biosynthetic processes, the primary transport of  $\text{NO}_3^-$  across the plasma membrane into the plant cell is mechanistically tied to a symport of 2 $\text{H}^+$  (McClure et al. 1990, Glass et al. 1992, Siddiqi and Glass 1993, Meharg and Blatt 1995, Mistrik and Ullrich 1996, Glass and Crawford 1998, Forde 2000), while the  $\text{NH}_4^+$  uptake mechanism is believed to occur by an electrogenic uniport (Raven and Farquhar 1981, Smith 1982, Ullrich et al. 1984, Wang et al. 1994, Howitt and Udvardi 2000, von Wirén et al. 2000, Cerezo et al. 2001). When the above primary transport and assimilation functions are summed, it emerges that the plant cell experiences an intracellular  $\text{H}^+$  appearance of 4 moles of  $\text{H}^+$  per mole of N taken up and assimilated, regardless of whether N is supplied as  $\text{NH}_4^+$  or  $\text{NO}_3^-$ . However, the analysis is further complicated by the intracellular buildup of  $\text{NO}_3^-$  or  $\text{NH}_4^+$  that has been transported but not metabolized; these pools magnify the contribution of proton fluxes associated with primary  $\text{NO}_3^-$  transport, but have no comparable effect with  $\text{NH}_4^+$  transport.

Another complication is the larger buildup of organic acids, especially malate, with  $\text{NO}_3^-$  as compared to  $\text{NH}_4^+$  nutrition (see above), although it has been suggested that, mechanistically, malate accumulation might respond to external pH rather than N source (Goodchild and Givan 1990). Malate production, however, further increases the  $\text{NO}_3^-$ -associated  $\text{H}^+$  load, rather than counteracting a presumed  $\text{OH}^-$ -load, as is commonly invoked in discussions of the role of malate as a biochemical "pH-stat". We propose an alternative explanation, that increased net malate synthesis during  $\text{NO}_3^-$  provision is driven by the greater need, relative to  $\text{NH}_4^+$  provision, for reduction equivalents in the root, rather than for pH balance. Interestingly, however, the synthesis of malate via PEP carboxylase, though not its accumulation, is often elevated under  $\text{NH}_4^+$  nutrition (Arnozis et al. 1988, Cramer and Lewis 1993, Lepoint et al. 1996), although this is not always the case (Goodchild and Givan 1990). It is likely that the increased PEP carboxylase activity serves an anapleurotic function in the provision of carbon skeletons for ammonium assimilation (Raab and Terry 1994).

The fact that  $\text{NO}_3^-$  is often reduced in the shoot illustrates that the resulting cellular acid burden, in the absence of the opportunity to offload protons to an external medium, poses no problem for the shoot in normally-functioning plant tissues, contrary to what is often stated (Kirkby 1968, Raven and Smith 1976, Salsac et al. 1987). The unloading of the proton burden imposed upon the cytosol by both nitrogen forms may be alternatively explained by biophysical pH stat mechanisms involving the pumping of  $\text{H}^+$  across the tonoplast and plasma membranes. The potency and rapidity of pH rectification effected by the tonoplast  $\text{H}^+$  ATPase is well established in the context of many other physiological phenomena (Siebke

et al. 1992, Heber et al. 1994, Yin et al. 1996 a, Dietz et al. 1998, Oja et al. 1999), and its significance in the context of  $\text{NH}_4^+$  toxicity should not be discounted. Moreover, the plasma-membrane  $\text{H}^+$  ATPase is well known to respond to both inorganic N sources (Troelstra et al. 1985, Siddiqi and Glass 1993, Yamashita et al. 1995, Venegoni et al. 1997).

In light of these considerations, changes in the amino acid or organic acid profiles of plants under  $\text{NH}_4^+$  nutrition, while loosely associated with  $\text{NH}_4^+$  supply (cf. Goodchild and Givan 1990) and even observed under conditions where  $\text{NH}_4^+$  does not suppress growth (van Beusichem et al. 1988, Chaillou et al. 1991), are unlikely to be directly related to the manifestation of the toxicity syndrome.

