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Ewald Schnug
Luit J. de Kok (Eds.)

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Bundesallee 50, 38116 Braunschweig, Germany

landbauforschung@fal.de

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Aspects of sulfur nutrition of plants; evaluation of China's current, future and available resources to correct plant nutrient sulfur deficiencies - report of the first Sino-German Sulfur Workshop

Ewald Schnug[1], Lanzhu Ji[2] and Jianming Zhou[3]

Abstract

Sulfur is an essential plant nutrient that must be supplemented through fertilizer application when quantities in the soil are insufficient or when other natural inputs are not available. Besides just being involved in producing yield, sulfur-containing compounds are responsible for numerous aspects of crop quality and the natural resistance of plants. As a result of increasing crop yields and removal, growing use of sulfur-free fertilizers and increased attention to air quality standards leading to continuing reductions in atmospheric sulfur contributions, the need for the application of plant nutrient sulfur is accelerating in China. In order to stimulate networking between plant sulfur-related research initiatives in China and Germany, the first Sino-German Workshop on "Aspects of sulfur nutrition of plants; evaluation of China's current, future and available resources to correct plant nutrient sulfur deficiencies", was held on May 24-29, 2004 in the Institute of Applied Ecology, Shenyang, China. During the workshop the China's current, future, and available resources to correct plant nutrient sulfur deficiencies were evaluated.

Keywords: Crop yield, crop quality, food quality, Sulfur deficiency, sulfur fertilization, sulfur metabolism, sulfur nutrition

Introduction

Sulfur is one of the mineral elements essential for plant life. Starting from the amino acid cysteine (Cys), higher plants synthesize a complex spectrum of S compounds with diverse physiological functions. Among these are the tripeptide glutathione (GSH), which is central to the response against abiotic stressors (reactive oxygen species, heavy metals). In addition, there are several sulfur-containing pathogen-directed defense compounds: Glucosinolates as secondary S metabolites, rich pathogenesis related (PR) proteins of the thionin-type, and H_2S released from Cys. As activated sulfate (APS) and Cys are also basic components of primary metabolism and structural compounds (sulfolipids, proteins), plants had to develop strategies to reconcile S availability and S demand during plant development with the requirements of different stress responses. A major goal of the recent research carried out by a DFG research group in Germany is to develop a model for the coordination of S assimilation with the synthesis of GSH, glucosinolates, S-rich defense proteins and H_2S, using an integrated approach based on the tools of molecular physiology and cell biology. The comparative approach with plants of different physiotype (*Arabidopsis thaliana, Brassica napus/juncea, Populus tremula/alba*) will allow to address general and species-specific mechanisms, in particular the role of a luxuriant secondary metabolism (glucosinolates) and the impact of different growth patterns (herbaceous versus non-herbaceous). The use of transgenic plants with changed expression of single genes will allow to assess their contribution to the overall stress response. The integration of field experiments will help to evaluate the relevance of S nutrition-mediated defense reactions for resistance under field conditions.

China accounts for one-fifth of the world population, but has only 7% of the world's agricultural land mass. Thus, the country faces a significant challenge to meet food demands for its 1.3 billion inhabitants. China's economic and agricultural policies have changed dramatically over the last 20 years. Seeking to expand its agricultural sector, China has increased importation of fertilizers as well as increased domestic production. Chinese consumption of the three major nutrients nitrogen (N), phosphorus (P), and potassium (K) has expanded significantly at annual growth rates averaging 4, 7, and 10 percent, respectively. Concurrently, agricultural production has made considerable gains. As N, P, and K demands have been increasingly met, deficiencies of other nutrients have arisen and sulfur has become of increasing interest since it is typically required in quantities ranking fourth behind N, P, and K.

This paper reports on the objectives, presentations and topics of the first Sino-German Workshop on "Aspects of sulfur nutrition of plants; evaluation of China's current, future and available resources to correct plant nutrient sulfur deficiencies", May 24-

[1] Institute of Plant Nutrition and Soil Science, Federal Agricultural Research Center (FAL), Braunschweig, Germany
[2] Institute of Applied Ecology, Chinese Academy of Science, Shenyang, China
[3] Institute of Soil Science, Chinese Academy of Science, Nanjing, China

29, 2004, Institute of Applied Ecology, Shenyang, China.

Objectives of the workshop

The goals of this workshop were:
- To discuss fundamental, agronomic and environmental aspects of sulfur in higher plants, to promote and better coordinate sulfur-related research in plants.
- To stimulate networking between plant sulfur-related research initiatives in China and Germany.
- To provide optimal training of young scientists (PhD students, post docs, junior group leaders) in a complex research field with state-of-the-art approaches in physiology, biochemistry and molecular biology of plants.
- To evaluate China's current, future, and available resources to correct plant nutrient sulfur deficiencies through the next 10 years.

List of speakers and participants (within groups alphabetical order):

The delegates came from German universities in Braunschweig, Frankfurt, Hanover, Hamburg, Mainz and Groningen, The Netherlands. Scientists from the Max Plank Institute and the German Agricultural Research Centre participated. The Chinese delegates came from the Institute of Applied Ecology, CAS, the Institute of Soil Sciences, CAS, the Chinese Academy of Agricultural Sciences, Jiangxi Academy of Agricultural Sciences, Tianjin Academy of Agricultural Sciences and Anhui Agricultural University. Scientists from The Sulfur Institute, Washington, DC, also participated in the workshop.

Speakers from Germany:

Dipl.-Geoecol. Anja Brauer, FAL-PB, Braunschweig, E-mail: anja.brauer@fal.de
Dr. Ingo Hagel, FAL-PB Braunschweig, E-mail: ingo.hagel@t-online.de
Dr. Beate Huber, GSF, Munich, E-mail: beate-huber@aol.com
Prof. Dr. Juergen Kesselmeier, MPI & University, Mainz, E-mail: jks@mpch-mainz.mpg.de
Dr. Hans-Marten Paulsen, FAL-OEL, Trenthorst, E-mail: hans.paulsen@fal.de
Prof. Dr. Dr. Ewald Schnug, FAL-PB, Braunschweig, E-mail: ewald.schnug@fal.de
Prof. Dr. Dirk Selmar, University, Braunschweig, E-mail: d.selmar@tu-bs.de

Speakers from the German DFG-Research Group 383 (Sulfur metabolism in plants):

Prof. Dr. Ruediger Hell, University, Heidelberg, E-mail: r.hell@bot.uni-hd.de
Dr. Cornelia Herschbach, University, Freiburg, E-mail: cornelia.herschbach@ctp.uni-freiburg.de
Prof. Dr. Thomas Rausch: University, Heidelberg, E-mail: trausch@bot.uni-hd.de
Dipl.-Chem. Joana Salac, FAL-PB, Braunschweig, E-mail: ioana.salac@fal.de
Prof. Dr. Ahlert Schmidt, University, Hannover, E-mail: ahlert.Schmidt@botanik.uni-hannover.de
Dipl.-Ing. Agr. Pia Wickenhäuser, FAL-PB, Braunschweig, E-mail: pia.wickenhaeuser@fal.de

Foreign speakers

Dr. Ming Xian Fan, TSI, Washington, E-mail: agmarkt@sulphurinstitute.org
Dr. Luit J. De Kok, Chairman of the European COST Action 829 "Fundamental, agronomical and ecological aspects of sulfur in plants", University, Groningen, E-mail: l.j.de.kok@rug.nl
Mr. Donald Messick, TSI, Washington, E-mail: DMessick@sulphurinstitute.org

Speakers from China:

Former holders of German research fellowships granted by the Max Planck Society
Dr. Fan Xiaohui, ISSAS, Nanjing, E-mail: xhfan@issas.ac.ch
Dr. Hu Zhengyi, ISSAS, Nanjng, E-mail: zhyhu@ns.issas.ac.ch

Former holder of a DAAD fellowship:
Dr. Ma Youhua, University, Anhui, E-mail: mayouhua@mail.hf.ah.cn

Former holder of several fellowships granted by NFSC and the bilateral cooperation programmes between MoAs:

Dr. Wang Shiping, IB-CAS, Beijing, E-mail: shiping.wang@95777.com

Other speakers from China:

