Reduced sulphur allocation from three-year-old needles of Norway spruce (Picea abies [Karst] L.)

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Abstract

The $^{35}$S-labelled sulphur compounds glutathione, cysteine, and $\gamma$-glutamylcysteine were fed to 6-year-old spruce trees via the cut surface of a single 3-year-old needle. After 1-3 h exposure, uptake of the radiotracer into the fed needle, export into other parts of the plant and distribution between needles, bark and wood along the transport path were analysed. Uptake of cysteine into the exposed needle was one order of magnitude and uptake of $\gamma$-glutamylcysteine two orders of magnitude lower than that of glutathione. Independent of the thiol applied, the current year’s sprouts were the preferential sinks of exported $^{35}$S. Transport towards basipetal parts of the twig amounted to less than 10% of total $^{35}$S export in all cases. After feeding $^{35}$S-cysteine and $^{35}$S-$\gamma$-glutamylcysteine, $^{35}$S-glutathione was found along the transport path, in particular in distant parts of the twig. This was also observed when $^{35}$S-GSH was fed. This result confirms the significance of glutathione as the major long-distance transport form of reduced sulphur in spruce twigs. In xylem sap of trunk sections of spruce, cysteine rather than glutathione was the main thiol. Cysteine concentrations in the xylem sap of the trunk amounted to 260–500 nmol l$^{-1}$. Glutathione concentrations were 2-5 times and $\gamma$-glutamylcysteine concentrations 4–16 times lower than those of cysteine.

Key words: Glutathione, $\gamma$-glutamylcysteine, cysteine, long-distance transport.

Introduction

Although the enzymes of assimilatory sulphate reduction are not only found in the leaves, but also in roots (Pate, 1965; Nieto-Sotelo and Ho, 1986; Rüegsegger and Brunold, 1992), sulphate reduction in the roots may be insufficient to meet the roots demand for reduced sulphur (Brunold, 1990). In herbaceous plants, the reduction of sulphate and the synthesis of GSH are thought to take place predominantly in the leaves (Brunold, 1990). Other parts of the plant with a demand for reduced sulphur in growth and development are supplied with GSH as a source of reduced sulphur by phloem transport (Rennenberg et al., 1979; Bonas et al., 1982; Rennenberg and Thoene, 1987; Rauser et al., 1991).

Since several studies suggested that, in trees, the leaves are also a main site of assimilatory sulphate reduction (Brunold, 1983; Brunold et al., 1983; Bosma et al., 1991; Schupp and Rennenberg, 1992; Suter et al., 1992), experiments were performed to study the export of sulphur compounds out of the leaves. For spruce it was shown that feeding of $^{35}$S-GSH via the cut surface of a single 1-year-old needle resulted in a significant transport of glutathione especially into the developing sprouts (Schupp et al., 1992; Schneider et al., 1994). Apparently, the current year’s sprouts are the preferential sinks of sulphur exported from 1-year-old needles during the whole growing season. In beech, glutathione was transported exclusively into basipetal parts of the tree axis after feeding $^{35}$S-cysteine to leaves (Herschbach and Rennenberg, 1995). Similar results were obtained in experiments with oak, where the feeding of $^{35}$S-glutathione resulted in the predominant export of reduced sulphur (GSH) along the trunk to the roots (Seegmüller et al., 1995).

Allocation and distribution of $^{14}$C-labelled photoassimilates in pine trees depended on needle age and the time of season (Ericsson, 1978; Gower et al., 1995). In spring, assimilates were allocated from 1-year-old needles into
the developing current year’s sprouts, whereas 2-year-old needles exported assimilates into basipetal parts of the tree (Ericsson, 1978). Therefore, it may be assumed that a similar mechanism holds true for sulphur nutrition in spruce trees: 1-year-old needles support the current year’s sprouts and older needle generations the basipetal parts of the tree by export of glutathione. Alternatively, sulphate reduction in the roots may contribute to the supply of roots and cambial trunk tissues with reduced sulphur. In order to address this question, 35S-labelled reduced sulphur compounds, i.e. 35S-GSH and its precursors, 35S-cysteine and 35S-γ-EC, was fed to 3-year-old needles and the allocation of these sulphur compounds was analysed. In addition, the thiol composition of the xylem sap of trunk segments was studied to obtain information about possible interactions between the thiol pools of roots and the shoot.