### 3. Energetics and primary $\text{NH}_4^+$ acquisition

Clearly, an understanding of ammonium toxicity in plants is contingent upon an understanding of the mechanisms of primary entry of  $\text{NH}_4^+$  into plant cells. An ongoing debate plaguing the discussion has been whether  $\text{NH}_4^+$  or its conjugate base,  $\text{NH}_3$  (ammonia), is the chemical species entering the plant from the external medium via the plasma membrane. There is no doubt that, under conditions of high external pH, close to the  $\text{pK}_a$  of  $\text{NH}_4^+$  ( $\sim 9.25$ ),  $\text{NH}_3$  can build up to concentrations large enough to facilitate its entry via passive diffusion (Yin et al. 1996 b, Kosegarten et al. 1997, Wilson et al. 1998, Gerendas and Ratcliffe 2000, Plieth et al. 2000), and the permeability coefficient for  $\text{NH}_3$  does appear to suggest that  $\text{NH}_3$  can readily penetrate some biological membranes (Kleiner 1981, Ritchie and Gibson 1987). This point of view appears additionally supported by the observation that a transient cytosolic alkalinization occurs with exposure of plant cells to ammonia/ammonium (Kosegarten et al. 1997, Wilson et al. 1998, Gerendas and Ratcliffe 2000; see also Mirabet et al. 1997 and Minelli et al. 2000 for similar analyses in animal tissues). We favor the alternative hypothesis that under normal external pH conditions, the plasma membrane  $\text{H}^+$ -ATPase immediately responds to  $\text{NH}_4^+$  exposure (see above). Furthermore, it is important to note that soils only rarely exhibit pH values at all close to the  $\text{pK}$  of  $\text{NH}_4^+$ , and indeed are frequently so low that  $\text{NH}_3$  is present in such small amounts that no appreciable flux into the plant could possibly be sustained (it should be noted that in marine ecosystems, with a  $\text{pH} > 8$ ,  $\text{NH}_3$  might be significant). Moreover, biological membranes *in situ* are undoubtedly more complex than simple lipid bilayer solubility and permeability models suggest. In the case of  $\text{NH}_4^+$ , this is dramatically illustrated in the lack of uncoupling of photophosphorylation in highly intact chloroplasts (Heber 1984, Kendall et al. 1986, Blackwell et al. 1988, Gerendas et al. 1997, Kandlbinder et al. 1997, Zhu et al. 2000; also see below). Indeed, it is fascinating to speculate what mechanisms plant membranes (especially the tonoplast) use to maintain sequestration, against often sizable gradients, of highly mobile, lipophilic materials whose tight compartmentation is critical to cell function. Incidentally, Raven and Farquhar (1981),

often incorrectly cited to support the idea that NH<sub>3</sub> is the principal membrane-permeating species, also conclude forcibly that NH<sub>4</sub><sup>+</sup>, and not NH<sub>3</sub>, is the membrane-permeating species. A second often-cited paper in this context (Kleiner 1981) in fact provides little evidence in favour of NH<sub>3</sub> penetration, presenting instead an equivocal case for fluxes across higher plant membranes; this uncertainty was due to the lack of experimental evidence available at the time. This lack has clearly been superseded by more recent work in the field; the preponderance of recent experimental evidence supports the notion that NH<sub>4</sub><sup>+</sup> is the principal chemical species traversing plant plasma membranes under most conditions (Walker et al. 1979 a, b, Smith 1982, Ullrich et al. 1984, Schlee and Komor 1986, Wang et al. 1993 b, 1994, Karasawa et al. 1994, Ninne-man et al. 1994, Ryan and Walker 1994, Herrmann and Felle 1995, Kronzucker et al. 1995 a, 1996, Nielsen and Schjoerring 1998, von Wirén et al. 2000, Britto et al. 2001 a, b, Cerezo et al. 2001), and that cytosolic accumulation of NH<sub>4</sub><sup>+</sup>, as measured by at least three different techniques (NMR, compartmental analysis, and micro-electrodes; see Lee and Ratcliffe 1991, Wang et al. 1993 a, Wells and Miller 2000, respectively, for examples of each), is substantial enough to indicate that loss of NH<sub>4</sub><sup>+</sup> via simple diffusion of NH<sub>3</sub> is not significantly high.