Prof. Lin Bao, ISSAS, Nanjing, E-mail: blin@caas.ac.cn
Prof. Liu Chongqun, ISSAS, Nanjing, E-mail: qzfan@issas.ac.cn
Prof. Luo Qixiang, Academy of Agricultural Sciences, Jiangxi,
Dr. Zhou Yiming, Soil and Fertilizer Institute, Tianjing, E-mail: ymzhou@public.tpt.tj.cn

Synopsis of the scientific contributions

Kesselmeier presented the auditorium a view to the global sulfur cycle and China's contribution to atmospheric sulfur loads. On Wednesday, March 20, 2002 "Peoples Daily" published a headline "China Fighting Acid Rain, Sulfur Dioxide". The article revealed that China has decreased the release of sulfur dioxide by 1.86 million tons over the past two years as a result of its efforts to combat acid rain and sulfur dioxide control. In 1998, the State Council designated 11.4 percent of China's land, covering 175 cities in 27 provinces, autonomous regions and municipalities as acid rain and sulfur dioxide control regions. The sulfur dioxide release in these regions accounted for 60 percent of China's total. Over the past two years, the number of Chinese cities that have met the national standards has increased from 81 to 98, and the amount of sulfur dioxide has decreased from 14.08 million tons to 11.14 million tons. Beijing and Shanghai have taken the lead to set up areas without coal burning. By the end of last year, the output of high sulfur coal decreased by 32 million tons. Some 250 thermoelectric generating sets were shut down. China plans to shut down another 4,000 high sulfur coalmines, 135 thermoelectric generating sets and 1,300 small-sized cement and glass production lines this year. The annual Chinese emissions projected for 2020 are 40-45 Tg yr^{-1} S by 2020. However, there are already trends towards a lower figure for emissions observed, which is due a reduction in industrial coal use and a slow-down of the Chinese economy and a closure of small and inefficient plants.

Lu made a downscaling of the global to the Chinese S situation**.** This contribution revealed that the total S content in soils of China ranges from 100-500 mg kg^{-1} S. The organic S in soils of southern China accounts for 86-94% of the total S. The inorganic sulfur is mostly the easily soluble and the adsorbed sulfur. The content of the total S, organic S and available S in the cultivated soils of southern China is 299, 266 and 34 mg kg^{-1} S respectively. In southern China the sulfur input into the soil comes mainly from sulfur fertilizers (28.2 kg ha^{-1} S), rainfall (13.4 kg ha^{-1} S), and irrigation water (9.2 kg ha^{-1} S), with a total input of 50.6 kg ha^{-1} S. Balanced with sulfur removed from the soil by crop uptake (25.3 kg ha^{-1} S), sulfur leaching (19.9 kg ha^{-1} S) and runoff.

As a general introduction to the biology of S compounds **De Kok** refreshed the knowledge of the auditorium concerning the basic facts of plants' S metabolism and the main steps in the regulation of uptake, transport and storage of S compounds. In addition, the significance of S in physiological functioning of plants was reviewed. For instance, S-containing metabolites as glutathione (GSH) plays a key role as an important redox-system and precursor for many other S containing metabolites.

Glucosinolates are a special metabolic pathway for S in a number of plant families like for instance cruciferous crops. **Selmar** explained in his lecture the metabolism and catabolism of glucosinolates. The significance of glucosinolates for the subject of the workshop have to be seen in their role as an active principle in chemical plant defense, which stimulation either by altered genetics or environment bears challenges for improving plant health without pesticides.

"Sulfur-rich Proteins" are also involved in stress resistance and supposed to be an important part of SIR. **Hell** demonstrated that thionins and defensins are ubiquitous elements of innate defense in plants, which are encoded by large gene families and are differentially expressed. The inducibility of at least some Thi and Def genes by pathogens depends on optimal sulfur supply. Membrane damage by sulfur-rich proteins can be exploited to enhance resistance to pathogenic fungi using transgenic approaches and possibly also breeding.

Not only agricultural crop plants but also forests may suffer from S deficiency. **Herschbach** explained their view to the sulfur nutrition of deciduous trees at the whole plant level during stress.

A new field for extended plant S research are aspects of so-called sulfur-induced resistance (SIR), which were brought to the attention of the auditorium by **Salac**. Because of a number of evidences on the interaction of S with plant health, research has been stimulated in this field in order to understand the relationship between the S status of plants and resistance mechanisms. The significance of S fertilization for crop resistance has coined the term Sulfur Induced Resistance, abbreviated SIR. The fungicidal effect of elemental S on pests and diseases is long known while the significance of soil-applied S for crop resistance became evident a century later. Nevertheless, the fungicidal effect of foliar applied S has to be distinguished strictly from the health promoting effect of soil-applied S. Therefore, in what follows the significance soil-applied S fertilization on plant health will be highlighted. These recent findings clearly indicate that S supply has a strong influence on plant resistance by stimulating directly the biochemical processes in the primary and secondary metabolism. Nevertheless, future research is necessary in order to understand the efficacy of individual S metabolites involved in the activation and strengthening of plant defenses by S fertilization.

As representatives of the S fertilizer industry **Messick** and **Fan** stressed the increasing demand for S fertilizers and their use in Chinese agriculture, a fact which provides significant benefits to both fertilizer manufacturers and farmers. The estimated annual need of S for plant nutrition in China is 1.7

million tons S. It has been estimated that 30% of Chinese farmland, mainly in the counties Ji and Baodi are responding to S fertilization. Yield losses in rice, wheat and corn caused by S deficiency are 6% - 24%, particularly S demanding crops like Chinese cabbage, garlic, turnips and scallion responded to S fertilization of 60 kg ha^{-1} S with yield increased around 20%. **Messick** and **Fan** expect a deficit in S supply from 2011 on. Assuming that 20% of the market is captured (340,000 tons S) and a price of t 180 US$ per ton S for fertilizer this corresponds to a financial volume of 61.2 Million US$. The average yield increase potential in Chinese crop production by sulfur fertilization is estimated to 10% on 40 million ha of S deficient land. The additional yield is estimated to a total of 24 million tons for which the additional sulfur fertilizer demand amounts to 1.2 million tons of S. At the same time with yield increases an improved efficiency for nitrogen fertilizers of at least 2% is expected which saves a minimum of 5.5 million tons of N from being lost to the environment.

Despite its distinctive effects of crop yield S fertilization also improves the quality of plant products. **Hagel** demonstrated this by the example of the baking-quality of bread-making wheat. He carried out that in modern breeding (unconsciously) varieties with a higher demand of vitalizing sulfur were selected. This affects primarily the content of high molecular weight (HMW-)glutenin. This not only affects the technological features of the dough prepared from S deficient wheat grain, but also the digestibility of the wheat bread in the human intestine.

Paulsen stressed the special role of S nutrition and S application in organic farming. Besides a plant nutrient, S in elemental form may have a negative impact on rice roots, which are sensitive to low levels of sulfide. H_2S can derive from superfluous S in rice soils due to the nocturnal decline in the degree of oxidation in the rhizosphere, since the stomata of the rice plants are closed at night.

Under severe S starvation plants develop more or less characteristically deficiency symptoms. **Brauer et al.** demonstrated the symptomatology of visual symptoms of S-deficiency. They showed that symptoms of S deficiency can occur in all crops and in all growth stages and they concluded that the identification of such symptoms are an important tool in crop management. S deficiency symptoms can be diagnosed comparatively reliable in oilseed rape, while in cereals (including corn) and sugar beet this is only possible together with hydrological and other site parameters.

Ma demonstrated that a combination of information technology, soil-fertilizer and plant-nutrition technology can be used as a tool for managing S fertilizers throughout larger regions. By this system, soil S-deficiency status, effects of S fertilizer application and soil S balance of input and output in Chinese different regions could be directly queried. With increasingly maturation and popularization of the internet technology, attention is paid to WebGis (World-Wide-Web Geography Information System). It not only solves the problem of expensive price for GIS software, but also reduces the cost of collecting geography spatial data and improves the sharing degree and extension of the geography information. Organic farming has its special requirements to the quality of fertilizers: no soluble P sources are allowed in fertilization. **Fan** demonstrated a technology where available P could be produced from compounds of elemental S and rock phosphate fertilizers in soils directly.

