Uptake of ammonium and nitrate ions from acid mist applied to Sitka spruce \([Picea sitchensis (Bong.) Carr.]\) grafts over the course of one growing season

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Abstract Cloned Sitka spruce grafts were subjected to \(\text{NH}_4\text{NO}_3\) mist (1 mm day\(^{-1}\) equivalent) acidified with \(\text{H}_2\text{SO}_4\), for 4 days a week, from April until November. Three N treatments at pH 5.0 used 0.01 mol m\(^{-3}\) \(15\text{NH}_4\) \(15\text{NO}_3\), 1.6 mol m\(^{-3}\) \(15\text{NH}_4\ \text{NO}_3\) or 1.6 mol m\(^{-3}\) \(\text{NH}_4\ \text{NO}_3\). At pH 2.5, the treatments were \(15\text{NH}_4\ \text{NO}_3\), \(15\text{NH}_4\) \(\text{NO}_3\) and \(\text{NH}_4\ \text{NO}_3\) all at 1.6 mol m\(^{-3}\). At the end of the growing season, \(15\text{N}\) was found in every part of the trees, even when \(\text{NH}_4\text{NO}_3\) was supplied at 0.01 mol m\(^{-3}\). It was shown that both \(\text{NH}_4^+\) and \(\text{NO}_3^-\) could enter needles to a similar degree and be transported to the roots. No differences between pH treatments or clones could be demonstrated. Entry of labelled N via the roots, in those trees with unprotected compost, was reflected in higher fine root \(15\text{N}\) content, but no change in shoot \(15\text{N}\) levels. Per cent incorporation expressed as labelled N as a percentage of total N, was generally at its highest in bark, and in older needles in all treatments, values ranging from 5 to 11%. When \(15\text{N}\) content was expressed as total content for each tree part on a per tree basis, 25–36% was found in current year needles, with a further 12% in current year bark. The most reproducible data was that for dry weight of tree parts as per cent of the whole tree, where proportions compared closely between treatments and the two clones used. The implications of the results for cuticular transport mechanisms, N storage and internal cycling are discussed.

Key words Wet N inputs • Labelled N • N partitioning • N accumulation • Per cent incorporation

Introduction

Atmospheric inputs of nitrogen have been identified as a major cause of conifer decline in Europe and North America. N inputs to acid soils can lead to growth stimulation and an imbalance between N and cations, resulting in decline symptoms and these effects may be exacerbated by further uptake of atmospheric nitrogen via the canopy (Schulze 1989). These considerations have led to many studies designed to establish and measure the entry of wet deposited ammonium and nitrate into leaves and bark. Early work was based on comparing throughfall and stemflow analyses with those for precipitation (reviewed by Parker 1983) and using the results to estimate the net effects of canopy exchange. This method suffers from a number of inaccuracies enumerated by Wilson (1992) and by Macklon and Armstrong (1994). More recently work has turned to short-term studies of ion fluxes in and out of individual leaves and needles treated with artificial rain water (Evans et al. 1986; Bowden et al. 1989; Klemm 1989; Garten and Hanson 1990; Katz 1991; Macklon and Armstrong 1994). The consensus from these experiments was that \(\text{NH}_4^+\) ions can enter leaves more readily than \(\text{NO}_3^-\) ions, specifically in \(Picea abies\) (L.) Karst and \(Pinus sylvestris\) L. (Katz 1991; Wilson 1992). In Sitka spruce \([Picea sitchensis (Bong.) Carr.]\) needles, observations suggest that \(\text{NO}_3^-\) can be taken up from \(\text{HNO}_3\) (Skiba et al. 1986) and \(\text{NH}_4\text{NO}_3\) (Bowden et al. 1989).

Presently we are unaware of any studies which have tried to combine the science associated with short term flux studies with realistic long term exposures. Such work is necessary if we are to address some of the unresolved questions. This study was set up to (1) quantify the role of foliage in the uptake of \(\text{NO}_3^-\) and \(\text{NH}_4^+\) ions for Sitka spruce, (2) show the extent of distribution of foliage absorbed N within the tree, (3) show whether the pH or ion concentration influences uptake or distribution. \(15\text{N}\) labelled solutions were applied to mature grafts of Sitka spruce growing either in a glasshouse or open-top chamber over the course of a growing season.
Table 1 Description of the ion labelling and concentrations of ammonium, nitrate and H\textsubscript{2}SO\textsubscript{4} in acid mist solutions, used in the glasshouse (GH) and open-top chambers (OTC).

