K. Omasa, I. Nouchi, L.J. De Kok (Eds.)

# Plant Responses to Air Pollution and Global Change

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# Plant Responses to Air Pollution and Global Change

With 100 Figures, Including 2 in Color

Springer

Kenji Omasa
Professor, Graduate School of Agricultural and Life Sciences
The University of Tokyo
1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan

Isamu Nouchi
Head, Agro-Meteorology Group
National Institute for Agro-Environmental Sciences
3-1-3 Kannondai, Tsukuba, Ibaraki 305-8604, Japan

Luit J. De Kok
Professor, Laboratory of Plant Physiology
University of Groningen
P.O. Box 14, 9750 AA Haren, The Netherlands

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# Preface

The main force behind climate change is the elevated concentration of $CO_2$ in the atmosphere. Carbon dioxide and air pollutants come mostly from the same industrial sources and diffuse globally, so that air pollution is also part of global change in the present era. The impacts on plants and plant ecosystems have complex interrelationships and lead to global change in a circular manner as changes in land cover and atmospheric and soil environments. Plant metabolism of $CO_2$ and air pollutants and their gas fluxes in plant ecosystems influence the global gaseous cycles as well as the impacts on plants.

The 6th International Symposium on Plant Responses to Air Pollution and Global Changes was held at the Tsukuba Center for Institutes and Epochal Tsukuba, in Tsukuba, Japan, October 19–22, 2004. The aim of the symposium series is to bring together scientists of various disciplines who are actively involved in research on responses of plant metabolism to air pollution and global change. The previous symposia were held in Oxford, UK, 1982 (1st), in Munich, Germany, 1987 (2nd), in Blacksburg, USA, 1992 (3rd), in Egmond aan Zee, The Netherlands, 1997 (4th), and in Pulawy, Poland, 2001 (5th).

This book is one of three publications (this volume and special issues of *Phyton* and the *Journal of Agricultural Meteorology*) coming out of the symposium and contains a selection of invited papers. It also includes current topics on plant metabolism of air pollutants and elevated $CO_2$, responses of whole plants and plant ecosystems, genetics and molecular biology for functioning improvement, experimental ecosystems and climate change research, global carbon-cycle monitoring in plant ecosystems, and remote sensing and modeling of climate change impacts, with additional topics in risk assessment and protection against air pollution and global change in East Asia. Because the authors are researchers from 18 countries, coming from Europe, the United States, Australia, and East Asia, readers can obtain information on current research in those regions as well as finding a source of expert knowledge about the topics that are included.

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Kenji Omasa
Isamu Nouchi
Luit J.De Kok

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# Contributors

**Afanasieva, Larisa V.**, Institute of General and Experimental Biology Siberian Branch of the Russian Academy of Sciences, Sahjanova, 6, Ulan-Ude, 670042, Russia

**An, Ping**, Arid Land Research Center, Tottori University, Hamasaka 1390, Tottori 681-0001, Japan

**Andersson, Inger**, Department of Molecular Biology, Swedish University of Agricultural Sciences, Uppsala Biomedical Centre, Box 590, S-751 24 Uppsala, Sweden

**Andralojc, P. John**, Crop Performance and Improvement Division, Rothamsted Research, Harpenden, Hertfordshire AL5 2JQ, UK

**Asipov, Leonid**, Department of Plant and Environmental Sciences, The Hebrew University of Jerusalem, 91904 Jerusalem, Israel

**Berezhnaya, Nadezhda S.**, Siberian Institute of Plant Physiology and Biochemistry, Siberian Branch of the Russian Academy of Sciences, Lermontova, 132, Irkutsk, 664033, Russia

**Bicarova, Svetlana**, Institute of Geophysics, Slovak Academy of Sciences, Meteorological Observatory, Stara Lesna, 059 60 Tatranska Lomnica, Slovakia

**Blumenröther, Manuela**, Pathology of Woody Plants, Technische Universität München, Am Hochanger 13, 85354 Freising, Germany

**Bolhar-Nordenkampf, Harald R.**, Institute of Ecology and Conservation Biology, University of Vienna, Althanstraße 14, A-1090 Vienna, Austria

**Bytnerowicz, Andrzej**, USDA Forest Service, Pacific Southwest Research Station, 4955 Canyon Crest Drive, Riverside, CA 92507-6090, USA

**Castro, Ana**, Laboratory of Plant Physiology, University of Groningen, P.O. Box 14, 9750 AA Haren, The Netherlands

**De Kok, Luit J.**, Laboratory of Plant Physiology, University of Groningen, P.O. Box 14, 9750 AA Haren, The Netherlands

**Durenkamp, Mark**, Laboratory of Plant Physiology, University of Groningen, P.O. Box 14, 9750 AA Haren, The Netherlands

**Eilertsen, Hans C.**, Norwegian College of Fisheries Science (NFH), University of Tromsø, N-9037 Tromsø, Norway

**Ellis, Brian E.**, Michael Smith Laboratories, University of British Columbia, Vancouver BC V6T 1Z4 Canada

**Eneji, A. Egrinya**, Arid Land Research Center, Tottori University, Hamasaka 1390, Tottori 681-0001, Japan

**Fabian, Peter**, Ecoclimatology, Technische Universität München, Am Hochanger 13, 85354 Freising, Germany

**Fan, Yi-Da**, National Disaster Reduction Center of China, Ministry of Civil Affairs, Bai Guang Lu No.7, Beijing 100053,China

**Fleischer, Peter**, Research Station of Tatra National Park, State Forest of TANAP, 059 60 Tatranska Lomnica, Slovakia

**Funada, Ryo**, Faculty of Agriculture, Tokyo University of Agriculture and Technology, Saiwai-cho 3-5-8, Fuchu, Tokyo 183-8509, Japan

**Gamo, Minoru**, Institute for Environmental Management Technology, National Institute of Advanced Industrial Sciences and Technology, Onogawa, 16-1, Tsukuba, Ibaraki 305-8506, Japan

**Godzik, Barbara**, Institute of Botany, Polish Academy of Sciences, Lubicz 46, 31 512 Krakow, Poland

**Grill, Dieter**, Institut für Pflanzenwissenschaften, Karl-Franzens-Universität Graz, Schubertstraße 51, A-8010 Graz, Austria

**Grimsrud, Anette**, Norwegian College of Fisheries Science (NFH), University of Tromsø, N-9037 Tromsø, Norway

**Grulke, Nancy E.**, USDA Forest Service, Pacific Southwest Research Station, 4955 Canyon Crest Drive, Riverside, CA 92507-6090, USA

**Haberer, Kris**, Forest Botany and Tree Physiology, Albert-Ludwigs Universität Freiburg i. Br., Georges-Köhler-Allee 53/54, 79110 Freiburg, Germany

**Häberle, Karl-Heinz**, Ecophysiology of Plants, Technische Universität München, Am Hochanger 13, 85354 Freising, Germany

**Hajima, Tomohiro**, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Yayoi 1-1-1, Bunkyo-ku, Tokyo 113-8657, Japan

**Haslam, Richard P.**, Crop Performance and Improvement Division, Rothamsted Research, Harpenden, Hertfordshire AL5 2JQ, UK

**Heerdt, Christian,** Ecoclimatology, Technische Universität München, Am Hochanger 13, 85354 Freising, Germany

**Herbinger, Karin,** Institut für Pflanzenwissenschaften, Karl-Franzens Universität Graz, Schubertstraße 51, 8010 Graz, Austria

**Herman, Friedl,** Federal Office and Research Centre for Forests, Seckendorff-Gudent Weg 8, A-1130 Vienna, Austria

**Hogsett, William E.,** US Environmental Protection Agency, National Health and Environmental Effects Laboratory, Western Ecology Division, 200 SW 35th Street, Corvallis, OR 97333, USA