Materials and methods

Plant material

The 6-year-old spruce trees (Picea abies [Karst.] L.) used for the long-distance transport experiments of this study were obtained from the forest management in Schongau (FRG). The plants (height: 52–89 cm) were grown outdoors in 151 plastic pots containing spruce forest soil from a clean air region (Garmisch-Partenkirchen, Germany). Pots were dug into the soil of a meadow next to the Institute of Forest Botany and Tree Physiology.

The spruce trees (Picea abies L.) used for analysis of trunk xylem sap originated from two forest sites in the Black Forest Mountains. The sites have previously been described as N-deficient (town forest near Villingen-Schwenningen) and N-sufficient (forest near lake Schluchsee) (Feger et al., 1992). Briefly, the site near Villingen-Schwenningen is located at an elevation level of 800–960 m ASL. The mean annual precipitation was 900–1200 mm and the mean annual temperature was 6.0 °C (soil: Pseudogley-Braunerde, Pseudogley, slope deposition was 900–1200 mm and the mean annual temperature was 4.5–6.0 °C). The forest near lake Schluchsee is characterized by a mean temperature of 4.5–6.0 °C (soil: Braunerde-Podsol, Podsol-Braunerde, slope deposition: north-west; Zottl et al., 1987). From the forest near Villingen-Schwenningen, 90–130-year-old spruce trees and from the forest near lake Schluchsee, 30–70-year-old spruce trees, were felled for the present experiments.

Application of 35S-labelled thios

Two weeks prior to the experiments all attached sprouts from one well-needled 4-year-old twig of a 6-year-old spruce tree were cut off, except for the terminal current year’s sprouts. To prevent infections, the cut planes were covered with vaseline grease. This treatment did not visually influence the vitality of the twigs. Trees were transferred to the laboratory for adaptation to indoor conditions 16–20 h before the beginning of the experiments. Feeding experiments were started between 8.30 and 12.30 a.m. Plants were illuminated at 700–1100 μE m−2 s−1 at twig level (Osram Powerstar HQI-T 400W/DH Daylight) and were exposed to room temperature (23–30 °C). 35S-Thios were fed via a 3-year-old needle. For this purpose, the tip of the needle was cut off with a razor blade as described by Schupp et al. (1992). The remaining stump was immediately immersed in 10 μl feeding solution. The following feeding solutions were applied: 250 μM 35S-GSH (specific radioactivity: 0.77 TBq mmol−1, pH 6.0), 3 μM 35S-γ-EC (4.93 TBq mmol−1, pH 6.0), and 15 μM 35S-cysteine (3.73 TBq mmol−1, pH 6.0). These thiol concentrations equalled the average thiol contents in spruce needles as previously determined (Schupp and Rennenberg, 1988, 1992). The stock solutions of 35S-γ-EC and 35S-cysteine contained 50% and 35% 35S-SO32−, respectively. The results presented are corrected for this contamination: to calculate γ-EC uptake (pmol h−1) total 35S-radioactivity recovered was divided by a factor of 2 and to calculate 35S-cysteine uptake (pmol h−1) a division by a factor of 1.54 was applied.

Harvest

The incubation with 35S-thiols was terminated when the feeding solution was taken up completely, but at the latest after 3 h, by separating the fed needle from the twig. The cut needle was washed twice with 1 ml aqua dest. to remove unspecifically bound radioactivity. The twig was excised from the stem, dissected into age-groups and further cut up into sections of 2–5 cm length. From these sections needles, bark and wood were separated. Each sample was weighed, frozen in liquid nitrogen and stored at −20 °C until analysis.