Finally **Schnug** highlighted the significance of S fertilization as a part of sustainable development of agriculture. Understanding "sustainable development" as development that meets the needs of the present without compromising the ability of future generations to meet their own needs (The Brundtland Commission, 1997) sulfur fertilization contributes to sustainability because it, improves production performance, reduces the environmental impact of nitrogen and pesticides, improves the efficiency of non renewable resources (P), improves crop quality.

General conclusions and further actions

All participants addressed the workshop as a great success. Both German and Chinese scientists discussed the content of future cooperative projects to introduce advanced research technologies and methods, genetic research on sulfur-induced crop resistance to stresses, aspects of sulfur fertilizer use in conventional and environmentally-sound agriculture, GIS technology and its use in diagnosis of sulfur deficiency and sulfur fertilizer recommendations in different regions.

Further actions will be the proposal of two workshops to the Center, addressing the specific interests of science and society in organic farming and genetic engineering. Individual research collaborations between partners have been initiated already and seeking for funding will also involve approaches to the Center.

Acknowledgements

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Pathways of plant sulfur uptake and metabolism - an overview

Luit J. De Kok[1], Ana Castro[1], Mark Durenkamp[1], Aleksandra Koralewska[1], Freek S. Posthumus[1], C. Elisabeth E. Stuiver[1], Liping Yang[2] and Ineke Stulen[1]

Abstract

The sulfur requirement of plants varies strongly between species and can be defined as relative growth rate times the plants' sulfur content. In general sulfate taken up by the roots is the major sulfur source for growth, which has to be reduced to sulfide prior to its metabolism into essential sulfur compounds. Plants are also able to metabolize foliarly absorbed sulfur gases as sulfur source for growth. The reduction of sulfur takes predominantly place in the shoot in the chloroplast. Cysteine is the precursor or sulfur donor for most other organic sulfur compounds in plants. Sulfur amino acids cysteine and methionine are of great significance in the structure, conformation and function of proteins and enzymes. Cysteine is the precursor of glutathione, a water-soluble thiol compound which functions in the protection of plants against oxidative stress, heavy metals and xenobiotics.

Key words: sulfate uptake, sulfate reduction, sulfate assimilation, cysteine, methionine, sulfate assimilation, sulfolipids, proteins, phytochelatins, secondary sulfur compounds

Introduction

Sulfur is an essential element for growth and physiological functioning of plants, however, its content strongly varies between species and it ranges from 0.1 to 6 % of the dry weight (0.03 to 2 mmol g^{-1} dry weight; De Kok et al., 2002a). Sulfate taken up by the roots is the major sulfur source for growth, though it has to be reduced to sulfide before it is further metabolized. Root plastids contain all sulfate reduction enzymes, however, the reduction of sulfate to sulfide and its subsequent incorporation into cysteine takes predominantly place in the shoot in the chloroplast (Figure 1). Cysteine is the precursor or reduced sulfur donor of most other organic sulfur compounds in plants. The predominant proportion of the organic sulfur is present in the protein fraction (up to 70 % of total S), as cysteine and methionine residues. In proteins cysteine and methionine are highly significant in the structure, conformation and function of proteins. Plants contain a large variety of other organic sulfur compounds, as thiols (glutathione), sulfolipids and secondary sulfur compounds (alliins, glucosinolates, phytochelatins), which play an important role in physiology and protection against environmental stress and pests (De Kok et al., 2002a). Sulfur compounds are also of great importance for food quality and for the production of phyto-pharmaceutics. Sulfur deficiency will result in the loss of plant production, fitness and resistance to environmental stress and pests. Plants may have to deal with temporary or prolonged periods of excessive sulfur or sulfur deficiency. Excessive sulfur from both pedospheric and atmospheric origin may be utilized as sulfur source for plants (De Kok et al., 2002a, b). On the other hand, it may cause physiological imbalances and negatively affect plant growth.

Plants' sulfur requirement for growth

The uptake of sulfate by the roots and its reduction and further assimilation in the shoots, is under normal conditions highly regulated on "a whole plant level" and it will be in tune with the actual sulfur requirement of a plant species for biomass production (De Kok et al., 2002a). The sulfur requirement strongly varies between species and it may strongly vary at different developmental stages of the plant (vegetative growth, seed production). The overall plants' sulfur requirement ($S_{requirement}$) can be estimated as follows (De Kok et al., 2002a; Durenkamp and De Kok, 2004):

$$S_{requirement}\ (\mu mol\ g^{-1}\ plant\ day^{-1}) = RGR\ (\%\ day^{-1})\ \times\ S_{content}\ (\mu mol\ g^{-1}\ plant)$$

where RGR represent the relative growth rate and $S_{content}$ the total plant tissue sulfur content. The RGR can be estimated as follows:

$$RGR = (\ln W_2 - \ln W_1)/(t_2 - t_1)$$

where W_1 and W_2 represent the total weight (g) at time t_1 and t_2, respectively, and $t_2 - t_1$ the time interval (days) between harvests. The rate of sulfate uptake by the roots necessary to meet the plants' sulfur requirement for growth can be estimated as follows:

$$Sulfate_{uptake}\ (\mu mol\ g^{-1}\ root\ day^{-1}) = S_{requirement}\ (\mu mol\ g^{-1}\ plant\ day^{-1})\ \times\ (S/R_{ratio} + 1)$$

[1] Laboratory of Plant Physiology, University of Groningen, P.O. Box 14, 9750 AA Haren, The Netherlands
[2] Soil and Fertilizer Institute, Chinese Academy of Agricultural Sciences (CAAS), Beijing, 100081, China

where S/R_{ratio} represents the shoot (S) to root (R) biomass partitioning of the plant.

At optimal growth conditions the sulfur requirement (equivalent to sulfur flux) of different crop species ranges from 2 to 10 μmol g^{-1} plant fresh weight day^{-1} (0.08 to 0.4 μmol g^{-1} plant fresh weight h^{-1}, Figure 1). Generally the major proportion of the sulfate taken up is reduced and metabolized into organic compounds essential for structural growth. However, seedlings of some plant species, e.g. *Brassica oleracea*, may contain relatively high sulfate contents and here the organic sulfur content might be used for the estimation of the sulfur requirement needed for structural growth (Castro et al., 2003).

Uptake and assimilation of sulfate

Sulfate is taken up by the roots with high affinity and the maximal sulfate uptake rate is generally already reached at pedospheric sulfate levels of 0.1 mM and lower (Hawkesford, 2000; Hawkesford and Wray, 2000; Hawkesford et al., 2003a, b). The uptake of sulfate by the roots and its transport to the shoot is strictly controlled and it appears to be one of the primary regulatory sites of sulfur assimilation (Figure 1).

Sulfate is actively taken up across the plasma membrane of the root cells, subsequently loaded into the xylem vessels and transported to the shoot by the transpiration stream. The uptake and transport of sulfate is energy dependent (driven by a proton gradient generated by ATPases) through a proton/sulfate (presumably $3H^+/SO_4^{2-}$) co-transport (Clarkson et al., 1993). In the shoot the sulfate is unloaded and transported to the chloroplasts where it is reduced. The remaining sulfate in plant tissue is predominantly present in the vacuole, since the cytoplasmatic concentrations of sulfate are kept rather constant.

Distinct sulfate transporter proteins mediate the uptake, transport and subcellular distribution of sulfate. According to their cellular and subcellular expression, and possible functioning the sulfate transporters gene family has been classified in up to 5 different groups (Davidian et al., 2000; Hawkesford 2000; Hawkesford et al. 2003a, b; Buchner et al., 2004). Some groups are expressed exclusively in the roots or shoots or expressed both in the roots and shoots. Group 1 are 'high affinity sulfate transporters', which are involved in the uptake of sulfate by the roots (Figure 2). Group 2 are vascular transporters and are 'low affinity sulfate transporters'. Group 3 is the so-called 'leaf group', however, still little is known about the characteristics of this group. Group 4 transporters may be involved in the transport of sulfate into the plastids prior to its reduction, whereas the function of Group 5 sulfate transporters is not known yet (Buchner et al., 2004).