**Hosoi, Fumiki,** Graduate School of Agricultural and Life Sciences, The University of Tokyo, Yayoi 1-1-1, Bunkyo-ku, Tokyo 113-8657, Japan

**Idso, Sherwood B.,** Center for the Study of Carbon Dioxide and Global Change, Tempe, AZ 85285, USA

**Ignatieva, Olga V.,** Siberian Institute of Plant Physiology and Biochemistry, Siberian Branch of the Russian Academy of Sciences, Lermontova, 132, Irkutsk, 664033, Russia

**Inanaga, Shinobu,** Arid Land Research Center, Tottori University, Hamasaka 1390, Tottori 681-0001, Japan

**Ishii, Takashi,** Environmental Science Research Laboratory, Central Research Institute of Electric Power Industry, Abiko 1646, Abiko City, Chiba 270-1194, Japan

**Iwaki, Toshio,** Department of Applied Biochemistry, Osaka Prefecture University, Gakuencho 1-1, Sakai, Osaka, 599-8531, Japan

**Izuta, Takeshi,** Institute of Symbiotic Science and Technology, Tokyo University of Agriculture and Technology, Saiwai-cho 3-5-8, Fuchu, Tokyo 183-8509, Japan

**Jehnes, Sascha,** Forest Botany and Tree Physiology, Albert-Ludwigs Universität Freiburg i. Br., Georges-Köhler-Allee 53/54, 79110 Freiburg, Germany

**Jia, Hai-Kun,** College of Resources Science and Technology, Beijing Normal University, No.19 Xinjiekouwai Street, Beijing 100875, China

**Kaplan, Aaron,** Department of Plant and Environmental Sciences, The Hebrew University of Jerusalem, 91904 Jerusalem, Israel

**Keys, Alfred J.,** Crop Performance and Improvement Division, Rothamsted Research, Harpenden, Hertfordshire AL5 2JQ, UK

**Kimball, Bruce A.,** U.S. Water Conservation Laboratory, USDA, Agricultural Research Service 4331 East Broadway Road, Phoenix, AZ 85040, USA

**Kitaya, Yoshiaki,** Graduate School of Life and Environmental Sciences, Osaka Prefecture University, Gakuen-cho 1-1, Sakai, Osaka 599-8531, Japan

**Koch, Nina,** Ecophysiology of Plants, Technische Universität München, Am Hochanger 13, 85354 Freising, Germany

**Kohno, Yoshihisa,** Environmental Science Research Laboratory, Central Research Institute of Electric Power Industry, Abiko 1646, Abiko City, Chiba 270-1194, Japan

**Koike, Takayoshi,** Field Science Center for Northern Biosphere, Hokkaido University, Kita-9, Nishi-9, Kita-ku, Sapporo, Hokkaido 060-0809, Japan

**Kosugi, Yoshiko,** Graduate School of Agriculture, Kyoto University, Kitashirakawa, Sakyo-ku, Kyoto 606-8502, Japan

**Law, Beverly,** College of Forestry, Oregon State University, Corvallis, OR 97331-5752, USA

**Lawlor, David W.,** Crop Performance and Improvement Division, Rothamsted Research, Harpenden, Hertfordshire, AL5 2JQ, UK

**Lee, E. Henry,** US Environmental Protection Agency, National Health and Environmental Effects Laboratory, Western Ecology Division, 200 SW 35th Street, Corvallis, OR 97333, USA

**Li, Xiao-Yan,** College of Resources Science and Technology, Beijing Normal University, No.19 Xinjiekouwai Street, Beijing 100875, China

**Lieman-Hurwitz, Judy,** Department of Plant and Environmental Sciences, The Hebrew University of Jerusalem, 91904 Jerusalem, Israel

**Liu, Lian-You,** College of Resources Science and Technology, Beijing Normal University, No.19 Xinjiekouwai Street, Beijing 100875, China

**Liu, Puling,** Institute of Soil and Water Conservation, Chinese Academy of Sciences and Ministry of Water Resources, 26# Xinong Rd. Yangling, Shaanxi 712100, China

**Loibl, Wolfgang,** ARC Systems Research, Austrian Research Centers, A-2444 Seibersdorf, Austria

**Lomsky, Bohumir,** VULHM, Jiloviste, Strnady, 15604 Zbraslav-Praha, Czechia

**Löw, Markus,** Ecophysiology of Plants, Technische Universität München, Am Hochanger 13, 85354 Freising, Germany

**Madgwick, Pippa J.**, Crop Performance and Improvement Division, Rothamsted Research, Harpenden, Hertfordshire AL5 2JQ, UK

**Marcus, Yehouda,** Department of Plant Sciences, Tel Aviv University, 69978 Tel Aviv, Israel

**Maruyama, Yutaka,** Forestry and Forest Products Research Institute, Matsunosato 1, Tsukuba, Ibaraki 305-8687, Japan

**Matsumura, Hideyuki,** Environmental Science Research Laboratory, Central Research Institute of Electric Power Industry, Abiko 1646, Abiko City, Chiba 270-1194, Japan

**Matyssek, Rainer,** Ecophysiology of Plants, Technische Universität München, Am Hochanger 13, 85354 Freising, Germany

**Mikhailova, Tatiana A.**, Siberian Institute of Plant Physiology and Biochemistry, Siberian Branch of the Russian Academy of Sciences, Lermontova, 132, Irkutsk, 664033, Russia

**Miles, Godfrey P.**, Michael Smith Laboratories, University of British Columbia, Vancouver BC V6T 1Z4 Canada

**Mori, Ikuko,** National Institute for Environmental Studies, Onogawa 16-2, Tsukuba, Ibaraki, 305-8506, Japan

**Mori, Shigeta,** Tohoku Research Center, Forestry and Forest Products Research Institute Nabeyashiki 92-25, Shimo-Kuriyagawa, Morioka, Iwate 020-0123, Japan

**Murayama, Shohei,** Institute for Environmental Management Technology, National Institute of Advanced Industrial Sciences and Technology, Onogawa 16-1, Tsukuba, Ibaraki 305-8569, Japan

**Nakano, Yoshihisa,** Department of Applied Biochemistry, Osaka Prefecture University, Gakuencho 1-1, Sakai, Osaka, 599-853, Japan

**Nishikawa, Masataka,** National Institute for Environmental Studies, Onogawa 16-2, Tsukuba, Ibaraki, 305-8506, Japan

**Nunn, Angela J.**, Ecophysiology of Plants, Technische Universität München, Am Hochanger 13, 85354 Freising, Germany

**Ohtani, Yoshikazu,** Forestry and Forest Products Research Institute, Matsunosato 1, Tsukuba, Ibaraki 305-8687, Japan

**Oksanen, Elina,** Department of Biology, University of Joensuu, POB 111, 80101 Joensuu, Finland

**Olszyk, David M.**, US Environmental Protection Agency, National Health and Environmental Effects Laboratory, Western Ecology Division, 200 SW 35th Street, Corvallis, OR 97333, USA

**Omasa, Kenji,** Graduate School of Agricultural and Life Sciences, The University of Tokyo, Yayoi 1-1-1, Bunkyo-ku, Tokyo 113-8657, Japan

**Osmond, Barry,** School of Biochemistry and Molecular Biology, Australian National University, P.O. Box 3252 Weston Creek ACT 2611, Australia

**Oßwald, Wolfgang,** Pathology of Woody Plants, Technische Universität München, Am Hochanger 13, 85354 Freising, Germany

**Paoletti, Elena,** IPP-CNR, Via Madonna del Piano, 50019 Sesto Fiorentino, Italy

**Parry, Martin A. J.**, Crop Performance and Improvement Division, Rothamsted Research, Harpenden, Hertfordshire AL5 2JQ, UK