Analytical procedures

Sample analysis was carried out as previously described by Schupp et al. (1992) and Schneider et al. (1994). Needles, bark and wood were powdered in a mortar under liquid nitrogen. By this procedure differentiation between individual tissues within these organs was not possible. For extraction of low molecular weight 35S compounds, an aliquot of 30–120 mg frozen powder was extracted three times with 1 ml 0.1 N HCl, 1 mM EDTA and 50 mg insoluble polyvinylpyrrolidone (PVP) in the cold for 30 min. After centrifugation (15 min, 4°C, 23,000 g; Hettich Universal 30 RF, FRG) supernatants were mixed with 5 ml scintillation fluid (Aquaasafe 300, Zinsser Analytik, FRG) for liquid scintillation counting (LSC; Wallac Oy, Turku, Finland). To determine the amount of 35S incorporated into acid-insoluble material, the remaining pellets of bark and wood were digested for 1 d at 40°C with 1 ml tissue solubilizer (Soluene 350, Packard, Netherland). Then 200 μl isopropanol were added, the samples were transferred into scintillation vials and were bleached with 300 μl 30% H2O2 for 1 d at RT. Pellets from needle extracts were bleached during drying with 200 μl 30% H2O2 for 5 d at 40°C and then digested with 1 ml tissue solubilizer for 1 d at RT. The fed needle was digested with 1 ml tissue solubilizer (Soluene 350, Packard, Netherland) and bleached with 200 μl 30% H2O2. Radioactivity of all samples was determined by LSC after addition of 15 ml scintillation fluid (Aquaasafe 300, Zinsser Analytik, FRG). For calculating uptake and recovery of 35S, aliquots of the feeding and washing solutions of the fed needle were analysed by LSC.

To identify soluble 35S-labelled compounds, aliquots of 400 μl supernatant of the first HCl extract were reduced with 15 mM DTT, derivatized with 30 mM mBBr and separated by reversed-phase HPLC as described by Schupp et al. (1992). Following this procedure, the total thiol pool was detected in its reduced form. Fractions of the eluate were collected at 1 min intervals. Radioactivity of each sample was measured after addition of
4 ml liquid scintillation fluid (Hi Safe 3, Wallac Oy, Turku, Finland) by LSC.

**Collection of xylem sap and determination of thiol composition and contents**

Xylem sap from 1 m trunk sections at 1 m high were collected as previously described by Gulpen et al. (1993). Trees were felled in July, August and September 1993. The bark of both cut ends was peeled off and the wood was washed with distilled water to avoid contamination with phloem sap. Xylem sap was forced out in the normal flow direction with a water column containing 0.01% berberichloride applied on the basipetal surface. Eight 200 ml fractions were collected from each segment and fraction 3-6 were used for thiol analysis. For this purpose 400 µl xylem sap were reduced with 15 mM DTT and derivatized with 30 mM mBBr. Thiol derivatives were separated by HPLC and analysed for fluorescence as described by Schupp et al. (1992). Identification and quantification of thiols was based on co-injection of standards of known concentrations and thiol composition. In one set of samples, free SH groups were blocked with 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) prior to mBBr derivatization (Schupp et al., 1992) to prevent reaction with mBBr. Such controls were used to identify fluorescence not originating from thiols. Recoveries of the thiols analysed were 85 ± 7% for cysteine, 97 ± 17% for γ-glutamylcysteine and 94 ± 14% for glutathione, respectively.

**Results**

**Uptake and transport of 35S-GSH**

When 3-year-old spruce needles were fed 35S-GSH, the uptake varied from 15–54% of the radioactivity applied (Table 1). Part of this variation was a consequence of different times of incubation between experiments. Similar results were obtained for relative export of 35S out of the needle (Table 1). The fresh weight (FW) of the twig containing the fed needle, more or less equivalent to its length, had no influence on uptake or export of the 35S-GSH applied (data not shown). The amount of acid soluble 35S-compounds varied between 10–71% (Table 1), but two distinguishable groups of results became apparent within this range. The first two experiments performed in July/August showed that only a small amount of radiolabel absorbed remained in the soluble fraction (10% and 12%). In the following six experiments performed between August and October, 65 ± 6% of the 35S applied remained in the soluble fraction. These results indicated a general change in sulphur metabolism. Apparently, the major portion of the radioactivity was incorporated into protein and other acid insoluble sulphur compounds in summer, but no longer in autumn.