<table>
<thead>
<tr>
<th>pH and Location</th>
<th>Ion concentration (mol m\textsuperscript{-3})</th>
<th>Labelled ion</th>
<th>\textsuperscript{15}N atom %</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH\textsubscript{4}\textsuperscript{+}</td>
<td>NO\textsubscript{3}\textsuperscript{−}</td>
<td>H\textsubscript{2}SO\textsubscript{4}</td>
<td></td>
</tr>
<tr>
<td>2.5, GH</td>
<td>1.6</td>
<td>1.6</td>
<td>1.6</td>
</tr>
<tr>
<td>5.0, GH</td>
<td>1.6</td>
<td>1.6</td>
<td>1.6</td>
</tr>
<tr>
<td>2.5, OTC</td>
<td>1.6</td>
<td>1.6</td>
<td>1.6</td>
</tr>
<tr>
<td>5.0, OTC</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
</tr>
</tbody>
</table>

\textsuperscript{a} For OTCs, pH 5.0 mist solution, of 0.01 mol m\textsuperscript{-3} NH\textsubscript{4}NO\textsubscript{3}, was achieved by dilution of pH 2.5 solution. For GH, a separate solution of NH\textsubscript{4}NO\textsubscript{3} was prepared and the pH adjusted to 5.0 with H\textsubscript{2}SO\textsubscript{4}.

Materials and methods

Plant material

In January 1989, terminal shoots from two clones of Sitka spruce, clones 141 and DF (Sheppard et al. 1994), were taken from ortems (mother trees growing in a clone bank) and grafted onto 1-year-old open pollinated seedlings. In the autumn of 1989, they were repotted into 7 l black polythene liners (70:20:10 peat:grit:loam), each fertilised with 7.0 g Osmocote (18% N, 6% P, 11% K) and placed outside until transfer to open-top chambers (OTCs) at the Institute of Terrestrial Ecology, Penicuik, Midlothian and a well ventilated glasshouse (GH) at the Macaulay Land Use Research Institute, Aberdeen, in April 1991.

Misting solutions

The solutions used for misting were made up from equimolar mixtures of ammonium nitrate and sulphuric acid. For use in the OTC experiment, both NH\textsubscript{4}\textsuperscript{+} and NO\textsubscript{3}\textsuperscript{−} were labelled with \textsuperscript{15}N to give an enrichment of 5.18 atom per cent. For pH 2.5 treatments the solution contained 1.6 mol m\textsuperscript{-3} NH\textsubscript{4}NO\textsubscript{3}. For pH 5.0 treatment this solution was diluted to contain 0.01 mol m\textsuperscript{-3} NH\textsubscript{4}NO\textsubscript{3} (Table 1).

For use in the GH experiment, both solutions contained 1.6 mol m\textsuperscript{-3} NH\textsubscript{4}NO\textsubscript{3}, acidified to pH 2.5 or 5.0 (Table 1). Two solutions were prepared at each pH, one with only the NH\textsubscript{4}\textsuperscript{+} ion \textsuperscript{15}N labelled, and one with only the NO\textsubscript{3}\textsuperscript{−} ion labelled, both enriched to 49.5 atom per cent (to provide sufficient \textsuperscript{15}N for needle elution experiments, to be reported elsewhere).

The higher concentration of NH\textsubscript{4}NO\textsubscript{3} used matched those adopted for frost hardiness studies (Sheppard et al. 1994) and were equivalent to 29.1 kg N ha\textsuperscript{-1} year\textsuperscript{-1} at the application rate used. This is about three times the input of each ion to forests in Scotland (Crossley et al. 1992). A wide range of N concentrations have been found in fogwater over north-eastern Bavaria, where NO\textsubscript{3}\textsuperscript{−} ranged from 0.04 to 5.6 mol m\textsuperscript{-3} and NH\textsubscript{4}\textsuperscript{+} from 0.01 to 19.5 mol m\textsuperscript{-3} (Schulze 1989). The concentrations we have used are within this range.

