Herschbach, C; Schulte, M; von Ballmoos, P; Brunold, C; Rennenberg, H
Sulfate and Nitrate Assimilation in Leaves of *Quercus ilex* and *Quercus pubescens* Grown Near Natural CO2 Springs in Central Italy
The effect of long-term exposure to elevated pCO(2) concentrations on sulfate and nitrate assimilation was studied under field conditions using leaves from *Quercus ilex* and *Quercus pubescens* trees growing with ambient or elevated CO2 concentrations in the vicinity of three natural CO2 springs, Bossoleto, Laiatico and Sulfatara, in Tuscany, Italy. The activity of the key enzymes of sulfate assimilation, adenosine 5'-phosphosulfate reductase (APR) and nitrate assimilation, nitrate reductase (NR), were measured together with the levels of acid soluble thiols, and soluble non-proteinogenic nitrogen compounds. Whereas NR activity remained unaffected in Q. ilex or increased Q. pubescence, APR activity decreased in the area of CO2 springs. The latter changes were often accompanied by increased GSH concentrations, apparently synthesized from H2S and SO2 present in the gas mixture emitted from the CO2 springs. Thus, the diminished APR activity in leaves of Q. ilex and Q. pubescence from spring areas can best be explained by the exposure to gaseous sulfur compounds. Although the concentrations of H2S and SO2 in the gas mixture emitted from the vents at the CO2 springs were low at the Bossoleto and Laiatico spring, these sulfur gases pose physiological effects, which may override consequences of elevated pCO(2).
SULFUR METABOLISM IN PLANTS: MECHANISMS AND APPLICATIONS TO FOOD SECURITY AND RESPONSES TO CLIMATE CHANGE 237-248, DOI: 10.1007/978-94-007-4450-9_29, 2012

Hesse, H; Trachsel, N; Suter, M; Kopriva, S; von Ballmoos, P; Rennenberg, H; Brunold, C
Effect of glucose on assimilatory sulphate reduction in *Arabidopsis thaliana* roots
With the aim of analysing the relative importance of sugar supply and nitrogen nutrition for the regulation of sulphate assimilation, the regulation of adenosine 5'-phosphosulphate reductase (APR), a key enzyme of sulphate reduction in plants, was studied. Glucose feeding experiments with *Arabidopsis thaliana* cultivated with and without a nitrogen source were performed. After a 38 h dark period, APR mRNA, protein, and enzymatic activity levels decreased dramatically in roots. The addition of 0.5% (w/v) glucose to the culture medium resulted in an increase of APR levels in roots (mRNA, protein and activity), comparable to those of plants kept under normal light conditions. Treatment of roots with d-sorbitol or d-mannitol did not increase APR activity, indicating that osmotic stress was not involved in APR regulation. The addition of O-acetyl-L-serine (OAS) also quickly and transiently increased APR levels (mRNA, protein, and activity). Feeding plants with a combination of glucose and OAS resulted in a more than additive induction of APR activity. Contrary to nitrate reductase, APR was also increased by glucose in N-deficient plants, indicating that this effect was independent of nitrate assimilation. [S-35]-sulphate feeding experiments showed that the addition of glucose to dark-treated roots resulted in an increased incorporation of [S-35] into thiols and proteins, which corresponded to the increased levels of APR activity. Under N-deficient conditions, glucose also increased thiol labelling, but did not increase the incorporation of label into proteins. These results demonstrate that (i) exogenously supplied glucose can replace the function of photoassimilates in roots; (ii) APR is subject to co-ordinated metabolic control by carbon metabolism; (iii) positive sugar signalling overrides negative signalling from nitrate assimilation in APR regulation. Furthermore, signals originating from nitrogen and carbon metabolism regulate APR synergistically.
JOURNAL OF EXPERIMENTAL BOTANY 54 (388): 1701-1709, DOI: 10.1093/jxb/erg177, JUL 2003

Kopriva, S; Suter, M; von Ballmoos, P; Hesse, H; Krähenbuhl, U; Rennenberg, H; Brunold, C
Interaction of sulfate assimilation with carbon and nitrogen metabolism in *Lemna minor*
Cysteine synthesis from sulfide and O-acetyl-L-serine (OAS) is a reaction interconnecting sulfate, nitrogen, and carbon assimilation. Using *Lemna minor*, we analyzed the effects of omission of CO2 from the atmosphere and simultaneous application of alternative carbon sources on adenosine 5'-phosphosulfate reductase (APR) and nitrate reductase (NR), the key enzymes of sulfate and nitrate assimilation, respectively. Incubation in air without CO2 led to severe decrease in APR and NR activities and mRNA levels, but ribulose-1,5-bisphosphate carboxylase/oxygenase was not considerably affected. Simultaneous addition of sucrose (Suc) prevented the reduction in enzyme activities, but not in mRNA levels. OAS, a known regulator of sulfate assimilation, could also attenuate the effect of missing CO2 on APR, but did not affect NR. When the plants were subjected to normal air after a 24-h pretreatment in air without CO2, APR and NR activities and mRNA levels recovered within the next 24 h. The addition of Suc and glucose in air without CO2 also recovered both enzyme activities, with OAS again influenced only APR. (SO42-)-S-35 feeding showed that treatment in air without CO2 severely inhibited sulfate uptake and the flux through sulfate assimilation. After a resupply of normal air or the addition of Suc, incorporation of S-35 into proteins and glutathione greatly increased. OAS treatment resulted in high labeling of cysteine; the incorporation of S-35 in proteins and glutathione was much less increased compared with treatment with normal air or Suc. These results corroborate the tight interconnection of sulfate, nitrate, and carbon assimilation.
PLANT PHYSIOLOGY 130 (3): 1406-1413, DOI: 10.1104/pp.007773, NOV 2002

Vauclare, P; Kopriva, S; Fell, D; Suter, M; Sticher, L; von Ballmoos, P; Krähenbuhl, U; den Camp, RO; Brunold, C
Flux control of sulphate assimilation in *Arabidopsis thaliana*: adenosine 5 '-phosphosulphate reductase is more susceptible than ATP sulphurylase to negative control by thiols
The effect of externally applied l-cysteine and glutathione (GSH) on ATP sulphurylase and adenosine 5'-phosphosulphate reductase (APR), two key enzymes of assimilatory sulphate reduction, was examined in *Arabidopsis thaliana* root cultures. Addition of increasing l-cysteine to the nutrient solution increased internal cysteine, gamma-glutamylcysteine and GSH concentrations, and decreased APR mRNA, protein and extractable activity. An effect on APR could already be detected at 0.2 mm l-cysteine, whereas ATP sulphurylase was significantly affected only at 2 mm l-cysteine. APR mRNA, protein and activity were also decreased by GSH at 0.2 mm and higher concentrations. In the presence of l-buthionine-S, R-sulphoximine (BSO), an inhibitor of GSH synthesis, 0.2 mm l-cysteine had no effect on APR activity, indicating that GSH formed from cysteine was the regulating substance. Simultaneous addition of BSO and 0.5 mm GSH to the culture medium decreased APR mRNA, enzyme protein and activity. ATP sulphurylase activity was not affected by this treatment. Tracer experiments using (35) SO4 (2-) in the presence of 0.5 mm l-cysteine or GSH showed that both thiols decreased sulphate uptake, APR activity and the flux of label into cysteine, GSH and protein, but had no effect on the activity of all other enzymes of assimilatory sulphate reduction and serine acetyltransferase. These results are consistent with the hypothesis that thiols regulate the flux through sulphate assimilation at the uptake and the APR step. Analysis of radioactive labelling indicates that the flux control coefficient of APR is more than 0.5 for the intracellular pathway of sulphate assimilation. This analysis also shows that the uptake of external sulphate is inhibited by GSH to a greater extent than the flux through the pathway, and that the flux control coefficient of APR for the pathway, including the transport step, is proportionately less, with a significant share of the control exerted by the transport step.
PLANT JOURNAL 31 (6): 729-740, DOI: 10.1046/j.1365-313X.2002.01391.x, SEP 2002

Kopriva, S; Buchert, T; Fritz, G; Suter, M; Benda, RD; Schunemann, V; Koprivova, A; Schürmann, P; Trautwein, AX; Kroneck, PMH; Brunold, C
The presence of an iron-sulfur cluster in adenosine 5 '-phosphosulfate reductase separates organisms utilizing adenosine 5 '-phosphosulfate and phosphoadenosine 5 '-phosphosulfate for sulfate assimilation
It was generally accepted that plants, algae, and phototrophic bacteria use adenosine 5'-phosphosulfate (APS) for assimilatory sulfate reduction, whereas bacteria and fungi use phosphoadenosine 5'-phosphosulfate (PAPS). The corresponding enzymes, APS and PAPS reductase, share 25-30% identical amino acids. Phylogenetic analysis of APS and PAPS reductase amino acid sequences from different organisms, which were retrieved from the GenBank(TM), revealed two clusters. The first cluster comprised known PAPS reductases from enteric bacteria, cyanobacteria, and yeast. On the other hand, plant APS reductase sequences were clustered together with many bacterial ones, including those from Pseudomonas and Rhizobium. The gene for APS reductase cloned from the APS-reducing cyanobacterium Plectonema also clustered together with the plant sequences, confirming that the two classes of sequences represent PAPS and APS reductases, respectively. Compared with the PAPS reductase, all sequences of the APS reductase cluster contained two additional cysteine pairs homologous to the cysteine residues involved in binding an iron-sulfur cluster in plants. Mossbauer analysis revealed that the recombinant APS reductase from Pseudomonas aeruginosa contains a [4Fe-4S] cluster with the same characteristics as the plant enzyme. We conclude, therefore, that the presence of an iron-sulfur cluster determines the APS specificity of the sulfate-reducing enzymes and thus separates the APS- and PAPS-dependent assimilatory sulfate reduction pathways.
JOURNAL OF BIOLOGICAL CHEMISTRY 277 (24): 21786-21791, DOI: 10.1074/jbc.M202152200, Jun 14 2002