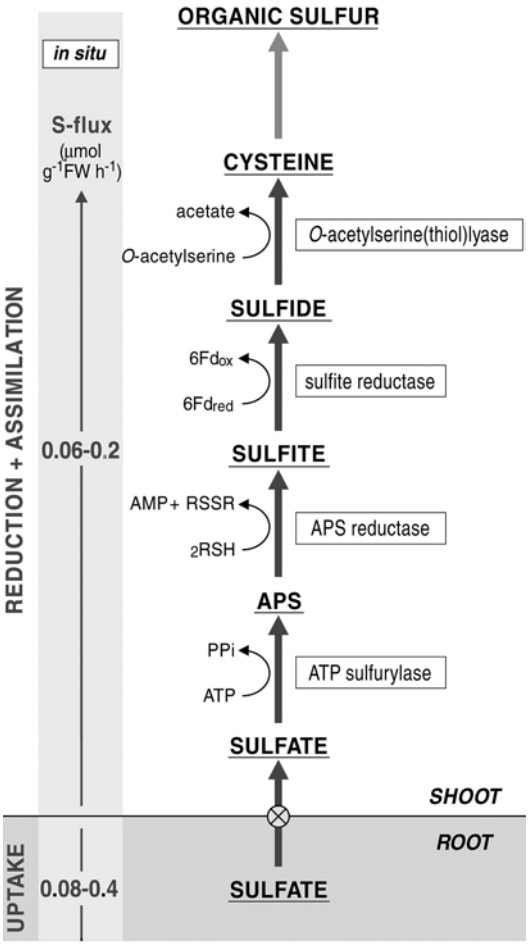

Figure 1:
An overview of sulfate reduction and assimilation in plants (APS, adenosine 5'-phosphosulfate; Fd_{red}, Fd_{ox}, reduced and oxidized ferredoxin; RSH, RSSR, reduced and oxidized glutathione) and the rates of sulfate uptake by the roots and its reduction and assimilation in the shoots of a variety of plant species grown under optimal sulfur supply (adapted from De Kok et al., 2002a).

Regulation and expression of the majority of sulfate transporters are controlled by the sulfur nutritional status of the plants. Upon sulfate deprivation, the rapid decrease in root sulfate is regularly accompanied by a strongly enhanced expression of most sulfate transporter genes (up to 100-fold), accompanied by a substantially enhanced sulfate uptake capacity (Hawkesford, 2000; Hawkesford and Wray, 2000; Hawkesford et al., 2003a, b; Buchner et al., 2004). It is still unresolved, whether sulfate itself or metabolic products of the sulfur assimilation (viz.

O-acetyl-serine, cysteine, glutathione) act as signals in the regulation of sulfate uptake by the root and its transport to the shoot, and in the expression of the sulfate tranporters involved (Davidian et al., 2000; Hawkesford, 2000; Hawkesford et al., 2003a, b; Buchner et al., 2004).

Even though root plastids contain all sulfate reduction enzymes, sulfate reduction takes predominantly place in the leaf chloroplasts. The reduction of sulfate to sulfide occurs in three steps (Figure 1). Sulfate needs to be activated to adenosine 5'-phosphosulfate (APS) prior to its reduction to sulfite. The activation of sulfate is catalyzed by ATP sulfurylase, which affinity for sulfate is rather low (K_m approximately 1 mM) and the *in situ* sulfate concentration in the chloroplast is most likely one of the limiting/regulatory steps in sulfur reduction (Stulen and De Kok, 1993). Subsequently APS is reduced to sulfite, catalyzed by APS reductase with likely glutathione as reductant (Leustek and Saito, 1999; Kopriva and Koprivova, 2003). The latter reaction is assumed to be one of the primary regulation points in the sulfate reduction, since the activity of APS reductase is the lowest of the enzymes of the sulfate reduction pathway and it has a fast turnover rate (Brunold, 1990, 1993; Leustek and Saito, 1999; Kopriva and Koprivova, 2003; Saito, 2003). Sulfite is with high affinity reduced by sulfite reductase with ferredoxin as a reductant and the formed sulfide is incorporated into cysteine, catalyzed by *O*-acetylserine(thiol)lyase, with *O*-acetylserine as substrate (Figure 1). The synthesis of *O*-acetylserine is catalyzed by serine acetyltransferase and together with *O*-acetylserine(thiol)lyase it is associated as enzyme complex named cysteine synthase (Droux et al., 1998; Hell, 2003). The formation of cysteine is the direct coupling step between sulfur and nitrogen assimilation in plants (Brunold, 1990, 1993; Brunold et al., 2003)

The remaining sulfate in plant tissue is transferred into the vacuole. The remobilization and redistribution of the vacuolar sulfate reserves appear to be rather slow and sulfur-deficient plants may still contain detectable levels of sulfate (Cram 1990; Davidian et al., 2000; Hawkesford, 2000; Buchner et al., 2004).

Metabolism of atmospheric sulfur gases

The rapid economic growth, industrialization and urbanization are associated with a strong increase in energy demand and emissions of gaseous pollutants including SO_2 (Shen et al., 1995; Feng et al., 2000; Emberson et al., 2001; Yang et al., 2002). As a consequence agricultural crop yields are at most risk from current levels of sulfurous air pollutants, viz. SO_2, since they are grown close to sources of emissions, where the annual average SO_2 concentrations may exceed 0.1 µl l^{-1}. However, the impact of sulfurous air pollutants on plant functioning is paradoxical, since they may both act as toxin and nutrient (De Kok, 1990; De Kok et al., 1998, 2000, 2002a, b; De Kok and Tausz, 2001). Plants even may benefit from elevated levels of atmospheric sulfur gases, since they contribute to plants' sulfur nutrition and exposure may result in enhanced yields, especially when sulfate is deprived in the root environment (Ernst, 1993; Van Der Kooij et al., 1997; De Kok et al., 1997, 2000).

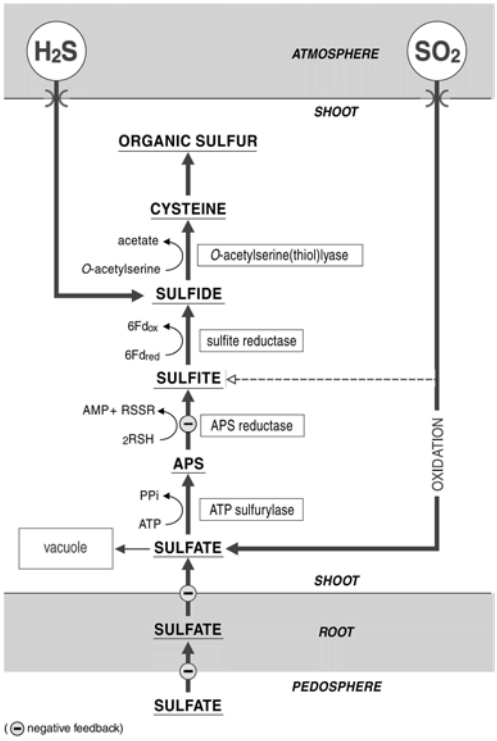

Figure 2:
Metabolism of SO_2 and H_2S in plant shoots and possible sites of feedback inhibition of sulfate uptake (adapted from De Kok et al. 2002a).