The low NH<sub>3</sub>-permeability of the plasma membrane is further substantiated by the observation that dramatic increases in the inwardly-directed NH<sub>3</sub> gradient are accompanied by even higher increases in the NH<sub>3</sub>/NH<sub>4</sub><sup>+</sup> flux in the opposite direction (i.e. efflux to the external medium); for example, Kronzucker et al. (1995 a) showed a 8-fold reduction in the gradient accompanied by a 105-fold increase in efflux. Clearly, this runs against the idea that NH<sub>3</sub> permeation plays a significant role in trans-plasma-membrane N fluxes under normal conditions. There has been some debate about the exact magnitude of cytosolic [NH<sub>4</sub><sup>+</sup>], but most studies agree that it lies in the low to medium millimolar range (see Kronzucker et al. 1995 a, Britto et al. 2001 a, and references therein). This agreement is found in spite of uncertainties relating to cellular heterogeneity (Henriksen et al. 1992, Taylor and Bloom 1998) which affect all these methods, and which points to the need for system verification (Kronzucker et al. 1995 b). One exception to the agreement in the above estimates consists of a short communication which did not report NH<sub>4</sub><sup>+</sup> measurements *per se*, but rather used an indirect method of analyzing <sup>31</sup>P- and <sup>13</sup>C-NMR signals (Roberts and Pang 1992) to infer that cytosolic NH<sub>4</sub><sup>+</sup> was in the micromolar range (2–438 μmol/L). A more recent study found cytosolic NH<sub>4</sub><sup>+</sup> concentrations in barley and rice plants to be several hundred millimolar, at the exceptionally high external concentration of 10 mmol/L NH<sub>4</sub><sup>+</sup> (Britto et al. 2001 b). These cytosolic values, it should be noted, were found under conditions intended to provoke NH<sub>4</sub><sup>+</sup> toxicity, and although unusually high, were nevertheless at, or below, concentrations predicted by the Nernst equation (see below).

At toxic external concentrations of NH<sub>4</sub><sup>+</sup>, the transport system responsible for NH<sub>4</sub><sup>+</sup> uptake into the plant is a “low-affi-

nity transport system” (LATS) the activity of which, surprisingly, is apparently not downregulated (unlike the high-affinity transport system), but rather produces higher fluxes with increased nitrogen status of the plant (Wang et al. 1993 b, Min et al. 1999, Rawat et al. 1999, Cerezo et al. 2001). The reasons for this lack of regulation are yet to be resolved, but a plausible explanation involves the likelihood that LATS transport is mediated by constitutively-expressed channel-type transporters possibly identical or very similar to those whose normal function is potassium uptake into the plant (Sokolik and Yurin 1986, Vale et al. 1988, Schachtman et al. 1992, White 1996, Nielsen and Schjoerring 1998; see also Mironova 1996, Hagen et al. 2000 for similar instances in animal systems), or belonging to a family of transporters identified as “non-selective cation channels” (Davenport and Tester 2000, Kronzucker et al. 2001). Given that K<sup>+</sup> tissue concentrations are reduced significantly under high NH<sub>4</sub><sup>+</sup> provision (Kirkby 1968), it may not be surprising that potassium channels are overexpressed in response to what essentially amounts to a K<sup>+</sup> starvation condition; the unfortunate side-effect is that it allows even more uncontrolled influx of NH<sub>4</sub><sup>+</sup> (itself competing with, and inhibiting, potassium suppression) into the plant.

Perhaps for this reason, plants that are susceptible to NH<sub>4</sub><sup>+</sup> toxicity display extraordinarily high plasma membrane fluxes of NH<sub>4</sub><sup>+</sup> in both directions (Feng et al. 1994, Nielsen and Schjoerring 1998, Rawat et al. 1999, Min et al. 1999, Britto et al. 2001 b, Cerezo et al. 2001). Given that such fluxes can be well in excess of the NH<sub>4</sub><sup>+</sup>-assimilation capacity of the plant, either tissue accumulation of NH<sub>4</sub><sup>+</sup> (Hecht and Mohr 1990, Lang and Kaiser 1994, Wieneke and Roeb 1997, Husted et al. 2000), and/or increased efflux of NH<sub>4</sub><sup>+</sup> from the plant must ensue.