Kopriva, S; Buchert, T; Fritz, G; Suter, M; Weber, M; Benda, R; Schaller, J; Feller, U; Schürmann, P; Schunemann, V; Trautwein, AX; Kroneck, PMH; Brunold, C
Plant adenosine 5 '-phospho sulfate reductase is a novel iron-sulfur protein
Adenosine 5'-phosphosulfate reductase (APR) catalyzes the two-electron reduction of adenosine 5'-phosphosulfate to sulfite and AMP, which represents the key step of sulfate assimilation in higher plants. Recombinant APRs from both *Lemna minor* and *Arabidopsis thaliana* were overexpressed in Escherichia coli and isolated as yellow-brown proteins. UV-visible spectra of these recombinant proteins indicated the presence of iron-sulfur centers, whereas flavin was absent. This result was confirmed by quantitative analysis of iron and acid-labile sulfide, suggesting a [4Fe-4S] cluster as the cofactor. EPR spectroscopy of freshly purified enzyme showed, however, only a minor signal at g = 2.01. Therefore, Mossbauer spectra of Fe-57-enriched APR were obtained at 4.2 K in magnetic fields of up to 7 tesla, which were assigned to a diamagnetic [4Fe-4S](2+) cluster. This cluster was unusual because only three of the iron sites exhibited the same Mossbauer parameters. The fourth iron site gave, because of the bistability of the fit, a significantly smaller isomer shift or larger quadrupole splitting than the other three sites. Thus, plant assimilatory APR represents a novel type of adenosine 5'-phosphosulfate reductase with a [4Fe-4S] center as the sole cofactor, which is clearly different from the dissimilatory adenosine 5'-phosphosulfate reductases found in sulfate reducing bacteria.
JOURNAL OF BIOLOGICAL CHEMISTRY 276 (46): 42881-42886, DOI: 10.1074/jbc.M107424200, Nov 16 2001

Kocsy, G; von Ballmoos, P; Rüegsegger, A; Szalai, G; Galiba, G; Brunold, C
Increasing the glutathione content in a chilling-sensitive maize genotype using safeners increased protection against chilling-induced injury
With the aim of analyzing their protective function against chilling-induced injury, the pools of glutathione and its precursors, cysteine (Cys) and gamma -glutamyl-Cys, were increased in the chilling-sensitive maize (*Zea mays*) inbred line Penjalinan using a combination of two herbicide safeners. Compared with the controls, the greatest increase in the pool size of the three thiols was detected in the shoots and roots when both safeners were applied at a concentration of 5 muM. This combination increased the relative protection from chilling from 50% to 75%. It is interesting that this increase in the total glutathione (TG) level was accompanied by a rise in glutathione reductase (GR; EC 1.6.4.2) activity. When the most effective safener combination was applied simultaneously with increasing concentrations of buthionine sulfoximine, a specific inhibitor of glutathione synthesis, the total gamma -glutamyl-Cys and TG contents and GR activity were decreased to very low levels and relative protection was lowered from 75% to 44%. During chilling, the ratio of reduced to oxidized thiols first decreased independently of the treatments, but increased again to the initial value in safener-treated seedlings after 7 d at 5 degreesC. Taking all results together resulted in a linear relationship between TG and GR and a biphasic relationship between relative protection and GR or TG, thus demonstrating the relevance of the glutathione levels in protecting maize against chilling-induced injury.
PLANT PHYSIOLOGY 127 (3): 1147-1156, DOI: 10.1104/pp.010107, NOV 2001

Kocsy, G; Galiba, G; Brunold, C
Role of glutathione in adaptation and signalling during chilling and cold acclimation in plants
Glutathione is an important component of the ascorbate-glutathione cycle, which is involved in the regulation of hydrogen peroxide (H2O2) concentrations in plants. During chilling and cold acclimation, i.e. exposure to temperatures between 0 and 15 degreesC, H2O2 may accumulate. Excess electrons from the photosynthetic and respiratory electron transport chains can be used for the reduction of oxygen, thus producing superoxide radicals (O-2(.-)); these are subsequently transformed to H2O2 via superoxide dismutase (SOD; EC 1.15.1.1). During the removal of excess H2O2, reduced glutathione (GSH) is converted to its oxidised form (GSSG), and GSH is regenerated by the activity of NADPH-dependent glutathione reductase (GR; EC 1.6.4.2). At low non-freezing temperatures, high GSH content and GR activity were detected in several plant species, indicating a possible contribution to chilling tolerance and cold acclimation. Changes in H2O2 concentration, and GSH/GSSG ratio alter the redox state of the cells and may activate special defence mechanisms through a redox signalling chain. The finding that several defence genes have antioxidant responsive elements or GSSG binding sites in their regulatory regions supports the idea that redox signalling is involved in regulating gene expression in response to low temperature.
PHYSIOLOGIA PLANTARUM 113 (2): 158-164, DOI: 10.1034/j.1399-3054.2001.1130202.x, OCT 2001

Koprivova, A; Melzer, M; von Ballmoos, P; Mandel, T; Brunold, C; Kopriva, S
Assimilatory sulfate reduction in C-3, C-3-C-4, and C-4 species of Flaveria
The activity of the enzymes catalyzing the first two steps of sulfate assimilation, ATP sulfurylase and adenosine 5'-phosphosulfate reductase (APR), are confined to bundle sheath cells in several C-4 monocot species. With the aim to analyze the molecular basis of this distribution and to determine whether it was a prerequisite or a consequence of the C-4 photosynthetic mechanism, we compared the intercellular distribution of the activity and the mRNA of APR in C-3, C-1-C-4, C-4-like, and C-4 species of the dicot genus Flaveria. Measurements of APR activity, mRNA level, and protein accumulation in six Flaveria species revealed that APR activity, cysteine, and glutathione levels were significantly higher in C-4-like and C-4 species than in C3 and C-3-C-4 species. ATP sulfurylase and APR mRNA were present at comparable levels in both mesophyll and bundle sheath cells of C-4 species Flaveria trinervia. Immunogold electron microscopy demonstrated the presence of APR protein in chloroplasts of both cell types. These findings, taken together with results from the literature, show that the localization of assimilatory sulfate reduction in the bundle sheath cells is not ubiquitous among C-4 plants and therefore is neither a prerequisite nor a consequence of C-4 photosynthesis.
PLANT PHYSIOLOGY 127 (2): 543-550, DOI: 10.1104/pp.010144, OCT 2001

Westerman, S; Stulen, I; Suter, M; Brunold, C; De Kok, LJ
Atmospheric H2S as sulphur source for Brassica oleracea: Consequences for the activity of the enzymes of the assimilatory sulphate reduction pathway
Short-term exposure of *Brassica oleracea* L. (curly kale) to atmospheric H2S levels (0.2-0.8 muL .L-1), which an sufficient to meet the plants sulphur requirement, resulted in a decrease in the activity of adenosine 5'-phosphosulphate reductase (APR) in the shoot. The reduction in APR activity was maximally 80 % and was already substantial after id exposure to 0.2 muL .L-1 H2S. The activity of APR in the roots remained unaffected upon exposure to all levels of H2S. The activities of ATP-sulphurylase (ATPS), serine acetyltransferase (SAT) and O-acetylserine(thiol)lyase (OAS-TL), in both shoot and roots were not affected upon exposure to H2S levels ranging from 0.2-0.8 muL .L-1. There was a rapid increase in the shoot thiol content, including cysteine, upon H2S exposure and a maximal 3-fold increase in thiol content occurred after 5 h exposure. In the roots, the thiol content was only slightly increased after 2 d H2S exposure. The relationship between the pattern of thiol accumulation and changes in sulphate assimilation upon H2S exposure is discussed. (C) 2001 Editions scientifiques et medicales Elsevier SAS.
PLANT PHYSIOLOGY AND BIOCHEMISTRY 39 (5): 425-432, DOI: 10.1016/S0981-9428(01)01258-X, MAY 2001

Kopriva, S; Jones, S; Koprivova, A; Suter, M; von Ballmoos, P; Brander, K; Fluckiger, J; Brunold, C
Influence of chilling stress on the intercellular distribution of assimilatory sulfate reduction and thiols in *Zea mays*
The effect of chilling on the intercellular distribution of mRNAs for enzymes of assimilatory sulfate reduction, the activity of adenosine 5'-phosphosulfate reductase (APR), and the level of glutathione was analysed in leaves and roots of maize (*Zea mays* L). At 25 degreesC the mRNAs for APR, ATP sulfurylase, and sulfite reductase accumulated in bundle-sheath only, whereas the mRNA for O-acetylserine sulfhydrylase was also detected in mesophyll cells. Glutathione was predominantly detected in mesophyll cells; however, oxidized glutathione was equally distributed between the two cell types. Chilling at 12 degreesC induced oxidative stress which resulted in increased concentrations of oxidized glutathione in both cell types and a prominent increase of APR mRNA and activity in bundle-sheath cells. After chilling, mRNAs for APR and sulfite reductase, as well as low APR activity, were detected in mesophyll cells. In roots, APR mRNA and activity were at higher levers in root tips than in the mature root and were greatly increased after chilling. These results demonstrate that chilling stress affected the levers and the intercellular distribution of mRNAs for enzymes of sulfate assimilation.
PLANT BIOLOGY 3 (1): 24-31, DOI: 10.1055/s-2001-11745, JAN 2001