Plant shoots form a sink for atmospheric sulfur gases, which can directly be taken up by the foliage. The foliar uptake of SO_2 is generally directly dependent on the degree of opening of the stomates, since the internal resistance to gas is low. SO_2 is highly soluble in the apoplastic water of the mesophyll, where it dissociates under formation of bisulfite (HSO_3^-) and sulfite (SO_3^{2-}). Sulfite may directly enter the sulfur reduction pathway and be reduced to sulfide, incorporated into cysteine, and subsequently into other sulfur compounds (Figure 3). Sulfite may also be oxidized to sulfate, extra- and intracellularly

by peroxidases or non-enzymatically catalyzed by metal ions or superoxide radicals and subsequently reduced and assimilated again. Excessive sulfate is transferred into the vacuole; enhanced foliar sulfate levels are characteristic for SO_2-exposed plants. The foliar uptake of H_2S appears to be directly dependent on the rate of H_2S metabolism into cysteine and subsequently into other sulfur compounds (De Kok et al., 1998, 2000, 2002a.b; Figure 2). There is strong evidence that *O*-acetyl-serine (thiol)lyase is directly responsible for the active fixation of atmospheric H_2S by plants. Plants are able to transfer from sulfate to foliar absorbed SO_2 or H_2S as sulfur source (De Kok, 1990, De Kok et al., 1998, 2000, 2002a,b, Yang et al., 2003) and levels of 0.06 $\mu l\ l^{-1}$ appear to be sufficient to cover the sulfur requirement of plants (Yang et al., 2003; Buchner et al., 2004). There is an interaction between atmospheric and pedospheric sulfur utilization. For instance, H_2S exposure resulted in a decreased activity of APS reductase and a depressed sulfate uptake in *Brassica oleracea* (Westerman et al., 2000, 2001; De Kok et al., 2002b). However, H_2S solely affected the expression of the different sulfate transporters in the shoot, but not in the roots (Buchner et al., 2004).

Synthesis and physiological functions of sulfur metabolites

Cysteine is sulfur donor for the synthesis of methionine, the major other sulfur-containing amino acid present in plants (Giovanelli, 1990; Noji and Saito, 2003). Both sulfur-containing amino acids are of great significance in the structure, conformation and function of proteins and enzymes, but high levels of these amino acids may also be present in seed storage proteins (Tabatabai, 1986). The thiol groups of the cysteine residues in proteins can be oxidized resulting in disulfide bridges with other cysteine side chains (and form cystine) and/or linkage of polypeptides. Disulfide bridges make an important contribution to the structure of proteins. The thiol groups are also of great importance in substrate binding of enzymes, in metal-sulfur clusters in proteins (e.g. ferredoxins) and in regulatory proteins (e.g. thioredoxins).

Sulfoquinovosyl diacylglycerol is the predominant sulfur-containing lipid present in plants. In leaves its content comprises up to 3 - 6 % of the total sulfur present (Heinz, 1993; Benning, 1998; Harwood and Okanenko, 2003). This sulfolipid is present in plastid membranes and likely is involved in chloroplast functioning. The route of biosynthesis and physiological function of sulfoquinovosyl diacylglycerol is still under investigation. From recent studies it is evident that sulfite it the likely sulfur precursor for the formation of the sulfoquinovose group of this lipid (Harwood and Okanenko, 2003).

Glutathione (γGlu-Cys-Gly; GSH) or its homologues, e.g. homoglutathione (γGlu-Cys-βAla) in *Fabaceae*; hydroxymethylglutathione (γGlu-Cys-βSer) in *Poaceae* are the major water-soluble non-protein thiol compounds present in plant tissue and account for 1-2 % of the total sulfur (De Kok and Stulen, 1993; Rennenberg, 1997; Grill et al., 2001). The content of glutathione in plant tissue ranges from 0.1 - 3 mM. Cysteine is the direct precursor for the synthesis of glutathione (and its homologues). First, γ-glutamylcysteine is synthesized from cysteine and glutamate catalyzed by γ-glutamylcysteine synthetase. Second, glutathione is synthesized from γ-glutamylcysteine and glycine (in glutathione homologues, β-alanine or serine) catalyzed by glutathione synthetase (2). Both steps of the synthesis of glutathione are ATP dependent reactions:

$$\text{cysteine} + \text{glutamate} + \text{ATP} \xrightarrow[\text{γ-glutamylcysteine synthetase}]{} \text{γ-glutamylcysteine} + \text{ADP} + \text{Pi} \quad (1)$$

$$\text{γ-glutamylcysteine} + \text{glycine} + \text{ATP} \xrightarrow[\text{glutathione synthetase}]{} \text{GSH} + \text{ADP} + \text{P} \quad (2)$$

$$\text{NADPH} + \text{H}^+ + \text{GSSG} \xrightarrow[\text{glutathione reductase}]{} 2\text{GSH} + \text{NADP}^+ \quad (3)$$

Glutathione is maintained in the reduced form by an NADPH-dependent glutathione reductase (3) and the ratio of reduced glutathione (GSH) to oxidized glutathione (GSSG) generally exceeds a value of 7 (Rennenberg, 1997; Foyer and Noctor, 2001; Tausz, 2001).

Glutathione fulfils various roles in plant functioning. In sulfur metabolism it functions as reductant in the reduction of APS to sulfite (Figure 1). It is also the major transport form of reduced sulfur in plants. Roots likely largely depend for their reduced sulfur supply on shoot/root transfer of glutathione via the phloem, since the reduction of sulfur occurs predominantly in the chloroplast (De Kok et al., 1993; Rennenberg, 1997; Grill et al., 2001). Glutathione is directly involved in the reduction and assimilation of selenite into selenocysteine (Andersen and McMahon, 2001). Furthermore glutathione is of great significance in the protection of plants against oxidative and environmental stress and it depresses/scavenges the formation of toxic reactive oxygen species, e.g. superoxide, H_2O_2 and lipid hydroperoxides (Grill et al., 2001; Tausz et al., 2003). Glutathione functions as reductant in the enzymatic detoxification of reactive oxygen species in the glutathione-ascorbate cycle and as thiol buffer in the protection of proteins via direct reaction with reactive oxygen species or by the formation of mixed disulfides. The potential of glutathione as protectant is related to the pool size of glutathione, its redox

state (GSH/GSSG ratio) and the activity of glutathione reductase. Glutathione is the precursor for the synthesis of phytochelatins ($(\gamma Glu\text{-}Cys)_nGly$), which are synthesized enzymatically by a constitutive phytochelatin synthase. The number of γ-glutamyl-cysteine residues $(\gamma Glu\text{-}Cys)_n$ in the phytochelatins may range from 2 - 5, sometimes up to 11. Despite the fact that the phytochelatins form complexes which a few heavy metals, viz. cadmium, it is assumed that these compounds play a role in heavy metal homeostasis and detoxification by buffering of the cytoplasmatic concentration of essential heavy metals (Rauser, 1993, 2000, 2001; Verkleij et al., 2003). Glutathione is also involved in the detoxification of xenobiotics, compounds without direct nutritional value or significance in metabolism, which at too high levels may negatively affect plant functioning. Xenobiotics may be detoxified in conjugation reactions with glutathione catalyzed by glutathione *S*-transferase, which activity is constitutive; different xenobiotics may induce distinct isoforms of the enzyme (Schröder, 1998, 2001; Gullner and Kömives, 2001). Glutathione *S*-transferases have great significance in herbicide detoxification and tolerance in agriculture and their induction by herbicide antidotes (safeners) is the decisive step for the induction of herbicide tolerance in many crop plants. Under natural conditions glutathione *S*-transferases are assumed to have significance in the detoxification of lipid hydroperoxides, in the conjugation of endogenous metabolites, hormones and DNA degradation products, and in the transport of flavonoids.

Some plant species contain so-called secondary sulfur compounds, viz. glucosinolates in *Brassica* (Schnug, 1990, 1993; Rosa, 1997; Graser et al., 2001, Glawisching et al., 2003) and γ-glutamyl peptides and alliins (*S*-alk(en)yl cysteine sulfoxides) in *Allium* (Randle et al., 1993, 1995; Randle, 2000; Randle and Lancaster, 2002; Coolong and Randle, 2003a, b). In shoot and roots of *Brassica* the glucosinolate content accounted for 1 - 2 % of the total sulfur, however, there is a great diversity in glucosinolates between cultivars based on differences in amino acid derived side chains and their elongated derivatives (Castro et al., 2004). Glucosinolates are composed of a ß-thioglucose moiety, a sulfonated oxime and a side chain. The synthesis of glucosinolates starts with the oxidation of the parent amino acid to an aldoxime, followed by the addition of a thiol group (through conjugation with cysteine) to produce thiohydroximate. The transfer of a glucose and a sulfate moiety completes the formation of the glucosinolates (Schnug, 1990; Rosa, 1997, 1999; Graser et al., 2001).