**Pfanz, Hardy,** Institut für Angewandte Botanik, Universität Duisburg-Essen, Campus Essen, Universitätsstraße 5, 45117 Essen, Germany

**Posthumus, Freek S.**, Laboratory of Plant Physiology, University of Groningen, P.O. Box 14, 9750 AA Haren, The Netherlands

**Rachmilevitch, Shimon,** Department of Plant and Environmental Sciences, The Hebrew University of Jerusalem, 91904 Jerusalem, Israel

**Raschi, Antonio,** IBIMET-CNR, Via Madonna del Piano, 50019 Sesto Fiorentino, Italy

**Reiter, Ilja M.**, Ecophysiology of Plants, Technische Universität München, Am Hochanger 13, 85354 Freising, Germany

**Rennenberg, Heinz,** Forest Botany and Tree Physiology, Albert-Ludwigs Universität Freiburg i. Br., Georges-Köhler-Allee 53/54, 79110 Freiburg, Germany

**Romagnoli, Simona,** Department of Microbiology, The Ohio State University, 484 West 12th Avenue, Columbus, OH 43210 USA

**Saigusa, Nobuko,** Institute for Environmental Management Technology, National Institute of Advanced Industrial Sciences and Technology, Onogawa 16-1, Tsukuba, Ibaraki 305-8506, Japan

**Samuel, Marcus A.**, Michael Smith Laboratories, University of British Columbia, Vancouver BC V6T 1Z4 Canada

**Sase, Hiroyuki,** Acid Deposition and Oxidant Research Center, Sowa 1182, Niigata 950-2144, Japan

**Shi, Pei-Jun,** College of Resources Science and Technology, Beijing Normal University, No.19 Xinjiekouwai Street, Beijing 100875, China

**Shigeoka, Shigeru,** Faculty of Agriculture, Kinki University, Nakamachi 3327-204, Nara 631-8505, Japan

**Shimizu, Atsushi,** National Institute for Environmental Studies, Onogawa 16-2, Tsukuba, Ibaraki, 305-8506, Japan

**Shimizu, Hideyuki,** National Institute for Environmental Studies, Onogawa 16-2, Tsukuba, Ibaraki 305-8506 Japan

**Shimizu, Yo,** Graduate School of Agricultural and Life Sciences, The University of Tokyo, Yayoi 1-1-1, Bunkyo-ku, Tokyo 113-8657, Japan

**Smidt, Stefan,** Federal Office and Research Centre for Forests, Seckendorff-Gudent Weg 8, A-1130 Vienna, Austria

**Song, Yang,** College of Resources Science and Technology, Beijing Normal University, No.19 Xinjiekouwai Street, Beijing 100875, China

**Stuiver, C. Elisabeth E.,** Laboratory of Plant Physiology, University of Groningen, P.O. Box 14, 9750 AA Haren, The Netherlands

**Stulen, Ineke,** Laboratory of Plant Physiology, University of Groningen, P.O. Box 14, 9750 AA Haren, The Netherlands

**Sugata, Seiji,** National Institute for Environmental Studies, Onogawa 16-2, Tsukuba, Ibaraki, 305-8506, Japan

**Sugimoto, Nobuo,** National Institute for Environmental Studies, Onogawa 16-2, Tsukuba, Ibaraki, 305-8506, Japan

**Tabita, Robert,** Department of Microbiology, The Ohio State University, 484 West 12th Avenue, Columbus, OH 43210 USA

**Tamoi, Masahiro,** Faculty of Agriculture, Kinki University, Nakamachi 3327-204, Nara 631-8505, Japan

**Tani, Makoto,** Graduate School of Agriculture, Kyoto University, Kitashirakawa, Sakyo-ku, Kyoto 606-8502, Japan

**Tausz, Michael,** Institut für Pflanzenwissenschaften, Karl-Franzens Universität Graz, Schubertstraße 51, A-8010 Graz, Austria

**Then, Christiane,** Ecophysiology of Plants, Technische Universität München, Am Hochanger 13, 85354 Freising, Germany

**Tian, Junliang,** Institute of Soil and Water Conservation, Chinese Academy of Sciences and Ministry of Water Resources, 26# Xinong Rd. Yangling, Shaanxi 712100, China

**Tingey, David T.,** US Environmental Protection Agency, National Health and Environmental Effects Laboratory, Western Ecology Division, 200 SW 35th Street, Corvallis, OR 97333, USA

**Totsuka, Tsumugu,** Acid Deposition and Oxidant Research Center, Sowa 1182, Niigata 950-2144, Japan

**Tsukamoto, Manabu,** Department of Applied Biochemistry, Osaka Prefecture University, Gakuencho 1-1, Sakai, Osaka, 599-8531, Japan

**Wadano, Akira,** Department of Applied Biochemistry, Osaka Prefecture University, Gakuencho 1-1, Sakai, Osaka, 599-8531, Japan

**Wang, Jing-Ai,** College of Geography and Remote Sensing Science, Beijing Normal University, No.19 Xinjiekouwai Street, Beijing 100875, China

**Wang, Lei,** College of Resources Science and Technology, Beijing Normal University, No.19 Xinjiekouwai Street, Beijing 100875, China

**Werner, Herbert,** Ecoclimatology, Technische Universität München, Am Hochanger 13, 85354 Freising, Germany

**Wieser, Gerhard,** Federal Office and Research Centre for Forests, Alpine Timberline Ecophysiology, Rennweg 1, 6020 Innsbruck, Austria

**Yamamoto, Susumu,** Institute for Environmental Management Technology, National Institute of Advanced Industrial Sciences and Technology, Onogawa 16-1, Tsukuba, Ibaraki 305-8569, Japan

**Yazaki, Ken'ichi,** Forestry and Forest Products Research Institute, Matsunosato 1, Tsukuba, Ibaraki 305-8687, Japan

**Yu, Yun-Jiang,** College of Resources Science and Technology, Beijing Normal University, No.19 Xinjiekouwai Street, Beijing 100875, China

**Zhao, Yanzhi,** College of Resources Science and Technology, Beijing Normal University, No.19 Xinjiekouwai Street, Beijing 100875, China

# I. Plant Responses to Air Pollution

# Metabolism of atmospheric sulfur gases in onion

Mark Durenkamp, Freek S. Posthumus, C. Elisabeth E. Stuiver, and Luit J. De Kok

Laboratory of Plant Physiology, University of Groningen, P.O. Box 14, 9750 AA Haren,
The Netherlands

**Summary.** The impact of atmospheric sulfur gases was studied in onion (*Allium cepa* L.). The occurrence of toxic effects of $H_2S$ in onion depended not only on the atmospheric $H_2S$ level but also on the duration of the exposure. Prolonged exposure of onion to $\geq 0.3\ \mu l\ l^{-1}$ $H_2S$ resulted in a strong reduction in shoot biomass production. $H_2S$ exposure resulted in a decrease in the organic N/S ratio at all levels (0.15 to 0.6 $\mu l\ l^{-1}$), which could be attributed to an increase in the pool of secondary sulfur compounds and not to changes in the sulfolipid content. The latter even decreased upon $H_2S$ exposure when expressed on a lipid basis. $SO_2$ exposure resulted in an enhanced content of sulfate and total sulfur in the shoot, whereas roots were not affected. In contrast to exposure to $H_2S$, $SO_2$ exposure did not result in an increase in non-protein organic (secondary) sulfur compounds, which showed that these compounds only were a sink pool for reduced atmospheric sulfur, when both the uptake of sulfate by the roots and its reduction in the shoot were bypassed.