Bovet, L; Kammer, PM; Suter, M; Brunold, C
Effect of mannitol and cold treatments on phosphate uptake and protein phosphorylation in *Lemna minor* (L.) plants
This report is aimed at elucidating the effect of mannitol and cold treatments on P uptake and protein phosphorylation in *Lemna minor* plants. Duckweed plants were incubated in the presence of [P-32] or [P-33]Pi, half-strength phosphate deprived E-medium under constant light regime for 1.5h. Total plant protein extracts (pellet and supernatant) were then prepared and subjected to IEF x SDS-PAGE. To analyse the effect of the stresses on P uptake and protein labelling, *Lemna minor* plants were preincubated with 0.1, 0.5 mol L-1 mannitol and at 4 degreesC respectively, for 4 hours, before adding labelled orthophosphate. The results show that the general protein phosphorylation (including LHCII) is related to the level of P uptake. Radioactive phosphate incorporation is stimulated by a low con centration of mannitol (0.1 mol L-1) but reduced by 0.5 mol.L-1 mannitol and cold stress in planta. The labelling into proteins is affected neither when stresses were applied to the plants after incubation with labelled orthophosphate, nor after in vitro protein phosphorylation. This indicates that general protein kinase activities in vivo are strictly limited by P uptake. A marked accumulation of soluble hexoses (mainly sucrose, glucose, and fructose) is observed under imposed stress, suggesting that the inhibition of P uptake in response to hyperosmotic and cold stresses is mediated by sugar accumulation in situ. However, metabolisable sugars like glucose did not alter the entry of phosphate at concentrations of 0,5 mol.L-1, showing that the chemical nature of the osmoticum influences P uptake.
JOURNAL OF PLANT PHYSIOLOGY 157 (4): 375-382; OCT 2000

Kocsy, G; von Ballmoos, P; Suter, M; Rüegsegger, A; Galli, U; Szalai, G; Galiba, G; Brunold, C
Inhibition of glutathione synthesis reduces chilling tolerance in maize
The role of glutathione (GSH) in protecting plants from chilling injury was analyzed in seedlings of a chilling-tolerant maize (*Zea mays* L.) genotype using buthionine sulfoximine (BSO), a specific inhibitor of gamma-glutamylcysteine (gamma EC) synthetase, the first enzyme of GSH synthesis. At 25 degrees C, 1 mM BSO significantly increased cysteine and reduced GSH content and GSH reductase (GR: EC 1.6.4.2) activity, but interestingly affected neither fresh weight nor dry weight nor relative injury. Application of BSO up to 1 mM during chilling at 5 degrees C reduced the fresh and dry weights of shoots and roots and increased relative injury from 10 to almost 40%. Buthionine sulfoximine also induced a decrease in GR activity of 90 and 40% in roots and shoots, respectively. Addition of GSH or gamma EC together with BSO to the nutrient solution protected the seedlings from the BSO effect by increasing the levels of GSH and GR activity in roots and shoots. During chilling, the level of abscisic acid increased both in controls and BSO-treated seedlings and decreased after chilling in roots and shoots of the controls and in the roots of BSO-treated seedlings, but increased in their shoots. Taken together, our results show that BSO did not reduce chilling tolerance of the maize genotype analyzed by inhibiting abscisic acid accumulation but by establishing a low level of GSH. which also induced a decrease in GR activity.
PLANTA 211 (4): 528-536, DOI: 10.1007/s004250000308, SEP 2000

Weber, M; Suter, M; Brunold, C; Kopriva, S
Sulfate assimilation in higher plants - Characterization of a stable intermediate in the adenosine 5 '-phosphosulfate reductase reaction
The enzyme catalysing the reduction of adenosine 5'-phosphosulfate (AdoPS) to sulfite in higher plants, AdoPS reductase, is considered to be the key enzyme of assimilatory sulfate reduction. In order to address its reaction mechanism, the APR2 isoform of this enzyme from *Arabidopsis thaliana* was overexpressed in Escherichia coli and purified to homogeneity. Incubation of the enzyme with [S-35]AdoPS at 4 degrees C resulted in radioactive labelling of the protein. Analysis of APR2 tryptic peptides revealed (SO32-)-S-35 bound to Cys248, the only Cys conserved between AdoPS and prokaryotic phosphoadenosine 5'-phosphosulfate reductases. Consistent with this result, radioactivity could be released from the protein by incubation with thiols, inorganic sulfide and sulfite. The intermediate remained stable, however, after incubation with sulfate, oxidized glutathione or AdoPS. Because truncated APR2, missing the thioredoxin-like C-terminal part, could be labelled even at 37 degrees C, and because this intermediate was more stable than the complete protein, we conclude that the thioredoxin-like domain was required to release the bound SO32- from the intermediate. Taken together, these results demonstrate for the first time the binding of (SO32-)-S-35 from [S-35]AdoPS to AdoPS reductase and its subsequent release, and thus contribute to our understanding of the molecular mechanism of AdoPS reduction in plants.
EUROPEAN JOURNAL OF BIOCHEMISTRY 267 (12): 3647-3653, DOI: 10.1046/j.1432-1327.2000.01394.x, JUN 2000

Harms, K; von Ballmoos, P; Brunold, C; Hofgen, R; Hesse, H
Expression of a bacterial serine acetyltransferase in transgenic potato plants leads to increased levels of cysteine and glutathione
The coding sequence of the wild-type, cys-sensitive, cysE gene from Escherichia coli, which encodes an enzyme of the cysteine biosynthetic pathway, namely serine acetyltransferase (SAT, EC 2.3.1.30), was introduced into the genome of potato plants under the control of the cauliflower mosaic virus 35S promoter. In order to target the protein into the chloroplast, cysE was translationally fused to the 5'-signal sequence of rbcS from *Arabidopsis thaliana*. Transgenic plants showed a high accumulation of the cysE mRNA. The chloroplastic localisation of the E. coli SAT protein was demonstrated by determination of enzymatic activities in enriched organelle fractions. Crude leaf extracts of these plants exhibited up to 20-fold higher SAT activity than those prepared from wild-type plants. The transgenic potato plants expressing the E. coli gene showed not only increased levels of enzyme activity but also exhibited elevated levels of cysteine and glutathione in leaves. Both were up to twofold higher than in control plants. However, the thiol content in tubers of transgenic lines was unaffected. The alterations observed in leaf tissue had no effect on the expression of O-acetylserine(thiol)-lyase, the enzyme which converts O-acetylserine, the product of SAT, to cysteine. Only a minor effect on its enzymatic activity was observed. In conclusion, the results presented here demonstrate the importance of SAT in plant cysteine biosynthesis and show that production of cysteine and related sulfur-containing compounds can be enhanced by metabolic engineering.
PLANT JOURNAL 22 (4): 335-343, DOI: 10.1046/j.1365-313x.2000.00743.x, MAY 2000

Koprivova, A; Suter, M; Op den Camp, R; Brunold, C; Kopriva, S
Regulation of sulfate assimilation by nitrogen in Arabidopsis
Using Arabidopsis, we analyzed the effect of omission of a nitrogen source and of the addition of different nitrogen-containing compounds on the extractable activity and the enzyme and mRNA accumulation of adenosine 5'-phosphosulfate reductase (APR). During 72 h without a nitrogen source, the APR activity decreased to 70% and 50% of controls in leaves and roots, respectively, while cysteine (Cys) and glutathione contents were not affected. Northern and western analysis revealed that the decrease of APR activity was correlated with decreased mRNA and enzyme levels. The reduced APR activity in roots could be fully restored within 24 h by the addition of 4 mM each of NO3-, NH4+, or glutamine (Gln), or 1 mM O-acetylserine (OAS). (SO42-)-S-35 feeding showed that after addition of NH4+, Gln, or OAS to nitrogen-starved plants, incorporation of S-35 into proteins significantly increased in roots; however, glutathione and Cys labeling was higher only with Gln and OAS or with OAS alone, respectively. OAS strongly increased mRNA levels of all three APR isoforms in roots and also those of sulfite reductase, Cys synthase, and serine acetyltransferase. Our data demonstrate that sulfate reduction is regulated by nitrogen nutrition at the transcriptional level and that OAS plays a major role in this regulation.
PLANT PHYSIOLOGY 122 (3): 737-746, DOI: 10.1104/pp.122.3.737, MAR 2000

Suter, M; von Ballmoos, P; Kopriva, S; den Camp, RO; Schaller, J; Kuhlemeier, C; Schurmann, P; Brunold, C
Adenosine 5 '-phosphosulfate sulfotransferase and adenosine 5 '-phosphosulfate reductase are identical enzymes
Adenosine 5'-phosphosulfate (APS) sulfotransferase and APS reductase have been described as key enzymes of assimilatory sulfate reduction of plants catalyzing the reduction of APS to bound and free sulfite, respectively. APS sulfotransferase was purified to homogeneity from *Lemna minor* and compared with APS reductase previously obtained by functional complementation of a mutant strain of Escherichia coli with an *Arabidopsis thaliana* cDNA library. APS sulfotransferase was a homodimer with a monomer M-r of 43,000, Its amino acid sequence was 73% identical with APS reductase. APS sulfotransferase purified from Lemna as well as the recombinant enzyme were yellow proteins, indicating the presence of a cofactor. Like recombinant APS reductase, recombinant APS sulfotransferase used APS (K-m = 6.5 mu M) and not adenosine 3'-phosphate 5'-phosphosulfate as sulfonyl donor. The V-max of recombinant Lemna APS sulfotransferase (40 mu mol min(-1) mg protein(-1)) was about 10 times higher than the previously published V-max of APS reductase. The product of APS sulfotransferase from APS and GSH was almost exclusively SO32-. Bound sulfite in the form of S-sulfoglutathione was only appreciably formed when oxidized glutathione was added to the incubation mixture. Because SO32- was the first reaction product of APS sulfotransferase, this enzyme should be renamed APS reductase.
JOURNAL OF BIOLOGICAL CHEMISTRY 275 (2): 930-936, DOI: 10.1074/jbc.275.2.930, Jan 14 2000