The physiological significance of glucosinolates is still ambiguous, though they are considered to function as sink compounds in situations of sulfur excess (Schnug, 1990, 1993; Ernst, 1993). However, when *Brassica* was exposed to H_2S (Westerman et al., 2001) and *Arabidopsis* to SO_2 (Van der Kooij et al., 1997), the sink capacity of the glucosinolate fraction seemed to be rather limited. Upon tissue disruption glucosinolates are enzymatically degraded by myrosinase and may yield a variety of biologically active products such as isothiocyanates, thiocyanates, nitriles and oxazolidine-2-thiones (Rosa, 1997, 1999; Kushad et al., 1999; Graser et al., 2001; Petersen et al., 2002; Reichelt et al., 2002; Wittstock and Halkier, 2002). The glucosinolate-myrosinase system is assumed to play a role in plant-herbivore and plant-pathogen interactions. Furthermore, glucosinolates are responsible for the flavor properties of *Brassicaceae* and recently have received attention in view of their potential anticarcinogenic properties (Kushad et al., 1999; Graser et al., 2001; Petersen et al., 2002; Reichelt et al., 2002).

The content of γ-glutamyl peptides and alliins in *Allium* species strongly depends on stage of development of the plant, temperature, water availability and the level of nitrogen and sulfur nutrition (Randle et al., 1993, 1995; Randle, 2000; Randle and Lancaster, 2002; Coolong and Randle, 2003a, b; Durenkamp and De Kok, 2002, 2003, 2004). In onion bulbs their content may account for up to 80 % of the organic sulfur fraction (Schnug, 1993). Less is known about the content of secondary sulfur compounds in the seedling stage of the plant. It is assumed that alliins are predominantly synthesized in the leaves, from where they are subsequently transferred to the attached bulb scale (Lancaster et al., 1986). The biosynthetic pathways of synthesis of γ-glutamylpeptides and alliins are still ambiguous. γ-Glutamylpeptides can be formed from cysteine (via γ-glutamylcysteine or glutathione) and can be metabolized into the corresponding alliins via oxidation and subsequent hydrolyzation by γ-glutamyl transpeptidases (Lancaster and Boland, 1990; Randle and Lancaster 2002). However, other possible routes of the synthesis of γ-glutamylpeptides and alliins may not be excluded (Granroth, 1970; Lancaster and Boland, 1990; Edwards et al., 1994; Randle and Lancaster, 2002). Alliins and γ-glutamylpeptides are known to have therapeutic utility and might have potential value as phytopharmaceutics (Haq and Ali, 2003). The alliins and their breakdown products (e.g. allicin) are the flavor precursors for the odor and taste of species. Flavor is only released when plant cells are disrupted and the enzyme alliinase from the vacuole is able to degrade the alliins, yielding a wide variety of volatile and non-volatile sulfur-containing compounds (Lancaster and Collin, 1981; Block, 1992). The physiological function of γ-glutamylpeptides and alliins is rather unclear (Schnug, 1993).

Various other sulfur metabolites, e.g. alliins, glucosinolates, phytoalexins, the release of volatile sulfur compounds as H_2S, the production of sulfur-rich proteins (thionins) and localized deposition of elemental sulfur are assumed to have significance in the resistance of plants against stress and pests (Schnug, 1997; Glawishnig et al., 2003; Haneklaus et al., 2003; Haq and Ali, 2003). Several aspects of sulfur metabolism and its possible significance in "sulfur-induced-resistance" need further evaluation (Schnug, 1997; Haneklaus et al., 2003).

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Advances in sulfur fertilizer requirement and research for Chinese agriculture: Summary of field trial data from TSI's China project from 1997 to 2003

Ming Xian Fan[1] and Donald L. Messick[1]

Abstract

Sulfur deficiency is increasingly becoming one of the limiting factors to further sustainable increases in agricultural production. The Sulphur Institute collaborated with 15 institutions throughout China to evaluate soil sulfur deficiency and sulfur fertilizer requirements from 1997 to 2003. A total of 535 field trials have been completed in 14 provinces, evaluating direct effects of sulfur fertilizer on major agricultural crops, over the six-year period. The data generated from field trials showed that sulfur fertilizer significantly increased crop yields in 468 trials, 87% of the total trials completed. Average yield increases achieved with sulfur fertilization varied from 7% to 30%, among different crops. About 30% of soils in China, equivalent to about 40 million hectares, are sulfur-deficient, especially in Anhui, Fujian, Heilongjiang, Henan, Hunan, Guangdong, Guangxi, Jiangxi, Shaanxi, and Yunnan Provinces. Based on the results of field trials, an average 30 kg ha^{-1} sulfur fertilizer is needed to maximize both crop yield and economic return in sulfur deficient soils. Therefore, a total of 1.2 million tons of sulfur is currently needed in Chinese agriculture. This sulfur deficit will increase to 2.4 million tons annually by 2013 unless correct measures are taken with inclusion of sulfur into fertilizer recommendation programs. With effective sulfur fertilizer strategies, China can increase by an average of 10% the yield in sulfur deficient soils (approximately 0.6 ton per hectare), adding about 24 million tons of grain in Chinese agricultural production every year. It will also improve crop quality and fertilizer efficiency through interaction of sulfur with other fertilizer nutrients and increase the economic return to farmers by approximately 36 billion yuan. The results generated from the six years' field trials provide further solid evidence that sulfur fertilizer is playing an important role in the sustainable development of Chinese agriculture through balanced fertilization, we encourage the Chinese government to recognize sulfur as an essential fertilizer nutrient like nitrogen, phosphorus, and potassium; to adopt favorable policies associated with sulfur fertilizer production, distribution and use; and to allow farmers to capitalize on the economic benefit with a relatively small input.

Key words: sulfur deficiency, sulfur fertilizer, sulfur requirement, Chinese agriculture, crop production

Introduction

In China, the rapid agricultural production growth during the last two decades (1980 to 2000) was closely linked with the increased use of mineral fertilizers, which was increased from 12.6 million tons to 41.5 million tons, averaged at about 300 kg ha^{-1}. It is estimated that 50 percent of farmer production costs in China go to fertilizer; and fertilizer also contributed about 45 to 50% increase in modern agricultural production (Chen Shoulun, 2002). In the high yield provinces of China the level of fertilizer use is over 400 to 500 kg ha^{-1} (China Agriculture Yearbook, 2000). According to Chinese governmental forecasts, China's population and grain production are expected to reach 1.4 billion and 560 million tons in 2010, respectively, based on the assumption of 400 kg grain consumption per capita per year. To meet the increasing demand of food and fiber for the increasing population and living standard, fertilizer use is projected to increase to 50 million tons in 2010 (Xiao Yunlai, 2001). However, with this high volume of fertilizer consumption improving fertilizer knowledge and technology is becoming even more important for optimizing its use for both economic and environmental considerations, like balanced fertilization, i.e. tailor fertilizer program based on crop demand and soil fertility status for both higher yield and economic returns. This requires increasing use of all essential plant nutrients in addition to traditional nitrogen, phosphate and potassium to achieve the maximum benefit possible from fertilizers through improved management practices that include all sources of nutrients and innovative technologies, while maintaining or improving soil fertility without harmful impacts on the environment. Like nitrogen, phosphorus, and potassium, sulfur is one of the major essential plant nutrients, and it contributes to an increase in crop yields in three different ways: 1) it provides a direct nutritive value, 2) it provides indirect nutritive value as soil amendments, especially for calcareous and saline alkali soils, 3) it improves the use efficiency of other