**Key words.** *Allium cepa*, $H_2S$, $SO_2$, Sulfolipids, Sulfur metabolism

## 1. Introduction

Generally, sulfate taken up by the roots is used as the main source of sulfur for plants and the uptake, transport and subcellular distribution of sulfate are mediated by specific sulfate transporter proteins (Hawkesford 2003; Hawkesford et al. 2003; Buchner et al. 2004). The uptake of sulfate by the roots and its transport to other plant parts are highly regulated and the affinity of the sulfate transporters towards sulfate is high; a maximum uptake and transport rate is generally already reached at ≤ 0.1 mM sulfate (Hawkesford and Wray 2000; Durenkamp and De Kok 2004; Buchner et al. 2004). The expression and activity of the sulfate transporter proteins, as well as the activity of the enzymes of the sulfate reduction pathway, strongly depend on the sulfur nutritional status of the plant (Buchner et al. 2004). Prior to its incorporation into organic compounds, sulfate needs to be reduced to sulfide, a process that primarily takes place in the chloroplasts. Subsequently, sulfide is incorporated into cysteine, the precursor for most other organic sulfur compounds (Fig. 1). In most plants the predominant proportion of the organic sulfur is present in the protein fraction as cysteine and methionine residues (up to 70 % of total S), however, species like onion also may contain high amounts of secondary sulfur compounds. Part of the organic sulfur is present in the lipid fraction; in general sulfoquinovosyldiacylglycerol (SQDG) appears to be the predominant plant sulfolipid and it accounts for 1 to 6 % of total S (Heinz 1993; De Kok et al. 1997; Benning 1998; Harwood and

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Okanenko 2003).

In spite of their potential phytotoxic effects, foliarly deposited atmospheric sulfur gases as $H_2S$ and $SO_2$ can also be used as sulfur source for growth, and they even may be beneficial if the sulfate supply to the roots is limited (De Kok et al. 2000, 2002a,b; Durenkamp and De Kok 2004). Due to the impermeability of the cuticle, $H_2S$ and $SO_2$ are taken up via the stomates and their uptake is both dependent on the stomatal conductance

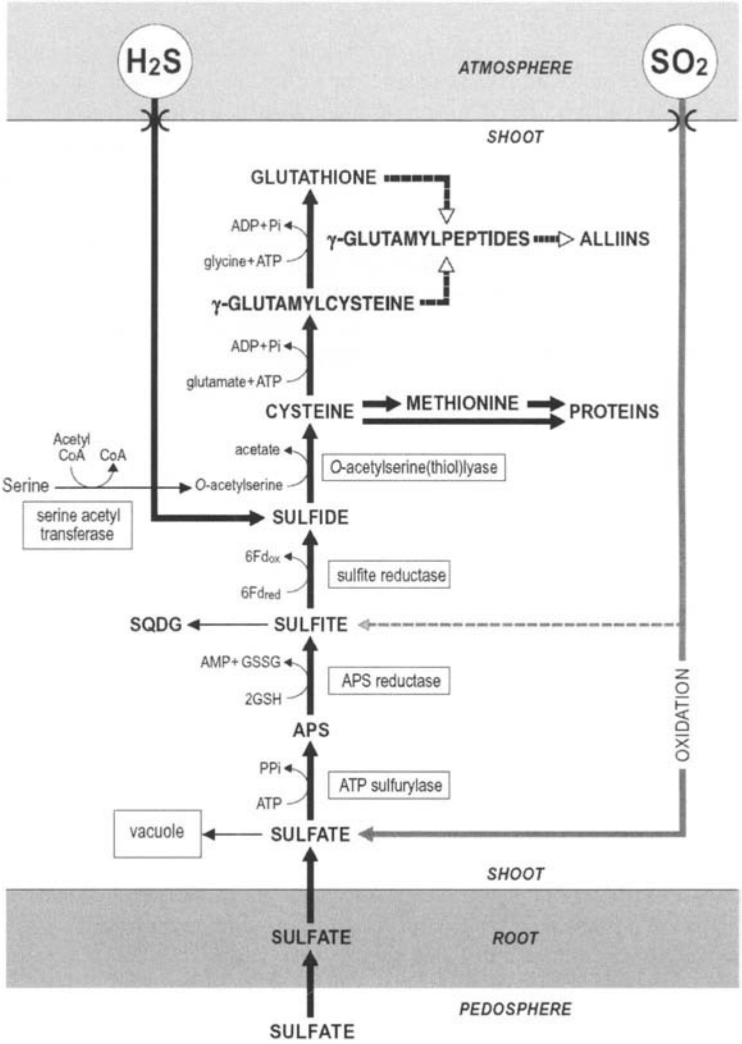

**Fig. 1.** Possible patterns of metabolism of atmospheric sulfur gases in onion (adapted from De Kok et al. 2002a). APS, adenosine 5'-phosphosulfate; Fd, ferredoxin; GSH, glutathione; SQDG, sulfoquinovosyldiacylglycerol.

and the internal (mesophyll) resistance towards these gases (De Kok et al. 1998, 2002a,b). The uptake of $H_2S$ is largely determined by the internal resistance, *viz.* the rate of metabolism of the absorbed sulfide into cysteine (Fig. 1). The rate of uptake depends on the activity of *O*-acetylserine(thiol)lyase and the availability of its substrate *O*-acetylserine (Stuiver and De Kok 2001) and it shows saturation kinetics with the atmospheric $H_2S$ level, which can be described by Michaelis-Menten kinetics (De Kok et al. 1998; Stuiver and De Kok 2001; Durenkamp and De Kok 2002). In contrast to $H_2S$, the uptake of $SO_2$ is largely determined by the stomatal conductance, since the internal resistance to $SO_2$ is low due to its high solubility and hydration in the cell sap. In general, there is a linear relation between the uptake of $SO_2$ and the level in the atmosphere (De Kok and Tausz 2001). Although $SO_2$, via sulfite, can directly be used in the sulfate reduction pathway, the greater part is oxidized to sulfate and transferred into the vacuole, especially at levels exceeding the sulfur requirement for growth (Fig. 1). Atmospheric sulfur gases have shown to be a useful tool to study sulfate uptake and sulfur assimilation by providing an extra source of sulfur taken up by the shoot, beyond the existing controls of sulfate uptake by the roots.

*Allium cepa* (onion) is one of the most important horticultural crops in the world. Secondary sulfur compounds (γ-glutamyl peptides and alliins) and their degradation products are responsible for the important role of *Allium* species in the food and phytopharmaceutical industry. The γ-glutamyl peptides are thought to act as precursors for the synthesis of alliins and they might have a function in the storage of sulfur and nitrogen (Randle and Lancaster 2002; Jones et al. 2004). The likely precursors for the synthesis of γ-glutamyl peptides and alliins are the thiol compounds γ-glutamyl cysteine and glutathione, which are products of the sulfur assimilation pathway (Fig. 1). In onion $H_2S$ exposure resulted in an increase in sulfate, thiols and other organic sulfur compounds in the shoot. The estimated N/S ratio of the latter compounds appeared to be 2 or less (Durenkamp and De Kok 2002, 2003, 2004), indicating that the increase could not be explained by an increase in the protein fraction (N/S ratio of proteins is generally around 40). It needs to be evaluated whether the increase in organic sulfur compounds upon $H_2S$ exposure was due to an accumulation of secondary sulfur compounds (γ-glutamyl peptides and alliins) and/or sulfolipids (Durenkamp and De Kok 2002, 2003, 2004). In addition, it needs to be assessed to what extent the observed accumulation of sulfur compounds is specific for $H_2S$ or the consequence of by-passing the regulatory control of the uptake of sulfate by the roots. In the present paper the impact of $H_2S$ and $SO_2$ on growth and sulfur metabolism has been compared. The significance of sulfolipids and secondary sulfur compounds as possible pool for excessive deposited atmospheric sulfur and the possible down-regulation of the sulfate reduction pathway upon $H_2S$ exposure are discussed.