Kopriva, S; Muheim, R; Koprivova, A; Trachsel, N; Catalano, C; Suter, M; Brunold, C
Light regulation of assimilatory sulphate reduction in *Arabidopsis thaliana*
Adenosine 5'-phosphosulphate reductase (APR) is considered to be a key enzyme of sulphate assimilation in higher plants. We analysed the diurnal fluctuations of total APR activity and protein accumulation together with the mRNA levels of three APR isoforms nf *Arabidopsis thaliana*. The APR activity reached maximum values 4h after light onset in both shoots and roots; the minimum activity was detected at the beginning of the night. During prolonged light, the activity remained stable and low in shoots, but followed the normal rhythm in roots. On the other hand, the activity decreased rapidly to undetectable levels within 24h of prolonged darkness both in shoots and roots. Subsequent re-illumination restored the activity to 50% in shoots and to 20% in roots within 8h. The mRNA levels of all three APR isoforms showed a diurnal rhythm, with a maximum at 2h after light onset. The variation of APR2 mRNA was more prominent compared to APR1 and APR3. (SO42-)-S-35 feeding experiments showed that the incorporation of S-35 into reduced sulphur compounds in vivo was significantly higher in light than in the dark. A strong increase of mRNA and protein accumulation as well as enzyme activity during the last 4h of the dark period was observed, implying that light was not the only factor involved in APR regulation. Indeed, addition of 0.5% sucrose to the nutrient solution after 38h of darkness led to a sevenfold increase of root APR activity over 6h. We therefore conclude that changes in sugar concentrations are also involved in APR regulation.
PLANT JOURNAL 20 (1): 37-44, DOI: 10.1046/j.1365-313X.1999.00573.x, OCT 1999

Ammann, M; Siegwolf, R; Pichlmayer, F; Suter, M; Saurer, M; Brunold, C
Estimating the uptake of traffic-derived NO2 from N-15 abundance in Norway spruce needles
The N-15 ratio of nitrogen oxides (NOx) emitted from vehicles, measured in the air adjacent to a highway in the Swiss Middle Land, was very high [delta(15)N(NO2) + 5.7 parts per thousand]. This high N-15 abundance was used to estimate lone-term NO2 dry deposition into a forest ecosystem by measuring delta(15)N in the needles and the soil of potted and autochthonous spruce trees [*Picea abies* (L.) Karst] exposed to NO2 in a transect orthogonal to the highway, delta(15)N in the current-year needles of potted trees was 2.0 parts per thousand, higher than that of the control after 4 months of exposure close to the highway, suggesting a 25% contribution to the N-nutrition of these needles. Needle fall into the pots was prevented by grids placed above the soil, while the continuous decomposition of needle litter below the autochthonous trees over previous years has increased delta(15)N values in the soil, resulting in parallel gradients of delta(15)N in soil and needles with distance from the highway. Estimates of NO2 uptake into needles obtained from the delta(15)N data were significantly correlated with the inputs calculated with a shoot gas exchange model based on a parameterisation widely used in deposition modelling. Therefore, we provide an indication of estimated N inputs to forest ecosystems via dry deposition of NO2 at the receptor level under field conditions.
OECOLOGIA 118 (2): 124-131, DOI: 10.1007/s004420050710, FEB 1999

von Ballmoos, P; Ammann, M; Egger, A; Suter, M; Brunold, C
NO2-induced nitrate reductase activity in needles of Norway spruce (*Picea abies*) under laboratory and field conditions
The induction of activity of the enzyme nitrate reductase (NR, EC 1.6.6.1, 1.6.6.2) in needles of Norway spruce (*Picea abies* [L.] Karst.) by nitrogen dioxide (NO2) was studied under laboratory and field conditions. In fumigation chambers an increase in nitrate reductase activity (NRA) was detected 4 h after the start of the NO2 treatment. During the first 2 days with 100 mu g NO2 m(-3), NRA reached a constant level and did not change during the following 4 days. At the same level of NO2, NRA was lower in needles from trees grown on NPK-fertilized soil than on non-fertilized soil. After the transfer of spruce trees from fertilized soil to NPK-rich nutrient solution, NRA was transiently increased. This effect was assigned to root injuries causing nitrate transport to the shoot and subsequent induction of NRA. Neither trees on fertilized soil nor trees transferred to NPK-poor nutrient solution had increased NRA unless NO2 was provided. The NO2 gradient in the vicinity of a highway was used to test the long-term effect of elevated levels of NO2 on needle NRA of potted and field-grown spruce trees. Compared with less polluted sites, permanently increased NRAs were detected when NO2 concentrations were above 20 mu g m(-3). Controls of field measurements some 10 years after the introduction of catalytic converters in cars showed no significant change neither in NO2 levels nor in the decreasing NRA of spruce needles with the distance from the highway.
PHYSIOLOGIA PLANTARUM 102 (4): 596-604, DOI: 10.1034/j.1399-3054.1998.1020415.x, APR 1998

Burgener, M; Suter, M; Jones, S; Brunold, C
Cyst(e)ine is the transport metabolite of assimilated sulfur from bundle-sheath to mesophyll cells in maize leaves
The intercellular distribution of the enzymes and metabolites of assimilatory sulfate reduction and glutathione synthesis was analyzed in maize (*Zea mays* L. cv LG 9] leaves. Mesophyll cells and strands of bundle-sheath cells from second leaves of 11-d-old maize seedlings were obtained by two different mechanical-isolation methods. Cross-contamination of cell preparations was determined using ribulose bisphosphate carboxylase (EC 4.1.1.39) and nitrate reductase (EC 1.6.6.1) as marker enzymes for bundle-sheath and mesophyll cells, respectively. ATP sulfurylase (EC 2.7.7.4) and adenosine 5'-phosphosulfate sulfotransferase activities were detected almost exclusively in the bundle-sheath cells, whereas GSH synthetase (FC 6.3.2.3) and cyst(e)ine, gamma-glutamylcysteine, and glutathione were located predominantly in the mesophyll cells. Feeding experiments using [S-35]sulfate with intact leaves indicated that cyst(e)ine was the transport metabolite of reduced sulfur from bundle-sheath to mesophyll cells. This result was corroborated by tracer experiments, which showed that isolated bundle-sheath strands fed with [S-35]sulfate secreted radioactive cyst(e)ine as the sole thiol into the resuspending medium. The results presented in this paper show that assimilatory sulfate reduction is restricted to the bundle-sheath cells, whereas the formation of glutathione takes place predominantly in the mesophyll cells, with cyst(e)ine functioning as a transport metabolite between the two cell types.
PLANT PHYSIOLOGY 116 (4): 1315-1322, DOI: 10.1104/pp.116.4.1315, APR 1998

Landolt, W; Bucher, JB; Schulin, R; Körner, C; Brunold, C
Effects of elevated CO2 concentration and N deposition on spruce-beech model ecosystems
The Swiss ICAT project, currently underway at the Birmensdorf open-top chamber facility, aims at investigating the biological effects of both elevated CO2 and nitrogen deposition on model ecosystems composed of provenances and clones of young spruce and beech trees as well as an understory of four herbaceous species and ivy. The plants are growing in natural unfertilized forest soils taken from two sites (acidic and calcareous) and transferred into 32 lysimeters, each with a ground area of 3 m(2). The model ecosystems were established in fall 1994. Beginning at the end of January 1995, these communities were exposed to either ambient or elevated CO2 (ambient + 200 mu l l(-1)) concentrations and 5 or 50 kg N ha(-1) a(-1) wet deposition. Emphasis is on understanding ecosystem level responses and their explanation by downscaling to plant and cell level reactions. The multidisciplinary project team will investigate the carbon, water and nutrient cycle.
IMPACTS OF GLOBAL CHANGE ON TREE PHYSIOLOGY AND FOREST ECOSYSTEMS 52 317-324; 1998

Koprivova, A; Brunold, C; Kopriva, S
Influence of nitrogen availability on sulfate assimilation pathway in *Arabidopsis thaliana*.
PHOTOSYNTHESIS: MECHANISMS AND EFFECTS, VOLS I-V 3629-3632; 1998