**Distribution of 35S-radioactivity from 35S-GSH between needles, bark and wood**

The distribution of exported 35S-radioactivity along the twig axis and between needles, bark and wood was similar in all experiments performed (Fig. 1). The greatest amount of radiolabel was always found in the twig section containing the fed needle (35%; n = 8) and in one of the current year's sprouts (34%; n = 8). Neither the two lateral sprouts, nor the axial twig sections were preferential sinks for the transported 35S. The high amount of 35S-label in one of the current year's sprouts was mainly due to 35S-radioactivity in the needles (Fig. 1). Basipetal transport towards the stem amounted to 7% maximum of exported radioactivity (Table 1). Especially radiolabel in the bark decreased rapidly with increasing distance from the fed needle in basipetal direction (Fig. 1). Close to the fed needle the greatest portion of 35S-radioactivity was found in the bark. In acropetal direction the relative amount of radiolabel in the bark decreased and highest values of 35S-radioactivity were found in the wood. This finding indicates that allocation of 35S initially was achieved by phloem transport. On the transport path, a transfer of the 35S from the bark into the wood occurred indicating a phloem to xylem exchange of 35S as previously observed by Schneider et al. (1994).

**Uptake and transport of 35S-γ-EC and 35S-cysteine**

When 35S-γ-EC or 35S-cysteine was fed to a 3-year-old spruce needle up to 55% of the thiols applied were taken up (Table 2). Because of different concentrations in the solutions fed (see Materials and methods), uptake of 35S-

### Table 1. Recovery, uptake and export of 35S-radioactivity applied as 35S-GSH via a single 3-year-old needle

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recovery</td>
<td>(% of 35S applied)</td>
<td>81</td>
<td>90</td>
<td>86</td>
<td>3</td>
</tr>
<tr>
<td>Uptake</td>
<td>(% of 35S applied)</td>
<td>15</td>
<td>54</td>
<td>37</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>(pmol GSH h⁻¹)</td>
<td>177</td>
<td>545</td>
<td>347</td>
<td>124</td>
</tr>
<tr>
<td>Export</td>
<td>(% of 35S absorbed)</td>
<td>8</td>
<td>88</td>
<td>37</td>
<td>27</td>
</tr>
<tr>
<td>Export to basipetal</td>
<td>(% of total export)</td>
<td>&lt;1</td>
<td>7</td>
<td>3.0</td>
<td>2.7</td>
</tr>
<tr>
<td>% Soluble 35S of total export</td>
<td></td>
<td>10</td>
<td>71</td>
<td>51</td>
<td>25</td>
</tr>
</tbody>
</table>
Table 2. Recovery, uptake and export of 35S fed as 35S-γ-EC and 35S-cysteine via a single 3-year-old needle

Experiments were carried out as described in Fig. 1 and recovery was calculated as described in Table 1. Uptake given as pmol h⁻¹ was calculated from the specific activity of the 35S-γ-EC (4.93 TBq mmol⁻¹) or 35S-cysteine (3.73 TBq mmol⁻¹) applied and the feeding time. The data were corrected for 35S-SO₄²⁻-contamination in the stock solutions (see Materials and methods) Export is defined as total radioactivity recovered excluding the application needle.