Taking into consideration plasma membrane electrical potentials, and the concentrations of NH<sub>4</sub><sup>+</sup> in the external medium and in the cytosol, a thermodynamic analysis reveals that under conditions of high NH<sub>4</sub><sup>+</sup> supply, known to induce toxicity, NH<sub>4</sub><sup>+</sup> transport into the plant is a passive process, while efflux of NH<sub>4</sub><sup>+</sup> from the cytosol to the external medium must be energetically active. Indeed, passive efflux transport could only occur if cytosolic NH<sub>4</sub><sup>+</sup> concentrations were to be much higher than measured by any technique to date (e.g. at an external concentration of 10 mmol/L, a realistic membrane potential of –120 mV would require a minimum, but unlikely, cytosolic concentration of 1 mol/L in order for passive efflux to occur). Although there is a debate about cytosolic concentrations of NH<sub>4</sub><sup>+</sup> (which need to be distinguished from vacuolar NH<sub>4</sub><sup>+</sup> concentrations), and therefore about the magnitude of the gradient against which such active efflux transport must work, all studies with the exception of one (Roberts and Pang 1992) have shown that cytosolic [NH<sub>4</sub><sup>+</sup>] can be in the millimolar range (see Britto et al. 2001 a). Along with detection of substantial (millimolar) NH<sub>4</sub><sup>+</sup> in the xylem stream (van Beusichem et al. 1988, Schjoerring et al. 2002), studies of plant-atmosphere NH<sub>3</sub>/NH<sub>4</sub><sup>+</sup> exchange (Farquhar et al. 1980, Schjoerring et al. 2000), and the inescapability of large endogenous cellular NH<sub>4</sub><sup>+</sup> production associated with protein

turnover under virtually all growth conditions, including growth on nitrate (Blackwell et al. 1987, Jackson et al. 1993, Feng et al. 1998), such cellular measurements belie the widely-held notion that free ammonium does not accumulate in plant tissues (Kafkafi and Ganmore-Neumann 1997, Tobin and Yamaya 2001 – but cf. Husted et al. 2000). Using measured cytosolic  $\text{NH}_4^+$  concentrations and membrane potentials in barley, Kronzucker et al. (2001) showed that the active efflux process is highly inefficient, which helps explain the high respiratory rates commonly, but not always (de Visser and Lambers 1983, Cruz et al. 1993), measured with  $\text{NH}_4^+$  nutrition in many plants (Haynes and Goh 1978, Matsumoto and Tamura 1981, Barneix et al. 1984, Blacquièrre and de Visser 1984, Cramer and Lewis 1993, Rigano et al. 1996; see also Kosenko et al. 1991, Martinelle and Haggstrom 1993, Hagen et al. 2000, Hagighat et al. 2000 a, b for similar examples in animal systems), even when  $\text{NH}_4^+$  assimilation is blocked by the glutamine synthetase inhibitor methionine sulfoximine (Britto et al. 2001 b). Consistent with this respiratory increase is a decline in cellular ATP levels (Kosenko et al. 1991, Rigano et al. 1996, Hagen et al. 2000, Hagighat et al. 2000 a, b). However, this is not a necessary outcome (e.g. Lang and Kaiser 1994), as increased energy utilization can occur in plant cells without concomitant declines in ATP or ATP/ADP ratios (Yan et al. 1992).

Based on the root respiratory increase with  $\text{NH}_4^+$  nutrition, and the decrease in root:shoot ratio, some workers have suggested that an excessively high carbon sink strength in root tissues, where most  $\text{NH}_4^+$  metabolism takes place (Schortemeyer et al. 1997, see Kronzucker et al. 1998 for additional references), is in part responsible for ammonium toxicity. Indeed, sugar and starch content of plants generally decrease with ammonium treatment (Kirkby 1968, Matsumoto et al. 1971, Breteler 1973, Lindt and Feller 1987, Lewis et al. 1989, Magalhaes and Huber 1989, Mehrer and Mohr 1989, Kubin and Melzer 1996), although some exceptions have been observed (Blacquièrre et al. 1987, Lang and Kaiser 1994). Contrarily, it has been suggested that tolerance to  $\text{NH}_4^+$  might be directly related to the capacity of the root glutamine synthetase/glutamate synthase (GS-GOGAT) enzyme system to assimilate  $\text{NH}_4^+$ , based on the assumption that free  $\text{NH}_4^+$  in the plant is itself toxic (Givan 1979, Magalhaes and Huber 1989, Monselise and Kost 1993, Fangmeier et al. 1994, Tobin and Yamaya 2001). However, it must be pointed out that even rice, an exceptionally  $\text{NH}_4^+$  – tolerant species with a very high GS capacity (Magalhaes and Huber 1989), can accumulate substantial amounts of free  $\text{NH}_4^+$  in the cytosol and vacuole, even at modest external concentrations (Wang et al. 1993 a, Kronzucker et al. 1999 a, Britto et al. 2001 b). These findings cast doubt on both the root-carbon-sink hypothesis, and the metabolic-detoxification hypothesis. Clearly,  $\text{NH}_4^+$  *per se* in the plant cell is not necessarily toxic, and carbon supply for root growth under  $\text{NH}_4^+$  nutrition is likely to be limiting only when capacity of the shoot to deliver photoassimilate via the phloem is impaired, and/or under conditions of