Kocsy, G; Owttrim, G; Brander, K; Brunold, C
Effect of chilling on the diurnal rhythm of enzymes involved in protection against oxidative stress in a chilling-tolerant and a chilling-sensitive maize genotype
The effect of chilling on diurnal changes in activity of adenosine 5'-phosphosulfate sulfotransferase, glutathione reductase (EC 1.6.4.2) and glutathione transferase CEC 2.5.1.18) was analysed in the second leaf of Z 7, a chilling-tolerant, and Penjalinan, a chilling-sensitive maize (*Zea mays* L.) genotype. Nitrate reductase (EC 1.6.6.1) was measured for comparison. All enzyme activities examined changed with a typical diurnal rhythm in both genotypes cultivated at 25 degrees C. Adenosine 5'-phosphosulfate sulfotransferase and nitrate reductase activity peaked during the light period, then decreased and reached lowest levels at the end of the dark period. Glutathione reductase activity increased in the dark and decreased during the light period. Maximum glutathione transferase activities were measured in the middle of the light period, minimal ones in the middle of the dark period. At 12 degrees C these diurnal changes were eliminated in all enzymes examined of both genotypes. The average adenosine 5'-phosphosulfate sulfotransferase and glutathione reductase activity were higher in the chilling-tolerant Z 7 than in the sensitive Penjanilan at 12 degrees C in the light. Increased levels of both enzymes may contribute in establishing increased levels of cysteine and reduced glutathione in the chilling-tolerant Z 7. Indeed it has been shown before that the chilling-tolerant maize genotypes contain higher levels of both compounds at low temperatures than chilling-sensitive ones.
PHYSIOLOGIA PLANTARUM 99 (2): 249-254, DOI: 10.1034/j.1399-3054.1997.990206.x, FEB 1997

Kocsy, G; Brunner, M; Rüegsegger, A; Stamp, P; Brunold, C
Glutathione synthesis in maize genotypes with different sensitivities to chilling
The effect of chilling on enzymes, substrates and products of sulfate reduction, gultathione synthesis and metabolism was studied in shoots and roots of maize (*Zea mays* L.) genotypes with different chilling sensitivity. At full expansion of the second leaf, chilling at 12 degrees C inhibited dry weight increase in shoots and roots compared to controls at 25 degrees C and induced an increase in adenosine 5'-phosphosulfate sulfotransferase and gamma-glutamylcysteine synthetase (EC 6.3.2.2) activity in the second leaf of all genotypes tested. Glutathione synthetase CEC 6.3.2.3) activity was about one order of magnitude higher than gamma-glutamylcysteine synthetase activity, but remained unchanged during chilling except for one genotype. During chilling, cysteine and glutathione content of second leaves increased to significantly higher levels in the two most chilling-tolerant genotypes. Comparing the most tolerant and most sensitive genotype showed that chilling induced a greater incorporation of S-35 from [S-35]sulfate into cysteine and glutathione in the chilling-tolerant than in the sensitive genotype. Chilling decreased the amount of S-35-label incorporated into proteins in shoots of both genotypes, but had no effect on this incorporation in the roots. Glutathione reductase (EC 1.6.4.2) and nitrate reductase (EC 1.6.6.1) activity were constitutively higher in the chilling-tolerant genotypes, but showed no changes in most examined genotypes during 3 d at 12 degrees C. Our results indicate that in maize glutathione is involved in protection against chilling damage.
PLANTA 198 (3): 365-370, DOI: 10.1007/BF00620052, MAR 1996

Furtig, K; Rüegsegger, A; Brunold, C; Brändle, R
Sulphide utilization and injuries in hypoxic roots and rhizomes of common reed (*Phragmites australis*)
The presented investigations have been carried out in order to estimate toxic sulphide levels and to examine detoxification capabilities in roots and rhizomes of the common reed (*Phragmites australis*). Underground organs of common reed are sensitive towards sulphide above 1 mM applied exogenously under hypoxia. However, certain tolerance may be achieved by sulphide detoxification. Accumulated sulphide is partially used for the synthesis of npn-toxic thiols, mainly glutathione: But the detoxification capacity of the fw in roots and 300 underground organs is limited. Maximum concentrations of thiols are about 60 nmol/g(-1) nmol/g(-1) fw in rhizomes. Energy metabolism is considerably affected by low sulphide concentrations of 1 mM for 4 days, and immediately disturbed by increased concentrations up to 6 mM sulphide. Adenylate energy charge, total adenylates, posthypoxic respiration, and fermentation capacity decrease significantly. Roots are more sensitive than rhizomes.
FOLIA GEOBOTANICA & PHYTOTAXONOMICA 31 (1): 143-151, DOI: 10.1007/BF02804003, 1996

Galli, U; Schuepp, H; Brunold, C
Thiols in cadmium- and copper-treated maize (*Zea mays* L)
Maize plants (Zen mays L. cv. Honeycomb F-l) were grown on quartz sand containing amounts of Cd or Cu which resulted in comparable internal contents in the roots. Fresh and dry weights and the content of Cd or Cu were measured in roots and shoots after eight weeks. In addition, cysteine, gamma-glutamylcysteine (gamma EC), glutathione (GSH) and the thiols in heavy-metal-binding peptides (HMBPs) were determined in the roots. At low internal contents, Cd and Cu inhibited root growth to the same extent. Inhibition by Cu was enhanced, however, at high internal contents, indicating that Cu was more toxic than Cd. Separation of extracts from roots of Cd- and Cu-treated plants on a Sephadex G-50 column resulted in HMBP complexes with relative molecular masses (M(r)s) of 6200 and 7300, respectively. Separation of these HMBP-complexes using HPLC resulted in a distinct pattern of thiol compounds for each heavy metal. The accumulation of HMBPs was linearly dependent on the content of Cd at all values examined. In Cu-treated roots, HMBP accumulation was linearly dependent on the internal Cu content only up to 7.1 mu mol . g(-1) dry weight. At internal contents which caused an enhanced inhibition of root growth, no further significant increase in the HMBP content was detected. At these internal Cu contents an increased transport of Cu to the shoot was measured. This result indicates that HMBPs are involved in reducing heavy-metal transport from roots to shoots.
PLANTA 198 (1): 139-143; JAN 1996

Ammann, M; von Ballmoos, P; Stalder, M; Suter, M; Brunold, C
Uptake and assimilation of atmospheric NO2-N by spruce needles (*Picea abies*): A field study
NO2 enters spruce needles by gas exchange through the stomata. Nitrate formed from NO2 is reduced in the cytosol by nitrate reductase (NR), the rate limiting enzyme of the nitrogen assimilatory pathway. A linear relationship was found between the nitrate reductase activity (NRA) NO2 concentration and the amount of N incorporated into amino acids and proteins, so that NRA was suggested as an estimate of NO2-uptake. In the present field study, 50 spruce trees (*Picea abies*) have been selected, which grow in a natural habitat in a NO2 concentration gradient in a forest crossed by a highway which is a major NO source. At part of the sites, the microclimatic conditions have been recorded, so that common models of local gas exchange of the needles could be used to estimate stomatal uptake of NO2. NRA was investigated as a function of radiation and stomatal uptake on the day before needle sampling. Close to the highway NRA was permanently elevated with a maximum in summer. As with the laboratory results, a linear relationship between stomatal uptake and NRA was found. Total N-content of current year shoots was not affected by the additional N-source provided by airborne NO2. The present study shows that the gas exchange models are consistent with the physiological reactions of spruce needles on a local level and therefore contribute to the validation of calculations of NO2 dry deposition to spruce forests.
WATER AIR AND SOIL POLLUTION 85 (3): 1497-1502, DOI: 10.1007/BF00477193, DEC 1995

AMMANN, M; STALDER, M; SUTER, M; BRUNOLD, C; BALTENSPERGER, U; JOST, DT; TÜRLER, A; GÄGGELER, HW
TRACING UPTAKE AND ASSIMILATION OF NO2 IN SPRUCE NEEDLES WITH N-13
For the first time, spruce shoots (*Picea abies* [L.] Karst,) were fumigated in vivo with N-13-labelled NO2 with the aim of elucidating the mechanism of NO2-trapping in the apoplast of the substomatal cavity. Uptake by the needles could be monitored on-line, and a quantitative analysis of the activity records delivered a deposition velocity in agreement with the common dry deposition estimates and ruled out rapid export processes, A fast extraction procedure was applied which revealed that NO2 did not produce any detectable traces of nitrite, In needles in which the enzymes of nitrate reduction were not induced by prior fumigation with NO2, incorporation of NO2 was partially inhibited as compared to the fully induced shoots which took up and assimilated NO2 as expected from a constant influx. The only labelled inorganic species found in the extracts was nitrate (60%), whereas the rest of the label (40%) was assimilated organic nitrogen, A quantitative analysis of the data shows that the reaction of NO2 in the apoplast yields at least three times more nitrate than nitrite, so that the existing models about the apoplastic trapping reaction, disproportionation or antioxidant scavenging, which both postulate substantial production of nitrite, have to be reconsidered.
JOURNAL OF EXPERIMENTAL BOTANY 46 (292): 1685-1691, DOI: 10.1093/jxb/46.11.1685, NOV 1995

KAST, D; STALDER, M; RÜEGSEGGER, A; GALLI, U; BRUNOLD, C
EFFECTS OF NO2 AND NITRATE ON SULFATE ASSIMILATION IN MAIZE
The effect of nitrogen dioxide (NO2) and nitrate on sulfate assimilation was studied in maize seedlings. The seedlings (*Zea mays* L. cv. LG 9) were grown on a N-free nutrient solution for 10 days and subsequently fumigated with 520 nLL(-1) NO2 or transferred to a nutrient solution containing 4 mM nitrate. Fresh weight, contents of protein, cysteine, gamma-glutamylcysteine and glutathione, and the extractable activity of adenosine 5'-phosphosulfate sulfotransferase, a key enzyme of sulfate assimilation, were measured in the second, third and fourth leaves during the following 7 days. For comparison, the activity of nitrate reductase was measured. The level of extractable proteins was higher in the leaves of fumigated or nitrate treated seedlings than in leaves of controls without NO2 and nitrate. Adenosine 5/-phosphosulfate sulfotransferase and nitrate reductase activities of the second and third leaves increased to a significantly higher level after 1 day of NO2 fumigation or nitrate treatment. Nitrate induced a significantly higher level of glutathione in the leaves, whereas its level at NO2 fumigation was not always significantly higher than that of the controls. Even though the quantitative changes in enzyme activities induced by nitrate were much greater, our results, in principle, show the same qualitative effects of nitrate and NO2, indicating that NO2 was used as a N-source and regulated sulfate assimilation in the same way as nitrate.
JOURNAL OF PLANT PHYSIOLOGY 147 (1): 9-14; OCT 1995