## 2. Atmospheric $H_2S$: toxin vs. nutrient

Atmospheric sulfur gases are potentially phytotoxic, however, there is a large variation between species in the susceptibility towards these gases and the mechanisms of toxicity are still not completely understood. Like cyanide, sulfide complexes with high affinity to metallo groups in proteins (for instance heme-containing NADH oxidizing enzymes) and this reaction is probably the primary biochemical basis for the phytotoxicity of $H_2S$ (Maas and De Kok 1988; De Kok et al. 1998, 2002b). Mutagenic effects of accumulated thiol compounds (Glatt et al. 1983) or sulfide itself might also be a cause for the phytotoxicity of $H_2S$, since exposure to $H_2S$ resulted in an increase in chromosomal aberrations in apical meristems and root tips (Wonisch et al. 1999a,b; Stulen et al. 2000). In general, dicotyledons are more susceptible to $H_2S$ than monocotyledons, since in the latter $H_2S$ hardly has direct access to the vegetation point (Stulen et al. 2000).

Onion and related *Allium* species, as monocotyledons, were not very susceptible to the toxic effects of $H_2S$ (Durenkamp and De Kok 2002, 2003, 2004). A one-week exposure up to 0.6 µl $l^{-1}$ $H_2S$, a level which by far exceeds the sulfur requirement for growth, did

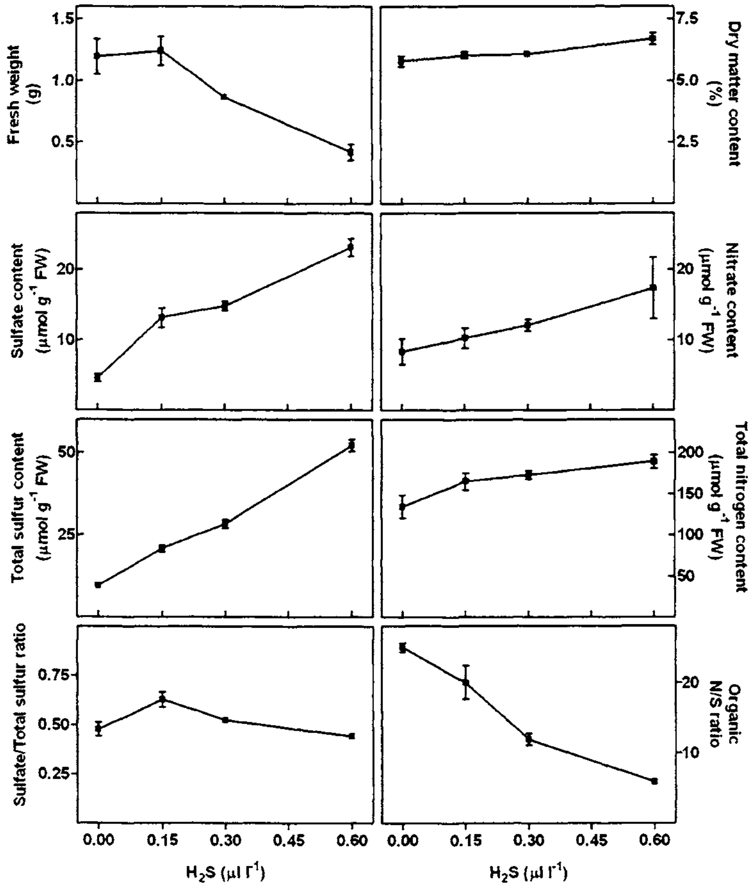

**Fig. 2.** Impact of prolonged $H_2S$ exposure on growth and sulfur and nitrogen metabolism of onion shoots (*Allium cepa* L.). Seedlings were grown in vermiculite for two weeks and subsequently transferred to a regular potting soil and exposed to 0, 0.15, 0.3 and 0.6 µl $l^{-1}$ $H_2S$ for 38 days. Fresh weight (g), dry matter content (%), metabolite contents (µmol $g^{-1}$ FW), sulfate/total sulfur ratio and organic N/S ratio of the shoot were determined as described in Durenkamp and De Kok (2002, 2004). Data represent the mean of three measurements with five plants in each (± SD).

not result in a reduction of growth in onion (Durenkamp and De Kok 2004). However, prolonged exposure to the same range of $H_2S$ levels for 38 days resulted in a substantial decrease in biomass production and a slight increase in dry matter content in onion shoots at levels $\geq 0.3$ µl $l^{-1}$ $H_2S$ (Fig. 2). Apparently, the occurrence of toxic effects of $H_2S$ in onion depended not only on the atmospheric $H_2S$ level but also on the duration of the exposure. The latter might be due to a cumulative effect of sulfide or produced toxic metabolites for instance in meristematic tissue. Prolonged exposure to $H_2S$ resulted in an increased content of sulfate and other sulfur-containing compounds, as illustrated by a maximal five-fold increase in the total sulfur content of the shoot upon exposure up to 0.6 µl $l^{-1}$ $H_2S$ (Fig. 2). The organic N/S ratio was decreased at all levels of $H_2S$ exposure, independent of the effects of $H_2S$ phytotoxicity (Fig. 2). The decrease in the organic N/S ratio could be attributed to an increase in non-protein organic (secondary) sulfur compounds, which pool might be a sink for reduced sulfur (Durenkamp and De Kok 2002, 2003, 2004). Prolonged $H_2S$ exposure also resulted in an enhancement of nitrogen-containing compounds in the shoot, which possibly was the consequence of a disturbed metabolism and/or an alteration in tissue and shoot development.

Atmospheric $H_2S$ could be used as a sulfur source for growth in onion, especially when the sulfate supply to the roots was deprived (Durenkamp and De Kok 2004). However, upon prolonged exposure $H_2S$ appeared to be phytotoxic and it reduced biomass production.

## 3. Impact of $H_2S$ exposure on sulfolipids

The main plant sulfolipid sulfoquinovosyldiacylglycerol (SQDG) is synthesized from UDP-sulfoquinovose and diacylglycerol with sulfite as the likely sulfur precursor (Sanda et al. 2001; Harwood and Okanenko 2003). Sulfite is synthesized from APS by APS reductase and this enzyme is the predominant site of regulatory control of the sulfate reduction pathway (De Kok et al. 2002a; Vauclare et al. 2002). The sulfolipid content of the shoot (expressed on a lipid basis) decreased upon exposure to $H_2S$ (Table 1), which could be caused by a down-regulation of the sulfate reduction pathway and by a subsequent decrease in sulfite production, the sulfur precursor of SQDG (Sanda et al. 2001). This suggestion is supported by observations in *Brassica oleracea*, where a similar decrease in sulfolipid content (expressed on a lipid basis) was observed upon $H_2S$ exposure (De Kok et al. 1997). The sulfate reduction pathway is known to be down-regulated via APS reductase upon $H_2S$ exposure in *B. oleracea* (Westerman et al. 2001b). Since the sulfolipid content was not increased upon exposure to $H_2S$, sulfolipids did not act as a sink pool for atmospheric reduced sulfur.