Glasshouse experiment

Grafted trees (<1.3 m high) of clone 141 were organised at random into 4 groups of 4. Each group was placed within a polyethylene screen 0.75 m square and 1.3 m high, to contain the acid spray and prevent drift between treatments. Spraying was undertaken 4 times a week to provide 1 mm rainfall equivalent per application. Two groups of 4 trees received pH 5.0 mist and 2 groups pH 2.5 mist. Within each pH treatment, 1 group of 4 trees received \textsuperscript{15}NH\textsubscript{4}NO\textsubscript{3} and the other received NH\textsubscript{4}\textsuperscript{+}NO\textsubscript{3} labelled mist.

Within each group of 4 grafts, 3 grafts had their root systems and compost enclosed in clear polyethylene bags taped round the stem above the graft. Leakage down the stem was minimized by wrapping the stem with an absorbent wad of tissue. The outer bags were left open between Friday afternoon and Monday morning when they were closed using fresh wadding, after watering the compost. The remaining graft in each group was left unprotected with roots and compost exposed to the mist. Treatments were applied using hand-held pressure sprayers (Hozelock Poly Sprayer) adjusted to the finest droplet size. The volume of mist, calculated on the basis of floor area, took about 20 min to deliver to each group. Spraying commenced as the trees were bursting bud (29 April) and finished on 8 November after 28 weeks during which 112 mm precipitation equivalent had been applied. As controls, two additional trees received no spray treatment so that the foliage remained dry throughout the experiment. These trees were also watered weekly.

Open-top chambers experiment

Twentyfour grafted trees (approx. 1.6 m high) comprising 12 of clone DF and 12 of clone 141 were treated in four open-top chambers. Three trees of each clone were allocated to each chamber, of which two gave mist treatments at pH 5.0 and two at pH 2.5. Mist was applied for 26 weeks, between 11 May and 11 November 1991. The open-top chambers are described in detail by Fowler et al. (1989). They have a floor area of 7.0 m\textsuperscript{2} and are supplied with charcoal filtered air at a rate comprising 2 changes min\textsuperscript{-1} which maintains internal temperatures within 1 or 2 °C of ambient. Solutions were applied as a mist, droplet size 40 μm, via a spinning disk droplet generator. Each application took approximately 20 min, and was scheduled for early morning to avoid scorching effects. For irrigation, tap water was supplied to the capillary matting on which the grafts stood. Rain was excluded from the chambers using polyethylene roofs suspended above the frustum.

Destructive harvests

In the week following the cessation of spraying, three samples of compost were removed from around the roots of each tree for analysis of total N and \textsuperscript{15}N. Samples were dried to constant weight at 70 °C and ground.

The trees were gently hosed down with tap water (twice to remove superficially adhering NH\textsubscript{4}NO\textsubscript{3}). Stems and branches of individual trees from the GH experiment were divided into year classes, bagged and frozen at −20 °C until further sub-division could be undertaken. Sub-division involved removing buds from the stem leaders, and separating needles, bark and wood from the current year's growth (year 0) and from 1- and 2-year-old growth. Bark and wood were separated from the seedling rootstocks between the soil and graft. Roots were washed to remove compost and divided into tap, coarse and fine roots (<1 mm diameter). Individual component parts were oven dried, weighed and milled (<0.4 mm) for chemical analysis.

To expedite progress with sample preparation, trees from the OTC experiment were separated into component parts after oven drying. Stem and bark were separated only in three trees, one from clone DF pH 2.5 and two from clone 141 pH 5.0.

Analysis for total N and atom% \textsuperscript{15}N

Milled samples were well mixed and sampled for analysis of total N, and atom% \textsuperscript{15}N using an ANA-SIRA mass spectrometer (VG Isogas, Middlewich, Cheshire, UK) equipped with a Carlo-Erba pre-analysers. Values for whole trees were obtained by addition of N, \textsuperscript{15}N or dry weight in component parts.

Units of uptake and content derived from \textsuperscript{15}N labelled mist are expressed as % total N, or as milligram per tree part on a whole tree basis. Labelled N entering from the mist was calculated using the following equations:

\[
\text{Uptake}_{\text{mist}} = \frac{\text{Iod}_{\text{mist}} \times \text{N}_{\text{io}}}{\text{Iod}_{\text{N}}} \times 100
\]

\[
\text{Content}_{\text{mist}} = \frac{\text{Iod}_{\text{mist}} \times \text{N}_{\text{io}}}{\text{Iod}_{\text{N}}} \times \text{M}_{\text{io}} \times 100
\]