BRUNNER, M; KOCSY, G; RÜEGSEGGER, A; SCHMUTZ, D; BRUNOLD, C
EFFECT OF CHILLING ON ASSIMILATORY SULFATE REDUCTION AND GLUTATHIONE SYNTHESIS IN MAIZE
The contents of cysteine, gamma-glutamylcysteine and glutathione, as well as the activity of enzymes of assimilatory sulfate reduction and glutathione synthesis in second leaves and root tips of maize seedlings (*Zea mays* L. cv. LG 11) cultivated at low temperatures, were compared with controls grown at 25 degrees C. In vitro nitrate reductase (EC 1.6.6.1) activity was measured for comparison. Compared with the controls, adenosine 5'-phosphosulfate sulfotransferase activity was 3.5- and 5.5-fold higher at 12 degrees C in second leaves and root tips, respectively. After 3 days of growth, the activity of gamma-glutamylcysteine synthetase (EC 6.3.2.2) and glutathione synthetase (EC 6.3.2.3) in the second leaves was also significantly higher at 12 degrees C compared with 25 degrees C. Consistent with these enzyme activities, there was an increase in cysteine and glutathione contents both in second leaves and root tips after 3 days of growth at 12 degrees C. Nitrate reductase activity of second leaves and root tips was not affected by growth at 12 degrees C, indicating that the increase in adenosine 5'-phosphosulfate sulfotransferase activity and of the enzymes of glutathione synthesis was specific. Our results demonstrate that the increased activity of a key enzyme of assimilatory sulfate reduction and of the enzymes of glutathione synthesis contribute to the increased glutathione levels measured at 12 degrees C.
JOURNAL OF PLANT PHYSIOLOGY 146 (05. Jun): 743-747; SEP 1995

GALLI, U; SCHÜEPP, H; BRUNOLD, C

THIOLS OF CU-TREATED MAIZE PLANTS INOCULATED WITH THE ARBUSCULAR-MYCORRHIZAL FUNGUS GLOMUS INTRARADICES

Mycorrhizal colonization of roots. fresh weight, content of cysteine, gamma-glutamylcysteine (gamma EC), glutathione (GSH), thiol groups in Cu-binding peptides (CuBP), and the uptake of Cu were measured in roots and shoots of maize (*Zea mays* L., cv. Honeycomb F-1) grown in quartz sand, with Cu at 0, 4.5, 9, 15 and 30 mu g g(-1) added with or without inoculum of the arbuscular-mycorrhizal fungus (AMF) Glomus intraradices. In control plants (no Cu added) AMF significantly reduced shoot growth, but did not affect root growth. At an external Cu supply of 9 mu g (g quartz sand)(-1) or higher, both mycorrhizal colonization and growth of roots and shoots of mycorrhizal and nonmycorrhizal plants were significantly reduced. With up to 9 mu g Cu g(-1), mycorrhizal colonization increased the content of cysteine, gamma EC and GSH in the roots. However, the amount of thiols in CuBPs was not increased by mycorrhizal colonization in Cu-treated plants and no differences in Cu uptake were detected between non-mycorrhizal and mycorrhizal plants. A CuBP-complex with a relative molecular mass of 7 300 and a SH:Cu ratio of 1.77:1 was separated on a Sephadex G-50 column from both non-inoculated and inoculated roots of Cu-treated plants. HPLC chromatography of the CuBPs of both non-inoculated and inoculated roots resulted in a similar peak pattern, indicating that no additional CuBPs were formed by the fungus. In conclusion, our results do not support the idea that AMF protects maize from Cu-toxicity.

PHYSIOLOGIA PLANTARUM 94 (2): 247-253, DOI: 10.1034/j.1399-3054.1995.940210.x, JUN 1995

WEBER, P; NUSSBAUM, S; FUHRER, J; GFELLER, H; SCHLUNEGGER, UP; BRUNOLD, C; RENNENBERG, H

UPTAKE OF ATMOSPHERIC (NO2)-N-15 AND ITS INCORPORATION INTO FREE AMINO-ACIDS IN WHEAT (*TRITICUM-AESTIVUM*)

Five-week-old wheat plants were exposed, under controlled environmental conditions, to 60 nl l(-1) (NO2)-N-15 or to purified air. After 48 and 96 h of exposure, leaves, stalks and roots were analysed for N-15-enrichment in alpha-amino nitrogen of soluble, free amino acids. In addition, the in vitro nitrate reductase (NR, EC 1.6.6.1) and nitrite reductase (NiR, EC 1.7.7.1) activities were determined in the leaves. NR activity in the leaves decreased continuously during the 96-h exposure to purified air. In the leaves exposed to (NO2)-N-15, NR activity increased within the first 24 h, then decreased, and reached the level of controls after 96 h. NiR activity in leaves exposed to purified air was almost constant during the 96-h exposure. In leaves exposed to (NO2)-N-15, NiR activity increased within the first 48 h, then decreased, and reached the level of controls after 72 h. Exposure to (NO2)-N-15 enhanced the total content of soluble, free amino acids in all tissues analysed. Most of this increase was attributed to Glu in the leaves and to Asn plus Gin in the stalks and the roots. After 48 h exposure to (NO2)-N-15 the highest N-15-enrichment in the a-amino group of soluble, free amino acids was observed in the leaves, the lowest enrichment in the roots. The main labelled amino compounds were Glu (with 8.0% N-15 enrichment compared to the control), gamma-aminobutyric acid (GABA; 7.9%), Ala (7.2%), Ser (6.8%), Asp (5.5%) and Gin (4.6%). Appreciable incorporation of N-15 into Asn was not found. After 96 h exposure to (NO2)-N-15 the N-15 enrichment in the a-amino group of soluble, free amino acids in the leaves declined as compared to the values obtained after 48 h fumigation. The possible pathway and the time course of N-15 incorporation into soluble, free amino acids from the (NO2)-N-15 absorbed are discussed.

PHYSIOLOGIA PLANTARUM 94 (1): 71-77, DOI: 10.1034/j.1399-3054.1995.940111.x, MAY 1995

FARAGO, S; BRUNOLD, C
REGULATION OF THIOL CONTENTS IN MAIZE ROOTS BY INTERMEDIATES AND EFFECTORS OF GLUTATHIONE SYNTHESIS
The regulation of the synthesis and the levels of GSH and other acid-soluble thiols was studied in maize roots by applying GSH, GSH monoethylester, OTC (L-2-oxothiazolidine-4-carboxylic acid), the GSH precursors cysteine and gamma-EC (gamma-glutamylcysteine), the gamma-EC synthetase (EC 6.3.2.2) inhibitor BSO (buthionine S,R-sulfoximine), and the herbicide safener benoxacor (4-dichloroacetyl-3,4-dihydro-3-methyl-2H-1,4-benzooxazine). OTC, GSH and GSH monoethylester did not affect the GSH content, whereas exogenous cysteine and gamma-EC augmented it 1.6- and 2.5-fold, respectively. Both cysteine and gamma-EC strongly increased their endogenous level. Benoxacor increased the activity of adenosine 5'-phosphosulfate sulfotransferase and gamma-EC synthetase within 2 and 4 h, respectively, but did not affecte GSH synthetase activity (EC 6.3.2.3). It rapidly induced a high level of gamma-EC and doubled the cysteine and the GSH contents. When benoxacor and gamma-EC were applied together an additional increase in GSH was detected. BSO alone or together with benoxacor resulted in decreased GSH levels. Both substances combined caused an accumulation of gamma-EC. These results are discussed on the basis of the localization of the GSH synthesizing enzymes in the proplastids and the cytoplasm and the intracellular site of action of benoxacor and BSO.
JOURNAL OF PLANT PHYSIOLOGY 144 (04. Mai): 433-437; OCT 1994

GALLI, U; SCHUEPP, H; BRUNOLD, C
HEAVY-METAL BINDING BY MYCORRHIZAL FUNGI
Ecto- and endomycorrhizal symbiosis can play a crucial role in protecting plant roots from heavy metals (HMs). The efficiency of protection, however, differs between distinct isolates of mycorrhizal fungi and different HMs. Fungal ecotypes from HM-contaminated sites seem to be more tolerant to HMs than reference strains from non-contaminated sites. The abundance of the extramatrical mycelium was shown to be important for HM binding by the fungus. Most of the HMs were demonstrated to be bound to cell wall components like chitin, cellulose, cellulose derivatives and melanins. The chemical nature of HM-binding substances in the fungal cells is not clear. Polyphosphate granules, which were proposed to have this function, seem to be artifacts of specimen preparation. The high N and S concentrations associated with the polyphosphate granules rather indicate the occurrence of HM-thiolate binding by metallothionein-like peptides.
PHYSIOLOGIA PLANTARUM 92 (2): 364-368, DOI: 10.1034/j.1399-3054.1994.920224.x, OCT 1994