excessive root respiration, that does not contribute to growth or maintenance (but rather to wasteful processes such as futile transmembrane  $\text{NH}_4^+$  cycling – see Britto et al. 2001 b), does not occur.

It is noteworthy that ammonium toxicity is frequently more pronounced at high light intensity (Goyal et al. 1982 a, b, Magalhaes and Wilcox 1983 a, 1984 a, Zornoza et al. 1987, Zhu et al. 2000, Bendixen et al. 2001). At first glance, this observation may appear to contradict the idea that increased carbon demand in the roots plays a role in  $\text{NH}_4^+$  toxicity, as the expectation might be that increased photosynthetic activity at higher light intensities could supply more carbon to the root. Indeed, it may be that the light optimum under  $\text{NH}_4^+$  (relative to  $\text{NO}_3^-$ ) nutrition is shifted to a higher intensity, to compensate for increased carbon utilization for respiration and amino acid production (a subject worthy of further study; see Givan 1979 and references therein; also see below for a discussion of root energy demands associated with  $\text{NH}_4^+$  nutrition). However, as in the case of plants suffering toxicity in a medium that is not pH-buffered, negative high-light effects are most likely to be an instance of the consequences of superimposed stresses. What is important here is that, in addition to the events occurring at the root level, plants susceptible to  $\text{NH}_4^+$  toxicity typically are afflicted by reduced rates of net photosynthesis (Takács and Técsi 1992, Claussen and Lenz 1999, cf. Raab and Terry 1994). More specifically, the decline in  $\text{CO}_2$  fixation (Puritch and Barker 1967, Ikeda and Yamada 1981, Mehrer and Mohr 1989) has been attributed to a decline in rubisco and NADP-dependent glyceraldehyde-3-phosphate dehydrogenase (Mehrer and Mohr 1989), impaired NADP reduction (Vernon and Zang 1960) or changes in chloroplast ultrastructure (Takács and Técsi 1992, Dou et al. 1999). It is important to reiterate here that uncoupling of plastidic energy gradients by  $\text{NH}_3$ , sometimes cited as the fundamental cause of  $\text{NH}_4^+$  toxicity, although demonstrated in early experiments with isolated chloroplasts (Krogmann et al. 1959, Puritch and Barker 1967, Crofts 1967, Izawa and Good 1972, Krause et al. 1982) has no basis in intact or suitably isolated systems (Heber 1984, Kendall et al. 1986, Blackwell et al. 1987, 1988, Gerendas et al. 1997, Kandlbinder et al. 1997, Zhu et al. 2000, Bendixen et al. 2001, our unpublished results).

In recent studies Zhu et al. (2000) and Bendixen et al. (2001) examined the possibility of direct effects of  $\text{NH}_4^+$  upon the photosystems of *Phaseolus vulgaris*. Somewhat surprisingly, chlorophyll fluorescence analysis revealed no significant differences in energy quenching ( $q_E$ ) or photoinhibition (as manifest in  $F_v/F_M$  ratios) between  $\text{NO}_3^-$ - and  $\text{NH}_4^+$ -grown plants (cf. Vanselow 1993, who did observe such differences in *Dunaliella*). However, significant depression in the ability of  $\text{NH}_4^+$ -grown plants to engage the violaxanthin-zeaxanthin cycle for photoprotection was observed (Bendixen et al. 2001), an effect due to the decline in ascorbate consistent with lower reduced carbon availability (see above), and with increased uronic acid levels (Kirkby 1968). Despite lack of fluorescence data to support changes in electron flow between PSII and