<table>
<thead>
<tr>
<th>Fed 35S-thiol</th>
<th>Feeding time (h:min)</th>
<th>Recovery (% of 35S applied)</th>
<th>Uptake (% of 35S applied)</th>
<th>Uptake (pmol h⁻¹)</th>
<th>Export (% of 35S absorbed)</th>
<th>Export to basipetal (% of total export)</th>
<th>% Soluble 35S of total export</th>
</tr>
</thead>
<tbody>
<tr>
<td>γ-EC</td>
<td>2:10</td>
<td>79</td>
<td>11</td>
<td>0.9</td>
<td>5</td>
<td>9</td>
<td>69</td>
</tr>
<tr>
<td></td>
<td>1:55</td>
<td>83</td>
<td>39</td>
<td>3.2</td>
<td>43</td>
<td>2</td>
<td>69</td>
</tr>
<tr>
<td></td>
<td>2:00</td>
<td>70</td>
<td>55</td>
<td>4.4</td>
<td>93</td>
<td>&lt;1</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td>1:55</td>
<td>90</td>
<td>48</td>
<td>3.9</td>
<td>3</td>
<td>4</td>
<td>74</td>
</tr>
<tr>
<td>Cysteine</td>
<td>3:00</td>
<td>101</td>
<td>28</td>
<td>9.1</td>
<td>7</td>
<td>2</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>1:00</td>
<td>66</td>
<td>55</td>
<td>54.6</td>
<td>50</td>
<td>&lt;1</td>
<td>73</td>
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<tr>
<td></td>
<td>1:10</td>
<td>51</td>
<td>35</td>
<td>57.9</td>
<td>57</td>
<td>1</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>3:00</td>
<td>91</td>
<td>49</td>
<td>31.2</td>
<td>49</td>
<td>&lt;1</td>
<td>66</td>
</tr>
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</table>

cysteine (pmol h⁻¹) was one order of magnitude lower than that of GSH and uptake of 35S-γ-EC (pmol h⁻¹) was one order of magnitude lower than that of 35S-cysteine (Tables 1, 2). The different thiol concentrations of the feeding solution reflected differences in the cellular concentrations of individual thiols and were applied in order to prevent artificial expansion of pool sizes upon feeding. The export of 35S-γ-EC or 35S-cysteine taken up varied considerably between experiments (Table 2), but proceeded almost exclusively in acropetal direction. Export to basipetal twig sections amounted to less than 10% of total 35S export in all experiments (Table 2). Radiolabel from both, cysteine and γ-EC, remained mainly in the acid soluble fraction (Table 2).

Distribution of 35S-radioactivity from 35S-cysteine or 35S-γ-EC between needles, bark and wood

The distribution of transported 35S-radioactivity along the twig axis was similar when 35S-cysteine or 35S-γ-EC was fed. In all experiments with high export, the highest amount of radiolabel was detected in the bark close to the needle fed (Table 2; Fig. 2B). Along the acropetal transport-path, major parts of radioactivity appeared in the wood sections but also in the needles. Simultaneously, the amount of radiolabel found in the bark decreased considerably (Fig. 2A, B). One of the current year's sprouts contained high amounts of radioactivity especially in the needles. As the experiments were performed in late autumn, this finding indicated that the needles were still...
preferential sinks for allocated sulphur compounds at that time of the year (Fig. 2A, B).

**Identification of transported $^{35}$S-labelled compounds**

Acid soluble, low molecular weight thiols were identified in tissue samples next to the fed needle and the highly labelled sprouts after feeding $^{35}$S-$\gamma$-EC or $^{35}$S-cysteine. $^{35}$S-SO$_4^{2-}$ was found in all samples (Figs. 3, 4). As to whether it originated exclusively from contamination of the stock solutions (see Materials and methods), or from oxidation of reduced $^{35}$S-sulphur (Rennenberg et al., 1982; Rauser et al., 1991) cannot be deduced from the present experiments.

When $^{35}$S-$\gamma$-EC was fed, the $^{35}$S-signal of thiols extracted from bark- and wood-segments close to the fed needle could not be assigned to $\gamma$-EC or cysteine because both thiols could not be resolved sufficiently by the HPLC (Figs 3A, B). In wood-extracts of current year's sprouts the $^{35}$S-label was partially due to glutathione and partially to $\gamma$-EC and cysteine (Fig. 3C). In current year's needles, glutathione was the only labelled thiol (Fig. 3D). When $^{35}$S-cysteine was fed, extracts of bark- and wood-segments close to the fed needle contained $^{35}$S-labelled cysteine, but also $^{35}$S-glutathione (Fig. 4A, B). In current year's needles, glutathione was the only labelled thiol compound detected (Fig. 4C, D).