FARAGO, S; BRUNOLD, C; KREUZ, K
HERBICIDE SAFENERS AND GLUTATHIONE METABOLISM
Herbicide safeners are chemicals which protect crop plants from injury by certain herbicides, without affecting weed control efficacy of the herbicides. The protective mechanism of herbicide safeners has not yet been fully elucidated, but there is increasing evidence that safeners act by selectively enhancing herbicide detoxification in crop plants. To date, two main detoxification pathways have been related to the mode of action of herbicide safeners. The first includes oxidation and subsequent glucose conjugation, mediated by cytochrome P450-dependent monooxygenases and UDP-glucosyltransferases, respectively. This pathway appears to be important predominantly in safener protection to aryloxyphenoxypropionate and sulfonylurea herbicides. The second pathway represents the conjugation of thiocarbamate sulfoxides and chloroacetanilide herbicides with glutathione. This mechanism is accomplished by either elevating the levels of reduced glutathione or the activity of glutathione S-transferase, or both. Since glutathione has been reported to be involved in several stress situations of plants its function associated with safener-induced herbicide tolerance will be discussed in more detail in this review.
PHYSIOLOGIA PLANTARUM 91 (3): 537-542; JUL 1994

UOTILA, M; AIOUB, AAA; GULLNER, G; KOMIVES, T; BRUNOLD, C
INDUCTION OF GLUTATHIONE TRANSFERASE-ACTIVITY IN WHEAT AND PEA-SEEDLINGS BY CADMIUM
The effect of the heavy metal cadmium on the glutathione transferase (GT) activity was studied in the shoots and roots of wheat and pea seedlings. The exposure to cadmium led to the reduction of plant growth rates and to a marked induction of GT activity in both plants. In wheat the induction was stronger in the roots than in the shoots at low cadmium concentrations (40-160 muM), but at 640 muM cadmium the effect was more pronounced in the shoots (4.0-fold increase of the activity as compared to control). In pea seedlings the induction rates were generally higher in the roots than in the shoots (at 640 muM cadmium the activity in the roots was 340% of the control).
ACTA BIOLOGICA HUNGARICA 45 (1): 11-16; 1994

KOMIVES, T; AIOUB, AAA; GULLNER, G; BRUNOLD, C
EFFECTS OF MERCURIC-CHLORIDE ON THE GLUTATHIONE TRANSFERASE ENZYME-ACTIVITY IN CORN (*ZEA-MAYS* L) PLANTS
Exposure of corn seedlings to different concentrations of the toxic heavy metal mercury(II) led to significantly altered activities of the enzyme glutathione transferase (GT, EC 2.5.1.18) important in glutathione utilization in this plant. Markedly elevated activities were found in the shoots. In the roots, however, the activities slightly decreased, induction was observed only after 3 days of exposure to 30 muM mercury concentration. The induction of GT activity was generally preceded by a decline. These responses are specific, as indicated by their time course and dose-dependence.
CEREAL RESEARCH COMMUNICATIONS 22 (01. Feb): 99-104; 1994

GALLI, U; MEIER, M; BRUNOLD, C
EFFECTS OF CADMIUM ON NONMYCORRHIZAL AND MYCORRHIZAL NORWAY SPRUCE SEEDLINGS [PICEA-ABIES (L) KARST] AND ITS ECTOMYCORRHIZAL FUNGUS LACCARIA-LACCATA (SCOP EX FR) BK AND BR - SULFATE REDUCTION, THIOLS AND DISTRIBUTION OF THE HEAVY-METAL
The effect of cadmium on assimilatory sulphate reduction and thiol content was studied in non-mycorrhizal and mycorrhizal Norway spruce seedlings (*Picea abies*) and its ectomycorrhizal fungus Laccaria laccata. The distribution of cadmium was also investigated. Isotope dilution experiments indicated that the fungus reduced sulphate via adenosine 9'-phosphate 5'-phosphosulphate sulphotransferase, whereas Norway spruce seedlings assimilated sulphate via adenosine 5'-phosphosulphate sulphotransferase in both roots and needles. In mycorrhizal roots only the plant sulphotransferase activity could be measured. Mycorrhizal and non-mycorrhizal roots and the mycelium of *Laccaria laccata* contained increased activities of sulphotransferase and more acid-soluble thiols when cultivated with cadmium. The increase in acid-soluble thiols was due to phytochelatins in roots and to glutathione in Laccaria laccata, where neither phytochelatins nor metallothioneins could be detected. Even though the cadmium content of mycorrhizal roots was slightly higher than that of non-mycorrhizal roots, concentrations of phytochelatin were only half as high as in non-mycorrhizal roots. Cadmium content of needles of mycorrhizal plants was significantly lower than that of non-mycorrhizal plants. Most of the cadmium in *Laccaria laccata* was associated with the cell walls and could be exchanged with Ni2+.
NEW PHYTOLOGIST 125 (4): 837-843, DOI: 10.1111/j.1469-8137.1993.tb03932.x, DEC 1993

NUSSBAUM, S; VON BALLMOOS, P; GFELLER, H; SCHLUNEGGER, UP; FUHRER, J; RHODES, D; BRUNOLD, C

INCORPORATION OF ATMOSPHERIC (NO2)-N-15-NITROGEN INTO FREE AMINO-ACIDS BY NORWAY SPRUCE *PICEA-ABIES* (L) KARST

During spring and autumn 1991, potted 6-year-old spruce trees (*Picea abies* (L.) Karst.) were fumigated with 60 nl . l-1 (NO2)-N-15 for 4 days under controlled conditions in constant light. Current and previous flush needles, the bark and the fine roots were analysed for total N-15 content and incorporation of N-15 into the alpha-amino nitrogen of free amino acids. In addition, in vitro nitrate reductase activity and stomatal conductance of the needles were measured. Nitrate reductase activity was significantly higher in the needles of fumigated trees compared to control trees exposed to filtered air. With an average of 9. 1 % N-15, free glutamate was the pool with the most label. Taking into account the time-course of the labelling of this pool, this figure can be taken as an estimate of the minimum contribution of NO2 to the N nutrition of the needles. N-15-labelled amino acids were also detected in the bark and the roots, indicating export from the needles.

OECOLOGIA 94 (3): 408-414, DOI: 10.1007/BF00317117, JUN 1993

RÜEGSEGGER, A; BRUNOLD, C

LOCALIZATION OF GAMMA-GLUTAMYLCYSTEINE SYNTHETASE AND GLUTATHIONE SYNTHETASE-ACTIVITY IN MAIZE SEEDLINGS

Fresh weight, protein, cysteine, gamma-glutamylcysteine, glutathione, and the extractable activity of the enzymes of glutathione biosynthesis, gamma-glutamylcysteine synthetase (EC 6.3.2.2) and glutathione synthetase (EC 6.3.2.3), were measured in roots, scutella, endosperms, and shoots of 3-, 7-, and 11-d-old maize (*Zea mays* L. cv LG 9) seedlings. In 3-d-old seedlings, the scutella represented 14% of the seedling fresh weight, containing 43% of total protein and 63 and 55% of the activity of gamma-glutamylcysteine synthetase and glutathione synthetase, respectively; in 11-d-old seedlings, the corresponding values were 4.5% for fresh weight, 8.0% for protein content, and 14 and 20% for the enzyme activities. The highest concentrations of thiols were found for cysteine (0.27 mm) in the roots, for glutathione (4.4 mm) in the shoots, and for gamma-glutamylcysteine (13 mum) in the scutella of 3-d-old seedlings. The enzyme activities of roots were localized in subcellular fractions after sucrose density gradient centrifugation. Nearly half of the gamma-glutamylcysteine synthetase activity was detected in the root proplastids of 4-d-old seedlings, whereas <10% of the glutathione synthetase activity was localized in this organelle. Our results demonstrate the importance of scutella in glutathione synthesis in the early stage of seedling development. Unlike chloroplasts, root plastids show only a small proportion of glutathione synthetase activity.

PLANT PHYSIOLOGY 101 (2): 561-566; FEB 1993

VON BALLMOOS, P; NUSSBAUM, S; BRUNOLD, C

THE RELATIONSHIP OF NITRATE REDUCTASE-ACTIVITY TO UPTAKE AND ASSIMILATION OF ATMOSPHERIC (NO2)-N-15-NITROGEN IN NEEDLES OF NORWAY SPRUCE (*PICEA-ABIES* [L] KARST)

Using (NO2)-N-15, relations between nitrate reductase activity and stomatal conductance, N-15-uptake and N-15-glutamate were studied in the two youngest needles flushes of potted Norway spruce (*Picea abies* [L.] Karst.). There were linear correlations between the stomatal conductance and the N-15-uptake and between the N-15-uptake and nitrate reductase (E.C. 1.6.6.1/1.6.6.2) activity. The N-15 labelling of free glutamate shows the assimilation of NO2 from the atmosphere in addition to the nitrogen from the soil. The portion of glutamate originating from (NO2)-N-15 was linearly related to nitrate reductase activity in spring experiments. This indicates that this enzyme activity reflected the rate of NO2-assimilation.