The total lipid content of the shoot was increased upon exposure to $H_2S$, which could not be explained by an increase in either sulfolipid or pigment content (Table 1). It needs to be evaluated to what extent this increase in lipid content upon $H_2S$ exposure can be attributed to changes in the overall structure and/or composition of membranes. Another option for the increase in total lipid content could be the formation of vesicles containing secondary sulfur compounds (as suggested by Turnbull et al. 1981). The possible enhancement of secondary sulfur compounds content in the shoot might be accompanied with a subsequent increase in vesicle formation resulting in an increase in the total lipid content. The latter was not observed in *Brassica oleracea* (De Kok et al. 1997), since in

**Table 1.** Impact of short-term $H_2S$ exposure on pigment content in shoot and lipid content in shoot and roots of onion (*Allium cepa* L.). Seedlings were grown in vermiculite for two weeks and subsequently transferred to a 25% Hoagland nutrient solution. Four-week-old seedlings were transferred to a fresh nutrient solution and exposed to 0.3 $\mu l\ l^{-1}$ $H_2S$ for one week. Total lipid content and sulfolipid content in shoot and roots were determined as described by De Kok et al. (1997) and the content of chlorophylls and carotenoids in the shoot was measured as described by Lichtenthaler (1987). Data represent the mean of three measurements with 12 plants in each (± SD).

| | 0 $\mu l\ l^{-1}$ $H_2S$ | 0.3 $\mu l\ l^{-1}$ $H_2S$ |
|---|---|---|
| **Shoot** | | |
| Total lipid content (mg $g^{-1}$ FW) | 3.60 ± 0.09 | 4.26 ± 0.17** |
| Sulfolipid content (nmol $g^{-1}$ FW) | 89.0 ± 6.1 | 86.6 ± 2.6 |
| Sulfolipid content (nmol $mg^{-1}$ total lipids) | 24.7 ± 2.1 | 20.7 ± 0.2* |
| Sulfolipid content (nmol $mg^{-1}$ chlorophyll) | 189 ± 5 | 187 ± 10 |
| Total chlorophyll content (mg $g^{-1}$ FW) | 0.47 ± 0.03 | 0.46 ± 0.01 |
| Total carotenoid content (mg $g^{-1}$ FW) | 0.11 ± 0.00 | 0.11 ± 0.00 |
| **Root** | | |
| Total lipid content (mg $g^{-1}$ FW) | 1.44 ± 0.12 | 1.52 ± 0.15 |
| Sulfolipid content (nmol $g^{-1}$ FW) | 36.5 ± 4.2 | 37.3 ± 4.2 |
| Sulfolipid content (nmol $mg^{-1}$ total lipids) | 25.3 ± 1.0 | 24.5 ± 0.3 |

*$P<0.05$; **$P<0.01$ *vs* 0 $\mu l\ l^{-1}$ $H_2S$; Student's *t*-test.

this species an accumulation of secondary sulfur compounds was absent upon $H_2S$ exposure (Westerman et al. 2001a).

The observed increase in the non-protein organic sulfur content upon $H_2S$ exposure (Durenkamp and De Kok 2002, 2003, 2004) could not be attributed to changes in the content of sulfolipids. Therefore, secondary sulfur compounds appeared to be the most likely pool for excessive deposited atmospheric sulfur in onion.

## 4. Impact of atmospheric $SO_2$ on sulfur metabolism: a comparison with $H_2S$

In general, plant exposure to $SO_2$ results in an increase in the sulfate content and a slight increase in the thiol content (mainly glutathione) of the shoot since part of the $SO_2$ can be assimilated into organic sulfur compounds via sulfite (De Kok and Tausz 2001; Tausz et al. 2003; Yang et al. 2003).

Growth of onion was not affected upon exposure to 0.3 $\mu l\ l^{-1}$ $SO_2$ (Table 2). An increase in the sulfate and total sulfur content of the shoot was observed upon exposure to $SO_2$ in both sulfate-sufficient and sulfate-deprived plants, whereas the content in the roots was not affected (Table 2). The increase in the total sulfur content of the shoot in sulfate-sufficient plants could solely be explained by an increase in the sulfate content (Table 2). Apparently, $SO_2$ was for the greater part oxidized to sulfate and transferred into the vacuole (Fig. 1). In contrast to exposure to $H_2S$, $SO_2$ exposure did not result in a significant

**Table 2.** Impact of sulfate nutrition and short-term $SO_2$ exposure on growth and sulfur metabolism in shoot and roots of onion (*Allium cepa* L.). Seedlings were grown in vermiculite for two weeks and transferred to a 25% Hoagland nutrient solution. Four-week-old seedlings were transferred to a fresh nutrient solution with 0 (-S) or 0.5 (+S) mM sulfate and exposed to 0 ($-SO_2$) or 0.3 ($+SO_2$) μl $l^{-1}$ $SO_2$ for one week. Fresh weight (g), sulfate and total sulfur content (μmol $g^{-1}$ FW) and sulfate/total sulfur ratio in shoot and roots were determined as described in Durenkamp and De Kok (2002, 2004). Data represent the mean of four measurements with 12 or 24 (initial) plants in each (± SD). Different letters indicate significant differences between treatments (P<0.05, Student's *t*-test).

| | Initial | -S | -S $+SO_2$ | +S | +S $+SO_2$ |
|---|---|---|---|---|---|
| **Shoot** | | | | | |
| Fresh weight | $0.48 \pm 0.05$ | $1.10 \pm 0.04^{a}$ | $1.12 \pm 0.06^{ab}$ | $0.98 \pm 0.23^{ab}$ | $1.27 \pm 0.13^{b}$ |
| Total sulfur content | $9.0 \pm 0.3$ | $4.0 \pm 0.3^{a}$ | $9.3 \pm 0.3^{b}$ | $8.5 \pm 1.2^{b}$ | $14.8 \pm 1.2^{c}$ |
| Sulfate content | $2.6 \pm 0.2$ | $0.6 \pm 0.0^{a}$ | $4.7 \pm 0.2^{c}$ | $3.6 \pm 0.5^{b}$ | $9.0 \pm 0.5^{d}$ |
| Sulfate/total sulfur | $0.29 \pm 0.03$ | $0.14 \pm 0.03^{a}$ | $0.50 \pm 0.02^{c}$ | $0.43 \pm 0.02^{b}$ | $0.61 \pm 0.03^{d}$ |
| **Root** | | | | | |
| Fresh weight | $0.23 \pm 0.02$ | $0.43 \pm 0.03^{a}$ | $0.42 \pm 0.06^{a}$ | $0.40 \pm 0.08^{a}$ | $0.46 \pm 0.03^{a}$ |
| Total sulfur content | $9.2 \pm 0.7$ | $4.1 \pm 0.2^{a}$ | $4.3 \pm 0.6^{a}$ | $8.9 \pm 0.3^{b}$ | $9.5 \pm 0.4^{b}$ |
| Sulfate content | $5.6 \pm 0.5$ | $0.9 \pm 0.3^{a}$ | $0.8 \pm 0.3^{a}$ | $5.1 \pm 0.2^{b}$ | $5.5 \pm 0.2^{c}$ |
| Sulfate/total sulfur | $0.61 \pm 0.05$ | $0.21 \pm 0.08^{a}$ | $0.18 \pm 0.06^{a}$ | $0.58 \pm 0.02^{b}$ | $0.58 \pm 0.01^{b}$ |

decrease in the organic N/S ratio of the shoot of sulfate-sufficient plants (27.7 ± 1.8 and 23.9 ± 3.5 at 0 and 0.3 μl $l^{-1}$ $SO_2$, respectively). As has been indicated above, a decrease in the organic N/S ratio upon $H_2S$ exposure could likely be attributed to an increase in secondary sulfur compounds (Durenkamp and De Kok 2002, 2003, 2004). These compounds only seemed to be a sink for reduced atmospheric sulfur like $H_2S$, via by-passing of the sulfate uptake in the roots and its reduction in the shoot, and not for oxidized (atmospheric) sulfur like $SO_2$. The reduction of sulfate is known to be highly regulated (De Kok et al. 2002a; Vauclare et al. 2002), in contrast to the uptake of $SO_2$, which resulted in an accumulation of sulfate upon $SO_2$ exposure. Sulfate accumulation was not observed when onion was subjected to increasing levels of pedospheric sulfate, since uptake of sulfate by the roots was strictly regulated (Hawkesford and Wray 2000; Durenkamp and De Kok, 2004; Buchner et al. 2004). A combination of $H_2S$ exposure and different levels of pedospheric sulfate nutrition will be used to further investigate the regulation of sulfate uptake, transport, subcellular distribution and reduction through APS reductase, since these processes predominantly control the assimilation of sulfate in plants.