**Thiol composition in trunk xylem sap**

Neither glutathione, nor cysteine or $\gamma$-glutamylcysteine were transported from the 3-year-old needles into basipetal sections of the stem. Therefore, it may be assumed that sulphate reduction in the roots and allocation to the trunk is required to meet the needs for reduced sulphur of meristematic and parenchymatic tissues in the trunk. As a first attempt to address this question, the thiol composition of the xylem sap of trunk sections of spruce trees was investigated. Despite the fact that the trees analysed originated from different forest stands, the thiol composition in the xylem sap of the trunk sections did not vary considerably (Table 3). The main thiol found

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**Fig. 2.** Distribution of transported $^{35}$S-radioactivity between needles, bark and wood along the twig axis of spruce trees fed $^{35}$S-$\gamma$-EC (A) and $^{35}$S-cysteine (B). Application was carried out via the cut surface of a single 3-year-old needle. After 1–3 h incubation the twig was cut off the stem, dissected between the age-groups into 2–5 cm sections and further separated into needles, bark and wood. The tissues were processed as described in Fig. 1. The figure shows the absolute values of radioactivity (Bq/FW of the section) of needles (■), bark (○) and wood (□), or bark plus wood (■□) and the total radioactivity of each twig section ( - - -). One of four experiments with similar results is shown for each feeding.
Identification of exported soluble $^{35}$S-compounds in twig sections. $^{35}$S-$\gamma$-EC was fed to a single 3-year-old needle. Harvest and processing of the tissues were performed as described in Fig. 1. Thiols were extracted with 0.1 N HCl, reduced with DTT, derivatized with mBBr and separated by HPLC analysis. The eluate was collected in 1 min fractions and radioactivity was determined by liquid scintillation counting. $^{35}$S-thiols in bark (A) and wood (B) of one application segment, and in wood (C) and needles (D) of a current year's twig were identified by the comparison of $^{35}$S-radioactivity in eluate-fractions and the fluorescence pattern of the HPLC analysis. Arrows mark the range in which individual compounds were eluted. Elution time varied by about 2 min between individual chromatograms.

was cysteine. Its concentration ranged from 260 nmol $^{-1}$ xylem sap in July to 500 nmol $^{-1}$ xylem sap in September. The glutathione concentrations determined were 2-5 times lower and the $\gamma$-glutamylcysteine concentrations 4-16 times lower as compared to the cysteine content of the xylem saps (Table 3).

Discussion

In the present experiments feeding of $^{35}$S-GSH to 3-year-old spruce needles resulted in uptake and export of $^{35}$S (Table 1) as previously observed with 1-year-old spruce needles (Schupp et al., 1992; Schneider et al., 1994). The uptake of $^{35}$S-GSH by the 3-year-old needles (37% of the $^{35}$S-GSH applied; Table 1) was significantly higher than that found after feeding via 1-year-old needles (Schupp et al., 1992: maximum 13%; Schneider et al., 1994: maximum 16%). However, the export of absorbed $^{35}$S-GSH out of 3-year-old needles (37%; Table 1) was relatively low as compared to 1-year-old needles (Schupp et al., 1992: 51%; Schneider et al., 1994: 61%). Thus, the 3-year-old needle class seemed to contribute to the sulphur nutrition of other tissues to a similar extent as the 1-year-old needle class.