ISOTOPENPRAXIS 29 (01. Feb): 59-70, DOI: 10.1080/10256019308046136, 1993

RÜEGSEGGER, A; BRUNOLD, C
GLUTATHIONE SYNTHESIS IN MAIZE SEEDLINGS
PROCEEDINGS OF THE FIRST EUROPEAN WORKSHOP ON GLUTATHIONE: REGULATION, CELLULAR DEFENCES & CLINICAL ASPECTS 241-247; 1993

KOCSY, G; BRUNOLD, C
EFFECT OF COLD TREATMENT ON GLUTATHIONE SYNTHESIS IN MAIZE SEEDLINGS
PROCEEDINGS OF THE FIRST EUROPEAN WORKSHOP ON GLUTATHIONE: REGULATION, CELLULAR DEFENCES & CLINICAL ASPECTS 248-249; 1993

BRUNOLD, C
REGULATORY INTERACTIONS BETWEEN SULFATE AND NITRATE ASSIMILATION
SULFUR NUTRITION AND ASSIMILATION IN HIGHER PLANTS: REGULATORY AGRICULTURAL AND ENVIRONMENTAL ASPECTS 61-75; 1993

SUTER, M; TSCHANZ, A; BRUNOLD, C
ADENOSINE 5'-PHOSPHOSULFATE SULFOTRANSFERASE FROM NORWAY SPRUCE - BIOCHEMICAL AND PHYSIOLOGICAL-PROPERTIES
Biochemical and physiological properties of adenosine 5'-phosphosulfate sulfotransferase, a key enzyme of assimilatory sulfate reduction, from spruce trees growing under field conditions were studied. The apparent K(m) for adenosine 5'-phosphosulfate (APS) was 29 +/- 5.5-mu-M, its apparent M(r) was 115,000. 5'-AMP inhibited the enzyme competitively with a K(i) of 1 mM, but also stabilized it. MgSO4 at 800 mM increased adenosine 5'-phosphosulfate sulfotransferase activity by a factor of 3, concentrations higher than 1000 mM were inhibitory. Treatment of isolated shoots with nutrient solution containing 1 or 2 mM sulfate, and 3 or 10 mM glutathione, respectively, induced a significant decrease in extractable adenosine 5'-phosphosulfate sulfotransferase activity over 24 h, whereas GSH as well as S2- and up to 5 mM cysteine and up to 200 mM SO-3(2-) had no effect on the in vitro activity of the enzyme. As with other enzymes involved in assimilatory sulfate reduction, namely ATP sulfurylase (EC 2.7.7.4), sulfite reductase (EC 1.8.7.1) and O-acetyl-L-serine sulfhydrylase (EC 4.2.99.8), adenosine 5'-phosphosulfate sulfotransferase was still detected at appreciable activities in 2- and 3-year-old needles. Adenosine 5'-phosphosulfate sulfotransferase activity was low in buds and increased during shoot development, parallel to the chlorophyll content. The enzyme activity was characterized by an annual cycle of seasonal changes with an increase during February and March.
BOTANICA ACTA 105 (3): 190-196; JUN 1992

RÜEGSEGGER, A; BRUNOLD, C
EFFECT OF CADMIUM ON GAMMA-GLUTAMYLCYSTEINE SYNTHESIS IN MAIZE SEEDLINGS
Cysteine, gamma-glutamylcysteine, and glutathione and the extractable activity of the enzymes of glutathione biosynthesis, gamma-glutamylcysteine synthetase (EC 6.3.2.2) and glutathione synthetase (EC 6.3.2.3), were measured in roots and leaves of maize seedlings (*Zea mays* L. cv LG 9) exposed to CdCl2 concentrations up to 200 micromolar. At 50 micromolar Cd2+, gamma-glutamylcysteine contents increased continuously during 4 days up to 21-fold and eightfold of the control in roots and leaves, respectively. Even at 0.5 micromolar Cd2+, the concentration of gamma-glutamylcysteine in the roots was significantly higher than in the control. At 5 micromolar and higher Cd2+ Concentrations, a significant increase in gamma-glutamylcysteine synthetase activity was measured in the roots, whereas in the leaves this enzyme activity was enhanced only at 200 micromolar Cd2+. Labeling of isolated roots with [S-35]sulfate showed that both sulfate assimilation and glutathione synthesis were increased by Cd. The accumulation of gamma-glutamylcysteine in the roots did not affect the root exudation rate of this compound. Our results indicate that maize roots are at least in part autonomous in providing the additional thiols required for phytochelatin synthesis induced by Cd.
PLANT PHYSIOLOGY 99 (2): 428-433, DOI: 10.1104/pp.99.2.428, JUN 1992

RÜEGSEGGER, A; BRUNOLD, C
EFFECT OF CADMIUM AND OR REMOVAL OF KERNELS OR SHOOTS ON THE LEVELS OF CYSTEINE, GAMMA-GLUTAMYL-CYSTEINE, GLUTATHIONE, AND TCA-SOLUBLE THIOLS IN MAIZE SEEDLINGS
Six day old maize seedlings (*Zea mays* L.) were exposed as intact plants (A), after the removal of kernels (B) or shoots (C) or after the removal of kernels and transfer into the dark (D) to 0 or 50 micromolar cadmium for 2 days. The roots were analyzed for fresh weight, total TCA-soluble thiols (including phytochelatins), glutathione, and its precursor compounds cysteine and gamma-glutamyl-cysteine. With all treatments, cadmium caused an increase in the contents of cysteine, gamma-glutamyl-cysteine and total TCA-soluble thiols and a decrease in glutathione content. Our data indicate that the roots are at least in part autonomous to provide the thiols required for phytochelatin synthesis.
PHYTON-ANNALES REI BOTANICAE 32 (3): 109-112; 1992

NEUENSCHWANDER, U; SUTER, M; BRUNOLD, C

REGULATION OF SULFATE ASSIMILATION BY LIGHT AND O-ACETYL-L-SERINE IN *LEMNA-MINOR*-L

The effect of 0.5 millimolar O-acetyl-L-serine added to the nutrient solution on sulfate assimilation of *Lemna minor* L., cultivated in the light or in the dark, or transferred from light to the dark, was examined. During 24 hours after transfer from light to the dark the extractable activity of adenosine 5'-phosphosulfate sulfotransferase, a key enzyme of sulfate assimilation, decreased to 10% of the light control. Nitrate reductase (EC 1.7.7.1.) activity, measured for comparison, decreased to 40%. Adenosine 5'-triphosphate (ATP) sulfurylase (EC 2.7.7.4.) and O-acetyl-L-serine sulfhydrylase (EC 4.2.99.8.) activities were not affected by the transfer. When O-acetyl-L-serine was added to the nutrient solution at the time of transfer to the dark, adenosine 5'-phosphosulfate sulfotransferase activity was still at 50% of the light control after 24 hours, ATP sulfurylase and O-acetyl-L-serine sulfhydrylase activity were again not affected, and nitrate reductase activity decreased as before. Addition of O-acetyl-L-serine at the time of the transfer caused a 100% increase in acid-soluble SH compounds after 24 hours in the dark. In continous light the corresponding increase was 200%. During 24 hours after transfer to the dark the assimilation of (SO4(2-))-S-35 into organic compounds decreased by 80% without O-acetyl-L-serine but was comparable to light controls in its presence. The addition of O-acetyl-L-serine to *Lemna minor* precultivated in the dark for 24 hours induced an increase in adenosine 5'-phosphosulfate sulfotransferase activity so that a constant level of 50% of the light control was reached after an additional 9 hours. Cycloheximide as well as 6-methyl-purine inhibited this effect. In the same type of experiment O-acetyl-L-serine induced a 100-fold increase in the incorporation of label from (SO4(2-))-S-35 into cysteine after additional 24 hours in the dark. Taken together, these results show that exogenous O-acetyl-L-serine has a regulatory effect on assimilatory sulfate reduction of L. minor in light and darkness. They are in agreement with the idea that this compound is a limiting factor for sulfate assimilation and seem to be in contrast to the proposed strict light control of sulfate assimilation.

PLANT PHYSIOLOGY 97 (1): 253-258, DOI: 10.1104/pp.97.1.253, SEP 1991

FARAGO, S; BRUNOLD, C
REGULATION OF ASSIMILATORY SULFATE REDUCTION BY HERBICIDE SAFENERS IN *ZEA-MAYS* L
Effects of the herbicide safeners N,N-diallyl-2,2-dichloroacetamide and 4-dichloroacetyl-3,4-dihydro-3-methyl-2H-1,4,benzooxazin (CGA 154281) on the contents in cysteine and glutathione, on the assimilation of (SO42-)-S-35, and on the enzymes of assimilatory sulfate reduction were analyzed in roots and primary leaves of maize (*Zea mays*) seedlings. Both safeners induced an increase in cysteine and glutathione. In labeling experiments using (SO42-)-S-35, roots of plants cultivated in the presence of safeners contained an increased level of radioactivity in glutathione and cysteine as compared with controls. A significant increase in uptake of sulfate was only detected in the presence of CGA 154281. One millimolar N,N-diallyl-2,2-dichloroacetamide applied to the roots for 6 days increased the activity of adenosine 5'-phosphosulfate sulfotransferase about 20- and threefold in the roots and leaves, respectively, compared with controls. CGA 154281 at 10 micromolar caused a sevenfold increase of this enzyme activity in the roots, but did not affect it significantly in the leaves. A significant increase in ATP-sulfurylase (EC 2.7.7.4) activity was only detected in the roots cultivated in the presence of 10 micromolar CGA 154281. Both safeners had no effect on the activity of sulfite reductase (EC 1.8.7.1) and O-acetyl-L-serine sulfhydrylase (EC 4.2.99.8). The herbicide metolachlor alone or combined with the safeners induced levels of adenosine 5'-phosphosulfate sulfotransferase, which were higher than those of the appropriate controls. Taken together these results show that the herbicide safeners increased both the level of adenosine 5'-phosphosulfate sulfotransferase activity and of the thiols cysteine and glutathione. This indicates that these safeners may be involved in eliminating the previously proposed regulatory mechanism, in which increased concentrations of thiols regulate assimilatory sulfate reduction by decreasing the activities of the enzymes involved.
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