PSI, the observation by Zhu et al. (2000) that NH<sub>4</sub><sup>+</sup> increased the reduction of molecular oxygen in the Mehler reaction indicates that such an impairment might have nevertheless occurred. This possibility is further supported by other studies in which an increased export of redox equivalents under NO<sub>3</sub><sup>-</sup>-feeding indicated a more efficient photosynthetic electron flow (Backhausen et al. 1994, Krömer 1995, Noctor and Foyer 1998). Zhu et al. (2000) observed increased lipid peroxidation, an important consequence of enhanced Mehler reaction activity with NH<sub>4</sub><sup>+</sup>. Interestingly, the Mehler reaction also appears to be favored by magnesium and potassium deficiencies (Cakmak and Marschner 1992, Polle et al. 1992, Cakmak 1994), conditions which are associated with NH<sub>4</sub><sup>+</sup> nutrition (see section III-2 above). It must be pointed out that the alleviation of overreduced photosystems via the Mehler reaction is insufficient to lend full protection against photoinactivation (Wiese et al. 1998) and, therefore, alternative means of photoprotection, especially in the absence of the zeaxanthin component, must be operating to maintain energy quenching, at least in the short term. In the absence of such mechanisms, photorespiration is a possible means of alleviating light stress (Heber et al. 1996), and indeed enhanced photorespiratory rates have been observed with NH<sub>4</sub><sup>+</sup> nutrition (Zhu et al. 2000). In the long term, a connection between the NH<sub>4</sub><sup>+</sup> - induced growth suppression at high light, and enhanced damage to the photosynthetic centers themselves, is very plausible.

#### 4. Hormonal balance

Ammonium-induced changes in growth and development are undoubtedly linked to alterations in hormonal balance, but there is much contradictory evidence in the literature regarding this, and it is important to point out here that, other than in the case of ethylene (see below), no explanations of NH<sub>4</sub><sup>+</sup> toxicity have been forthcoming from such studies. In the case of a recent review (Gerendas et al. 1997), a string of arguments, mostly speculative, were presented to link increased auxin transport to the roots with increased cytokinin production in roots. It was suggested that more prolific root branching results from the increased strength of the root tissue as a carbon sink under NH<sub>4</sub><sup>+</sup> nutrition, which would facilitate more auxin delivery to the root (Ziegler 1975, Torrey 1976, Sattelmacher and Thoms 1995). The increased number of root tips, which has been often observed, could then lead to increased production of cytokinins in ammonium-grown plants, and in turn, could shift root : shoot ratios in favor of increased shoot growth (Gerendas et al. 1997). However, there is little evidence to support the notion of increased cytokinin production under NH<sub>4</sub><sup>+</sup> provision conditions. In fact, the highest levels of cytokinins are observed on NO<sub>3</sub><sup>-</sup>/NH<sub>4</sub><sup>+</sup> mixtures, not on NH<sub>4</sub><sup>+</sup> alone (Singh et al. 1992, Smiciklas and Below 1992, Wang and Below 1996, Chen et al. 1998, Walch-Liu et al. 2000), with a specific role for induction by NO<sub>3</sub><sup>-</sup> having been invoked in cytokinin synthesis (Samuelson and Larsson 1993, Sakaki-

bara et al. 1998). Moreover, ammonium feeding, in at least one case, has been shown to lead to a suppression of root auxin content (Kudoyarova et al. 1997).

In a series of studies with tomato, A. V. Barker and co-workers investigated the role of ethylene in the development of the NH<sub>4</sub><sup>+</sup> toxicity syndrome (Feng and Barker 1992 a-d, Barker and Corey 1991, Barker 1999 a, b). Ethylene production is a more or less universal response to physiological stresses in plants, to the extent that it is often used as a plant stress indicator (Barker 1999 a, b), but in these studies a more specific role in ammonium toxicity was implicated. Ethylene evolution from leaf tissue was shown to increase linearly with tissue ammonium content once a threshold value of 0.2 mg NH<sub>4</sub><sup>+</sup>-N g<sup>-1</sup> (fresh wt.) was reached (Barker 1999 a), regardless of external pH. Importantly, it was further shown that ammonium accumulation preceded ethylene evolution (Barker 1999 b). Ammonium accumulation was high enough under urea feeding to trigger ethylene evolution, while nitrate nutrition increased ammonium accumulation only slightly, and did not trigger ethylene evolution (Feng and Barker 1992 c). The application of amino-oxyacetic acid (although problematic as it is also an aminotransferase inhibitor – Oaks 1994) and silver thiosulfate, inhibitors of ethylene synthesis and action, ameliorated symptoms of ammonium toxicity (Barker and Corey 1991, Feng and Barker 1992 b, d). Clearly, the role of ethylene in NH<sub>4</sub><sup>+</sup> toxicity deserves further attention.