Many plants analysed so far contain cysteine and $\gamma$-EC in appreciable amounts in addition to GSH in the xylem (Schupp et al., 1991; Rennenberg et al., 1994a, b; Table 3 in this study). Therefore, these compounds seem to play an important role in the allocation of reduced sulphur and, hence, in sulphur nutrition. The present feeding experiments with $^{35}$S-cysteine and $^{35}$S-$\gamma$-EC revealed an uptake and export of $^{35}$S similar to that obtained after feeding $^{35}$S-GSH (Tables 1, 2). From the distribution of $^{35}$S-label along the transport path a clear difference between feeding glutathione and its metabolic precursors, $\gamma$-EC and cysteine, was found. The $^{35}$S-GSH fed was predominantly allocated to the current year's sprouts, in particular to the needles (Fig. 1) as previously found by Schupp et al. (1992) and Schneider et al. (1994). Feeding of $^{35}$S-cysteine or $^{35}$S-$\gamma$-EC resulted in a high $^{35}$S-labelling
of the bark close to the insertion of the fed needle (Fig. 2B). This result indicated an export of the radio-labeled γ-EC and cysteine, but only minute loading for long-distance transport. A preferential resorption of cysteine and γ-EC by bark parenchyma cells as compared to GSH may be responsible for this observation. Alternatively, an inhibition of phloem to xylem transfer of cysteine and γ-EC may reduce the allocation of these compounds. Such an exchange has been documented for GSH (Schneider et al., 1994) and enables effective transport in both xylem and phloem. When 35S-cysteine and 35S-γ-EC were fed, 35S-GSH was, in particular, detected in twig sections distant to the fed needle (Figs. 3, 4). Similar results were obtained when 35S-cysteine was fed to beech leaves (Herschbach and Rennenberg, 1995). Apparently, GSH plays a dominant role as the long-distance transport pool of reduced sulphur in both coniferous and deciduous trees.

Basipetal transport of glutathione was found to be essential to meet the demand for reduced sulphur of the stem and the root of herbaceous plants (Rennenberg et al., 1979; Bonas et al., 1982; Rauser et al., 1991) and deciduous trees (Herschbach and Rennenberg, 1995; Seegmüller et al., 1995). In contrast, only a minute basipetal transport of reduced sulphur was observed when 35S-GSH was fed via a 1-year-old spruce needle (Schupp et al., 1992; Schneider et al., 1994). This was supported by the present study with 3-year-old spruce needles where basipetal transport never exceeded 10% of total 35S export (Tables 1, 2). Thus, it appears unlikely that the growth of the roots and those parts of the stem not covered with needles is supported by allocation of reduced sulphur from 1- and 3-year-old needles. The xylem sap of spruce trunks contained considerable amounts of reduced sulphur, mainly as cysteine (Table 3). This led to the assumption that spruce roots might be self-sufficient in sulphate reduction and, presumably, supply the cambial and parenchymatic tissue of the stem with reduced sulphur by allocation of cysteine. In spruce twigs, however, glutathione was the dominant thiol in xylem sap (Schupp, 1991). This difference may be caused by the export of GSH from older needle classes to supply the current year’s sinks as demonstrated in this and previous studies (Schupp et al., 1992; Schneider et al., 1994).

From investigations with herbaceous plants a cycling pool of reduced sulphur has been proposed to meet the needs of the whole plant in sulphur nutrition (Rennenberg, 1995). Glutathione may be fed into this pool by mature leaves and may be removed by growing tissues of the shoot and the roots. An individual cycling pool of reduced sulphur is unable to explain the present observations in spruce. In green parts of the tree, reduced sulphur allocation seemed to proceed exclusively in an acropetal direction as glutathione; in non-green parts of the tree, however, cysteine transport in an acropetal direction has been observed. Reallocation of cysteine in the xylem into a basipetal direction can not be excluded from the present experiments. Furthermore, as demonstrated by the distribution of 35S between soluble and insoluble fractions along the transport path (data not shown), a metabolization of reduced sulphur compounds during long-distance transport must be taken into account. A cycling pool of reduced sulphur in herbaceous plants is considered as a signal in the inter-organ regulation of sulphur nutrition (Rennenberg and Lamoureux, 1990; Herschbach and Rennenberg, 1991). The whole plant regulation of sulphur nutrition in spruce seemed to be achieved by different regulatory mechanisms as proposed by Rennenberg and Herschbach (1995).

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References