[1] The Sulphur Institute, Washington, DC. USA

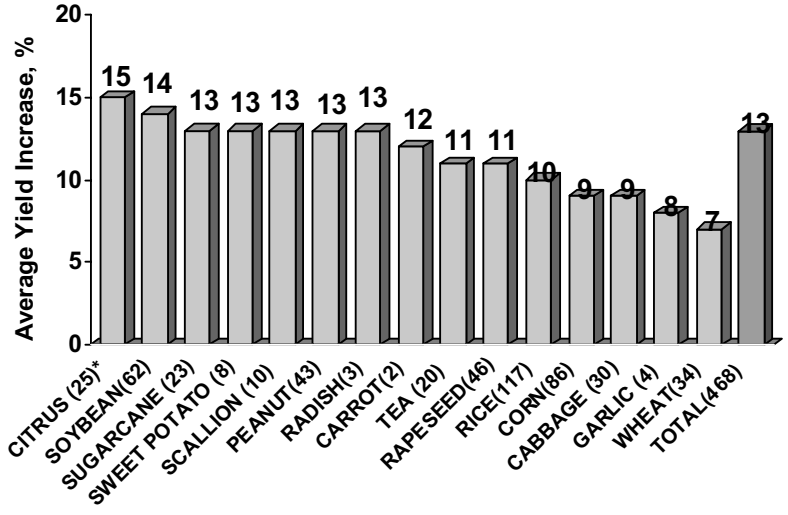

Figure 1:
Average crop yield responses from sulfur fertilization within China during 1997 to 2003 (values in the parentheses represent the total number of field trials).

essential plant nutrients, particularly nitrogen and phosphorus. However, its importance as a fertilizer nutrient and its requirements in agriculture were unrecognized in the past. Sulfur deficiencies were masked by the depletion of soil sulfur and sulfur input through precipitation, irrigation water, manures, and sulfur-containing fertilizers, such as ammonium sulfate and single superphosphate (SSP). According to The Sulphur Institute's model analysis on plant nutrient sulfur demand in the world, Asia is the most sulfur deficient region in the world, with an annual 5.8 million ton sulfur fertilizer deficit predicted by 2011(The Sulphur Institute 2003). China and India represent the largest sulfur demand countries in the region, with annual sulfur deficits of 2.3 and 1.9 million tons, respectively. Sulfur deficiency is increasingly becoming one of the limiting factors to further sustainable increases in agricultural production in China, as agricultural production intensifies and high-analysis fertilizers, containing little or no sulfur, are increasingly used.

Sulfur fertilizer effect on crop yield

From 1997 through 2003, The Sulphur Institute (TSI) collaborated with 15 institutions throughout China as a cooperative network to evaluate soil sulfur fertility status and sulfur fertilizer requirements. A total of 535 field trials have been completed in 14 provinces, evaluating direct effects of sulfur fertilizer on major agricultural crops, over the six-year period. The data generated from field trials showed that sulfur fertilizer significantly increased crop yields in 468 trials, 87% of the total trials completed. Average yield increases achieved with sulfur fertilization varied from 7% to 30%, among different crops (Figure 1). Among the crops tested, chili, tomato, citrus, sugarcane, sweet potato, soybean, cauliflower, scallion, rapeseed, and peanut had the highest yield response at 10% or greater. Eighteen field trials were conducted to examine the residual effect of sulfur fertilizer. Crop yields were increased by sulfur fertilizers applied in the preceding crops in15 field trials, ranging from 4% to 7%.

Crop yield responses to sulfur fertilizer were also different among the tested provinces. Differences were observed due to soil sulfur fertility status, cropping system and fertilizer use history. Generally, better crop responses to sulfur fertilization were obtained in the southern provinces. The average yield increases over the six-year time period, 1997 to 2002, in the tested provinces are presented in Figure 2.

Over sixty field trials were conducted to evaluate crop responses to different sulfur fertilizers, including ammonium sulfate, elemental sulfur, gypsum, phosphogypsum and SSP in all fourteen provinces. No significant difference was obtained in crop yield

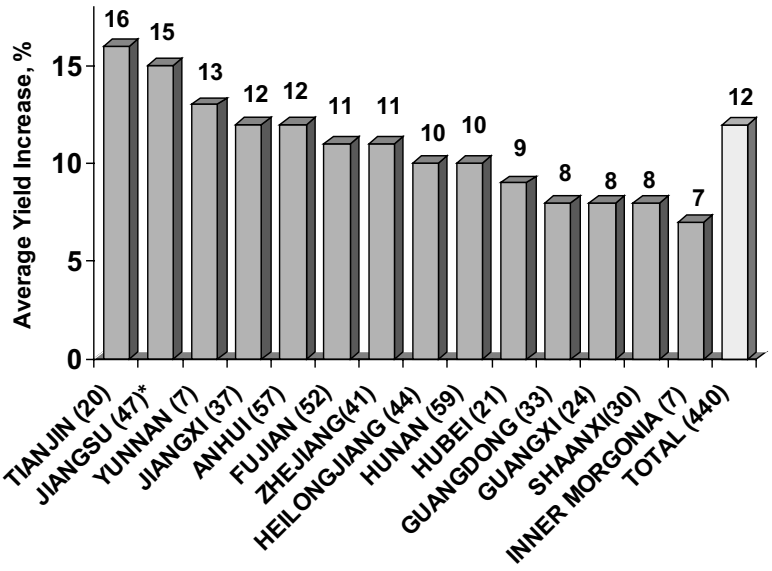

Figure 2:
Average sulfur fertilization effect on crop yield in different provinces of China from 1997 to 2002 (Yunnan, Tianjin and Jiangsu are the mean of three year's data).

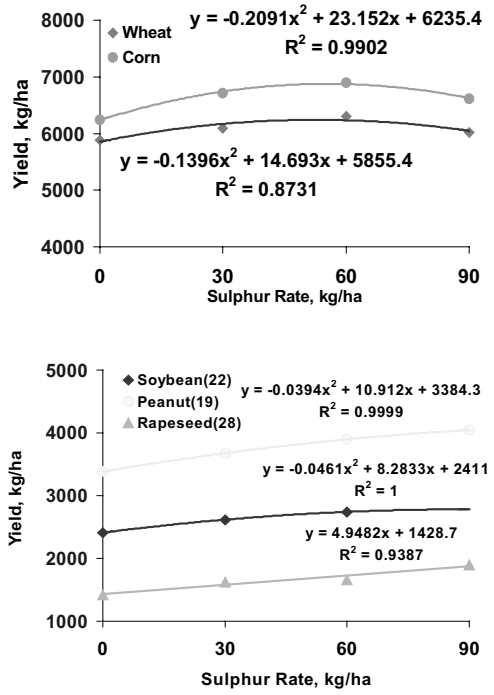

Figure 3:
Average yield responses of cereal (123 field trials) and oil crops (69 field trials) to sulfur fertilizer rates in China from 1997 to 2002.

due to sulfur sources, though in some field trials, higher yield increases were found with ammonium sulfate and SSP fertilizers in northeastern China, as compared to elemental sulfur.

Crop yield increased significantly with increasing sulfur rates to 60 kg sulfur ha^{-1} for cereal crops, and to 90 kg sulfur ha^{-1} for oil, sugar and vegetable crops in over 200 field trials conducted in 14 provinces with different soils and fertilizer managements. The optimum sulfur fertilizer rates for maximum yield ranged from 40 to 60 kg ha^{-1} for cereal crops; and from 60 to 90 kg ha^{-1} for oil, sugar, vegetable and cash crops (Figure 3).

Sulfur fertilizer effect on crop quality

Sulfur is a constitute of three essential amino acids, vital to protein production and enzyme activity, and participates in the synthesis of many secondary compound in plants. Sulfur fertilization has a decisive role in improving crop quality and increasing its market value, particularly in the case of wheat, rapeseed, sugarcane, fruits, vegetables and tea. According to the results of field trials, sulfur fertilizer increased crude protein content in rice and wheat by 10% to 27% in Anhui and Jiangsu provinces; oil

Table 1:
Sulfur fertilizer effect on tea leaf quality and orange quality in Southern China Provinces in 2002.