#### IV. Alleviation of NH<sub>4</sub><sup>+</sup> toxicity

As mentioned above (section III.2), NH<sub>4</sub><sup>+</sup> toxicity can be alleviated in certain cases by buffering external pH such that the acidification of the rhizosphere associated with ammonium uptake is counteracted. Maintaining neutral to slightly alkaline pH can also prevent the precipitous fall in cellular malate typically associated with provision of ammonium (Goodchild and Givan 1990). In addition, optimization of light regimes so as to avoid high light effects (section III.3) is more critical with ammonium-grown plants than with plants grown with nitrate or organic N. It is also very important to maintain high levels, in nutrient solutions, of cations known to be depressed in plant tissue when NH<sub>4</sub><sup>+</sup> is used as a sole N source (section III.2). In particular, the supply levels of K<sup>+</sup> have been shown to alleviate toxicity both in solution culture experiments and in the field (Barker et al. 1967, Lips et al. 1990, Zhang et al. 1990, Feng and Barker 1992 a, Barker 1995). At present, it is not known whether the normally homeostatically-controlled cytosolic concentrations of potassium, or only the vacuolar pools (Walker et al. 1996, and references therein), are affected by high NH<sub>4</sub><sup>+</sup> supply. Our preliminary results (unpublished) suggest that in NH<sub>4</sub><sup>+</sup>-sensitive species such cytosolic displacement does indeed occur. In the case of calcium, it is interesting to speculate whether the much-depressed vacuolar (and possibly other intracellular) pools of this universal signaling ion (Berridge 1997), under NH<sub>4</sub><sup>+</sup> nutrition, could result in a

dampening of the amplitude of  $\text{Ca}^{2+}$ -spike responses to various stimuli, as a result of diminished gradients.

One of the most fascinating aspects of  $\text{NH}_4^+$  nutrition is that, while toxicity is observed in many species when  $\text{NH}_4^+$  is provided alone, it can be alleviated by co-provision of nitrate (Goyal et al. 1982 a, b, Below and Gentry 1987, Deignan and Lewis 1988, Hecht and Mohr 1990, Feng and Barker 1992 a, c, Adriaanse and Human 1993, Cruz et al. 1993, Gill and Reisenauer 1993, Schortemeyer et al. 1997). Furthermore, co-provision induces a synergistic growth response that can surpass maximal growth rates on either N-source alone by as much as 40 to 70 % in solution culture (Weissman 1964, Cox and Reisenauer 1973, Heberer and Below 1989), though by somewhat less in soil (Hagin et al. 1990, Gill and Reisenauer 1993). Interestingly, the synergistic response is observed even in species such as conifers, where nitrate uptake is very small (van den Driessche 1971, van den Driessche and Dangerfield 1978, Kronzucker et al. 1997). However, in a few cases, such as some Ericaceae plants, a synergistic response is absent, and some plants even experience growth inhibition on nitrate (Dijk and Eck 1995). Several proposals have been put forth which attempt to explain the phenomenon of nitrate-ammonium synergism. Pivotal to many of these is the possible role of nitrate as a signal that stimulates (or optimizes) a multitude of biochemical responses (Stitt and Krapp 1999, Tischner 2000). One possibility is that cytokinin synthesis is maximized when  $\text{NO}_3^-$  and  $\text{NH}_4^+$  are provided together (Smiciklas and Below 1992, Chen et al. 1998; also see section III.4). Another is that the rhizospheric alkalization effect of nitrate uptake by plants may help to limit the acidification associated with  $\text{NH}_4^+$  nutrition (Imsande 1986, Marschner 1995, also see section III.2). However, this effect can at best be partial or require very high  $\text{NO}_3^- : \text{NH}_4^+$  ratios in the nutrient solution, because  $\text{NO}_3^-$  uptake is significantly inhibited, often by as much as 50 %, by ammonium (Kronzucker et al. 1999 a, b, and references therein), while  $\text{NH}_4^+$  uptake can be moderately stimulated by nitrate (Rideout et al. 1994, Saravitz et al. 1994, Kronzucker et al. 1999 a). Given that nitrogen efflux is also substantially lowered with co-provision, the net result of the plant's use of the two separate N sources together is that total N uptake can be significantly (up to 75 %) higher than with the same N concentration presented in the form of either N source alone (Kronzucker et al. 1999 a).