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# Impact of atmospheric $NH_3$ deposition on plant growth and functioning – a case study with *Brassica oleracea* L.

Ana Castro, Ineke Stulen, and Luit J. De Kok

Laboratory of Plant Physiology, University of Groningen, P.O. Box 14, 9750 AA Haren, The Netherlands

**Summary.** *Brassica oleracea* L. (curly kale) was exposed to 0, 2, 4, 6 and 8 $\mu l\ l^{-1}$ $NH_3$ during one week and the impact on growth and N compounds was determined. Exposure to $NH_3$ increased shoot biomass production at 2 and 4 $\mu l\ l^{-1}$, but resulted in an inhibition of shoot and root growth at 6 and 8 $\mu l\ l^{-1}$. Shoot to root ratio was not affected up to 4 $\mu l\ l^{-1}$, but decreased at higher levels. Shoot total N content was increased at all levels, mainly due to the increase in free amino acids. Even at atmospheric $NH_3$ levels, at which the foliarly absorbed $NH_3$ would cover a limited proportion of N requirement there was already an enhancement of the nitrogen content of the shoots and roots. Apparently there was no direct regulatory control of and/or interaction between atmospheric and pedospheric nitrogen utilization in *B. oleracea.* It needs to be evaluated to what extent foliarly absorbed $NH_3$ is used as nitrogen source for growth.

**Key words** Ammonia, *Brassica oleracea*, Nitrogen pollutants, Nutrient, Toxin

## 1. Atmospheric N deposition in Europe

$NH_3$ is a major air pollutant, which accounts for up to 80% of the total N deposition in central Europe (Fangmeier et al. 1994; Gessler and Rennenberg 1998; Krupa 2003). Atmospheric $NH_3$ pollution is the consequence of intensive farming activities (animal manure and fertilizer use), and to a lesser extent to anthropogenic sources and natural background emissions (Leith et al. 2002; Krupa 2003; Pitcairn et al. 2003; Erisman and Schaap 2004). High $NH_3$ emissions and consequently, excessive N deposition will lead to direct phytotoxic effects, eutrophication and acidification (Stulen et al. 1998; Rennenberg and Gessler 1999; Krupa 2003). The toxic effect of $NH_3$ has often been ascribed to nutrient imbalances due to cation release (Wollenweber and Raven 1993).

While the impact of atmospheric N deposition on ecosystems such as heathlands (Van der Eerden et al. 1991; Leith et al. 2002; Sheppard and Leith 2002) and forests (Högberg et al. 1998; Rennenberg and Gessler 1999; Bassirirad 2000) has been studied in detail, fewer studies have dealt with its impact on crop plants (Van der Eerden 1982; Clement et al. 1997). In addition, there are hardly any data available on the contribution of foliar uptake of atmospheric $NH_3$ to the plant's N requirement for growth (Pérez-Soba and Van der Eerden 1993; Stulen et al. 1998).

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## 2. Foliar uptake and metabolism of $NH_3$

The uptake of $NH_3$ shows a diurnal variation and is dependent on the water status of the plant, temperature, light intensity, internal $CO_2$ level and nutrient availability (Hutchinson et al. 1972; Rogers and Aneja 1980; Van Hove et al. 1987; Husted and Schjoerring 1996; Schjoerring et al. 1998). The foliar uptake of $NH_3$ is determined by the stomatal conductance and the internal (mesophyll) resistance to the gas and its uptake via the cuticle surface can be neglected (Krupa 2003). The internal resistance of the mesophyll cells appears to be the limiting factor for foliar uptake of $NH_3$ (Hutchinson et al. 1972). The internal resistance to $NH_3$ is low, since this gas is highly water-soluble and in addition it is rapidly converted into $NH_4^+$ in the aqueous phase of the mesophyll cells (Fangmeier et al. 1994). $NH_3$ uptake takes place as long as the atmospheric level exceeds the internal $NH_4^+$ level (Husted and Schjoerring 1996).

The $NH_4^+$ formed in the mesophyll cells may be assimilated by the glutamine synthetase/glutamate synthase cycle (Lea and Mifflin 1974; Pérez-Soba et al. 1994; Pearson and Soares 1998). Foliar $NH_3$ uptake may affect plant metabolism in various ways and result in changes in parameters as metabolic compounds, enzyme activity, root uptake and plant growth (Pérez-Soba et al. 1994; Gessler and Rennenberg 1998; Pearson and Soares 1998). Metabolic changes related to the $NH_3$ assimilatory capacity of the plant generally lead to an increase in the pool of N-containing metabolites, such as amino acids and total N content (Van Dijk and Roelofs 1988; Pérez-Soba et al. 1994; Clement et al. 1997; Gessler and Rennenberg 1998). Visible symptoms, such as black spots and necrosis in the leaves, arise when $NH_3$ uptake by the shoot exceeds the assimilation capacity of the plant (Van der Eerden 1982; Fangmeier et al. 1994).

## 3. Impact of $NH_3$ on growth and N metabolism of *Brassica oleracea*

The present case study was aimed at investigating the impact of a range of $NH_3$ levels on growth and N metabolism of *Brassica oleracea* L. Plants were grown on a Hoagland nutrient solution containing 3.75 mM nitrate (for experimental details see Castro et al. 2004). *B. oleracea* was chosen because it is an economically important crop plant with a relatively high RGR, and it is a suitable species because of its preference for nitrate (Pearson and Stewart 1993) as well as its sensitivity to $NH_4^+$ (Britto and Kronzucker 2002). *Brassica* species originate from saline, sulfur-rich environments and are considered to have a high S requirement for growth (Westerman et al. 2000). Therefore, the impact of $NH_3$ on S compounds was measured as well.

Upon $NH_3$ exposure the shoot biomass production was slightly increased at levels up to 4 $\mu l\ l^{-1}$, whereas it was decreased at levels ≥ 6 $\mu l\ l^{-1}$ $NH_3$. Root biomass production was decreased significantly at 6 and 8 $\mu l\ l^{-1}$ $NH_3$, showing that exposure of the shoot to $NH_3$ had a negative effect on root growth (in the used experimental conditions, the formation of $NH_4^+$, by dissolution of atmospheric $NH_3$ into the nutrient solution, was prevented). Relative growth rate (RGR), calculated on a plant basis was only significantly decreased at 8 $\mu l\ l^{-1}$ $NH_3$. Exposure to 6 and 8 $\mu l\ l^{-1}$ $NH_3$ affected root biomass production relatively more than shoot biomass production, resulting in a higher shoot to root ratio (S/R,

**Table 1.** Impact of $NH_3$ on growth of *Brassica oleracea*. Seedlings (26 days old) were exposed for 7 days. Shoot and root growth (g FW) was calculated by subtracting the final fresh weight from the initial fresh weight. RGR, relative growth rate (g $g^{-1}$ $day^{-1}$) on a plant basis. S/R, shoot to root ratio on a fresh weight basis. DMC, dry matter content (%). Data represent the mean of 2 experiments, with 3 measurements per experiment with 3 plants in each (±SD). Means followed by different letters are statistically different at $p< 0.01$. Statistical analysis was performed by using an unpaired Student's t-test. For further experimental details see Castro et al. (2004).