An interesting aspect of this analysis is that, at least in rice, a 50/50 mixture of  $\text{NO}_3^-$  and  $\text{NH}_4^+$  results in a more or less equal concentration of  $\text{NO}_3^-$  and  $\text{NH}_4^+$  in the cytosol of root cells (Kronzucker et al. 1999 a), attenuating the requirement for charge balancing of either N source, at least in the cytosol. Possibly the most important synergistic response of co-provision of  $\text{NO}_3^-$  and  $\text{NH}_4^+$  lies in the enhanced transport of nitrogen to the shoot. This is an issue of high agronomic importance, since nitrogen stored in shoot tissue can be remobilized during the critical period of grain-filling and fruit development, when N-delivery via roots can become impaired due to the onset of senescence (Mae et al. 1985). A significant

proportion of the xylem N flux is unmetabolized  $\text{NO}_3^-$ , while the remainder consists mostly of products of ammonium assimilation (Kronzucker et al. 1999 a). Enhanced root assimilation in the presence of nitrate is supported by several studies (Goyal et al. 1982 b, Ota and Yamamoto 1989), and can be mechanistically explained by the induction by nitrate of the GS-GOGAT pathway specifically localized in the proplastids of roots (Redinbaugh and Campbell 1993), opening up a pathway not available to ammonium assimilation in the absence of nitrate. In addition to these dramatic effects, the presence of nitrate may help to alleviate  $\text{NH}_4^+$  toxicity through its ability to be reduced in the shoot, moderating the differential carbon drain between roots and shoots, and improving electron flow between photosystems I and II (section III.3). Obviously, the synergistic response to co-provision of  $\text{NH}_4^+$  and  $\text{NO}_3^-$ , in addition to providing a promising avenue for agronomic improvements, has also yielded insights into the mechanisms of ammonium toxicity, and is an area in need of further exploration.

## V. Conclusions

The suppression of growth and yield in  $\text{NH}_4^+$ -sensitive species can be severe, and for this reason  $\text{NH}_4^+$  toxicity is of major importance in agricultural and ecological settings. Certain plant species, and even families, are particularly sensitive to, or tolerant of,  $\text{NH}_4^+$  as the sole nitrogen source. However, the symptoms of, mechanisms underlying, and means of alleviating, ammonium toxicity, are diverse. Explanations of the mechanisms underlying  $\text{NH}_4^+$  toxicity have been hampered by numerous misconceptions regarding this subject, and many often-cited possibilities have more recently been shown to be at best insufficient, partial explanations, or even incorrect. These latter include the uncoupling of photophosphorylation by  $\text{NH}_4^+$  *in planta*; the effects of external pH declines resulting from  $\text{NH}_4^+$  acquisition; the role of biochemical pH-stat mechanisms in cells accounting for differences in the internal  $\text{H}^+$  balance associated with differences in  $\text{NH}_4^+$  and  $\text{NO}_3^-$  metabolism; the accumulation *per se* of free  $\text{NH}_4^+$  in plant tissues (including, specifically, the cytosol); and the higher root carbon allocation to amino acid synthesis under  $\text{NH}_4^+$  nutrition. More plausible explanations include the involvement of ethylene synthesis and action as a key plant response to  $\text{NH}_4^+$  stress; the role of  $\text{NH}_4^+$  membrane flux processes, particularly the energy-demanding active efflux of cytosolic  $\text{NH}_4^+$ ; photosynthetic effects, particularly with respect to photoprotection; and displacement of essential cation concentrations from homeostatic set points in subcellular compartments. These possibilities deserve more research attention. In addition, much could be learned about ammonium toxicity mechanisms by examining its alleviation through various means, particularly through the co-presence of nitrate.

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