| [$NH_3$] | **0 $\mu l\ l^{-1}$** | **2 $\mu l\ l^{-1}$** | **4 $\mu l\ l^{-1}$** | **6 $\mu l\ l^{-1}$** | **8 $\mu l\ l^{-1}$** |
|---|---|---|---|---|---|
| **Shoot growth** | $1.90±0.07^c$ | $2.04±0.04^b$ | $2.45±0.25^b$ | $1.78±0.45^a$ | $1.69±0.30^a$ |
| **Root growth** | $0.55±0.20^b$ | $0.36±0.06^b$ | $0.48±0.08^b$ | $0.10±0.07^a$ | $0.20±0.11^a$ |
| **RGR** | $0.20±0.01^a$ | $0.20±0.01^a$ | $0.20±0.01^a$ | $0.16±0.04^a$ | $0.15±0.03^b$ |
| **S/R** | $3.3±0.6^a$ | $4.2±0.3^a$ | $4.1±0.4^a$ | $5.8±1.1^b$ | $5.9±1.5^b$ |
| **Shoot DMC** | $14.1±1.2^a$ | $14.2±1.5^a$ | $13.1±1.0^a$ | $13.0±1.2^a$ | $14.0±0.9^a$ |
| **Root DMC** | $6.4±1.2^a$ | $6.1±0.8^a$ | $7.2±0.5^a$ | $11±0.4^c$ | $9.1±0.9^b$ |

Table 1). Shoot dry matter content (DMC) was not affected upon exposure to $NH_3$, whereas root dry matter content was decreased at 6 and 8 $\mu l\ l^{-1}$ $NH_3$ (Table 1).

Exposure to $NH_3$ resulted in a substantial increase in shoot total N content at all atmospheric levels (Fig. 1a). This was mainly due to an increase in the soluble N fraction (amino acids, amides and $NH_4^+$), viz. 1.5 fold and 5.6-fold at 4 $\mu l\ l^{-1}$ and at 8 $\mu l\ l^{-1}$, respectively, compared to that of the control (0 $\mu l\ l^{-1}$, results not shown). Root total N content was only increased at 2 $\mu l\ l^{-1}$ $NH_3$ (Fig. 1a). Shoot nitrate content was increased at all $NH_3$ levels, but most at 4 $\mu l\ l^{-1}$. Root nitrate content was increased at 2 $\mu l\ l^{-1}$, not affected at 4 $\mu l\ l^{-1}$, and decreased at 8 $\mu l\ l^{-1}$ (Fig. 1c). The free amino acid content in the shoot increased with increasing $NH_3$ levels (8% and 15% at 4 $\mu l\ l^{-1}$ and 8 $\mu l\ l^{-1}$, respectively), while no effect was observed in the roots (Fig. 1e).

Shoot sulfur content was not affected by exposure to 2 $\mu l\ l^{-1}$ $NH_3$, but decreased at higher levels. Root total sulfur was increased at 2 to 6 $\mu l\ l^{-1}$, and decreased at 8 $\mu l\ l^{-1}$ (Fig. 1b). Shoot sulfate content was increased at 4 $\mu l\ l^{-1}$, and decreased at 6 and 8 $\mu l\ l^{-1}$. Root sulfate content was increased at 2 $\mu l\ l^{-1}$, not changed at 4 $\mu l\ l^{-1}$, and decreased at 6 and 8 $\mu l\ l^{-1}$ (Fig. 1d).

The impact of atmospheric $NH_3$ on total S and sulfate (Fig. 1b,d) can be explained by changes in RGR (Table 1), rather than by a direct effect of $NH_3$ exposure on S compounds. Noteworthy is the relatively high sulfate content found in this species. Other experiments with *Brassica* seedlings also showed that a high percentage (90%) of total S is present as sulfate, and only 10% as organic S (Castro et al. 2003). Therefore, for this species the definition of "sulfur requirement for growth" may have to be redefined, as "organic sulfur need for growth" (Castro et al. 2003). In the shoot, the organic N/S ratio increased with increasing $NH_3$ levels, which correlates well with the increase in free amino acid content. Changes in the organic N/S ratio in the root were minor.

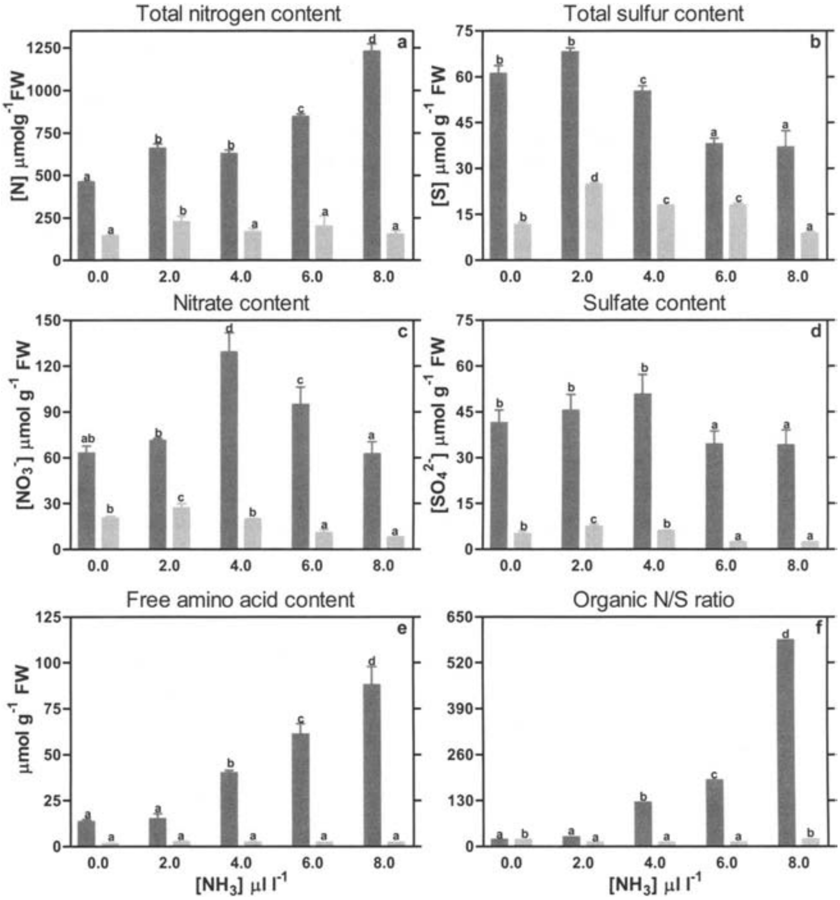

**Fig. 1.** Impact of $NH_3$ on N and S compounds in *Brassica oleracea*. Seedlings (26 days old) were exposed for 7 days. Shoot data is given in dark-grey bars, root data in light-grey bars. Data on total nitrogen, nitrate and free amino acids contents represent the mean of 2 experiments, with 3 measurements per experiment with 3 plants in each (±SD). Data on total S and sulfate content represent the mean of 3 measurements with 3 plants in each (±SD). The organic N/S ratio, a parameter was calculated by subtracting the nitrate and sulfate contents from total nitrogen and sulfur contents, respectively. Different letters indicate significant differences at $p < 0.01$. Statistical analysis was performed by using an unpaired Student's t-test. For experimental details see Castro et al. (2004).

## 4. Impact of $NH_3$ on nitrate uptake by *Brassica oleracea*

The net nitrate uptake rate (NNUR) was not affected at 2 µl $l^{-1}$ but was reduced by 25% upon exposure to ≥4 µl $l^{-1}$ $NH_3$ (Table 2). It has been suggested that a decrease in NNUR upon $NH_3$ may be due to a down-regulation of the nitrate transporters by reduced N