Tea (average of four field trials)					
	Yield (kg ha^{-1})	Phenols (%)	Amino Acid (%)	Caffeine (%)	Water extract (%)
Control	1691	21.1	2.14	2.76	35.7
ES 60 kg ha^{-1}	1842 (8.9%)	21.9 (3.8%)	2.27 (6.2%)	2.92 (5.6%)	36.1 (1.2%)
Orange (average of six field trials)					
	Yield (t ha^{-1})	Vitamin C (mg 100 ml^{-1})	Sugar (g 100 ml^{-1})	Acidity (g 100 ml^{-1})	Soluble Solid (%)
Control	33.2	34.2	8.0	0.89	10.1
ES 60 kg ha^{-1}	38.5 (16%)	35.7 (4.4%)	8.3 (3.8%)	0.95 (6.7%)	10.2 (1.0%)

content of peanut by 6.5% and methionine content in peanut by 40% in Fujian and Jiangxi Provinces; sugar content in sugarcane and banana by 10% to 23% in Guangdong, Guangxi, and Jiangxi provinces. Amino acid and polyphenol contents in tea leaves and Vitamin C and sugar content in orange juice are important indexes in evaluating tea leaf and orange quality and market value. The results from three years' field trials conducted in Hunan, Zjhejiang and Anhui Provinces from 1999 to 2002, sulfur fertilizer increased amino acid content of tea leaves by 6.6% and Vitamin C content in orange juice by 4.4% (Table 1), thereby greatly improving green tea and orange quality. Sulfur fertilization also reduced nitrate concentration in various leaf vegetables by 10% to 50% in Anhui, Fujian and Guangdong Provinces.

Economic benefits of sulfur fertilization

Sulfur fertilizer increased crop yield, improved crop quality, and also significantly increased economic return to the producers. According to the Value Increase: Input Cost Ratio (VCR) calculated from the field trial results for the seven years (Figure 4), for high yield cash crops like banana, vegetables, citrus, sugarcane, sweet potato and tea, the economic returns from sulfur fertilizer investment (VCR) were very high, ranging from 18 to 40. The average VCRs for oil and grain crops were from 10 to 15. Considering that a VCR of 2 to 2.5 is generally accepted as profitable and conducive to fertilizer application, sulfur fertilization is viewed as highly profitable in soils having inadequate sulfur due to its lower cost as compared to that of other fertilizer nutrients, like nitrogen, phosphorus and potassium.

Sulfur fertilizer increased crop yield, and also increased nutrient uptake and nutrient use efficiency, such as nitrogen, which resulted in less likelihood of nutrient loss to the environment due to leaching and/or runoff. This effect has been demonstrated by large number of data generated from sulfur interaction with nitrogen field trials on different crops in China. Total nitrogen uptake by rice was increased by 13 kg ha^{-1} and 19 kg ha^{-1} by applying 30 and 60 kg sulfur ha^{-1} with 120 kg nitrogen ha^{-1} in one rice field trial in Jiangxi, which resulted in a 7 % and 10% increase in nitrogen use efficiency. In most field trials studying the interaction of sulfur with nitrogen on rice, adding 30 kg sulfur ha^{-1} with the low rate of nitrogen (120 kg ha^{-1}) resulted in higher yield than the high rate of nitrogen (180 kg ha^{-1}) without sulfur (Figure 5). With the increasing concerns about nitrogen fertilizer cost and the potential impact on environment, the beneficial effect of sulfur fertilizer on nitrogen uptake and utilization by plant is critical in precise farming and fertilizer management.

Soil sulfur deficiency in China

Combining with the field trials evaluating crop response to sulfur fertilizers, over 20,000 soil samples have been taken from major agricultural soils to determine the soil sulfur fertility status. The results show that about 30% of soils in China, equivalent to about 40 million hectares, are sulfur-deficient, especially in Anhui, Fujian, Guangdong, Guangxi, Heilongjiang, Henan, Hunan, Jiangxi, Shaanxi, and Yunnan Provinces (Figure 6). Based on the results of several years' field trials, an average 40 kg ha^{-1} sulfur fertilizer is needed to maximize both crop

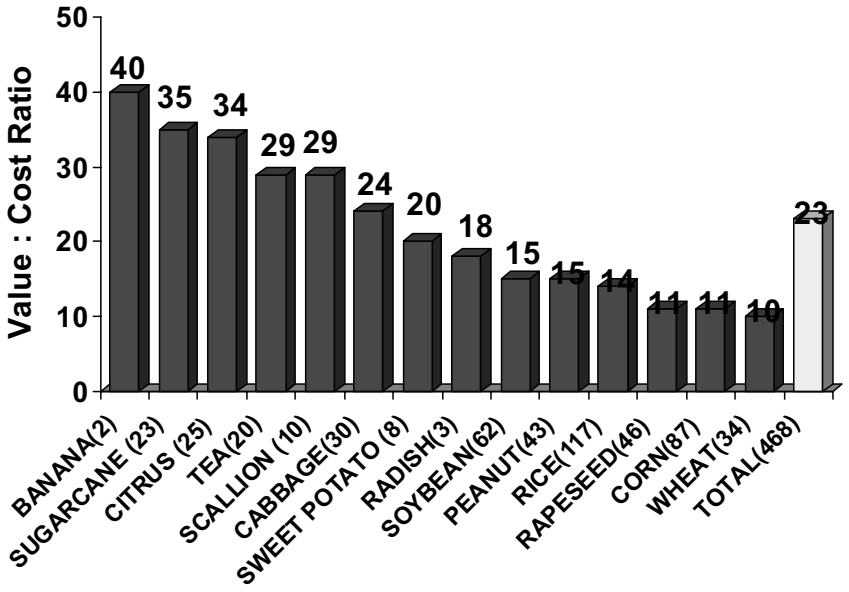

Figure 4:
Average economic profit obtained from crop response to sulfur fertilizer in the field trials from 1997 to 2003 (values in the parentheses represent the total number of field trials).

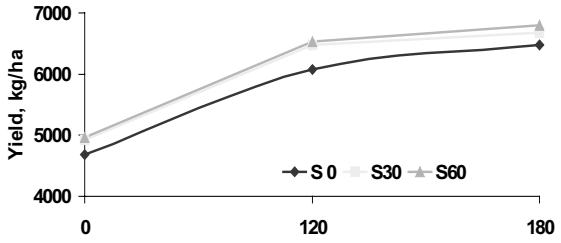

Figure 5:
Average rice yield responses to interactions of three sulfur rates and three nitrogen rates in ten field trials in southern China (1999 to 2002).

yield and economic return, while maintaining soil fertility in sulfur deficient soils. Therefore, a total of 1.6 million tons sulfur fertilizer is needed in Chinese agriculture.

Demonstration and Extension Activities in China

To further increase the awareness of the importance of sulfur in balanced fertilization and promote sulfur fertilizer use in balanced fertilization in China, more than 10 large-scale demonstration projects were established in sulfur deficient, intensively cultivated regions in Anhui, Fujian, Guangdong, Guangxi, Henan, Jiangxi, Tianjing, and Zhejiang Provinces on major agricultural crops such as rice, corn, peanut, soybean, sugarcane, tea, vegetables. These demonstration projects showed sulfur fertilizer increased both crop yield and economic returns. For example, Jiangxi Academy of Agricultural Sciences, collaborating with TSI, established two large scale demonstration projects in Xia Jiang County, located in the southern Jiangxi province with >60% of sulfur deficient soils, equivalent to 20,000 ha; and Xing Guo County, located in the center of Jiangxi province with 46% of sulfur deficient soils, equivalent to 15,000 ha in 2001. Each demonstration project includes 750 ha demonstration area, and three simple comparison fields. The site-measured yield in the "Harvest Day" showed sulfur fertilizer increased rice yield by 10.6%, compared with the rice yield in the plots without sulfur fertilizer; and 15.4% increase, compared with the previous three years' average yield. Meanwhile, combined with these demonstration projects, numerous regional site-workshops and extension activities, such as field tours and "Harvest Day" events have been organized

in these regions to show sulfur's beneficial effects on crop production and promote sulfur use through balanced fertilization. Through these extension activities and other education publication materials, the latest research achievements and sulfur fertilizer technology were disseminated to a large number of Chinese farmers, agricultural extension workers as well as government officials, who are responsible for conversion of research achievements to farmers' practices within their province, which greatly increased their awareness of the importance of sulfur in agriculture, and helped to accelerate the interest in sulfur fertilization in China.

Conclusions

The results generated from the six years' field trials conducted in the major agricultural provinces of China provide further solid evidence that sulfur deficiency is limiting crop production, affecting crop yield and quality as well as economic return. Sulfur fertilization is playing an important role in the sustainable development of Chinese agriculture, given the now extensive database of information demonstrating sulfur fertilization benefits.

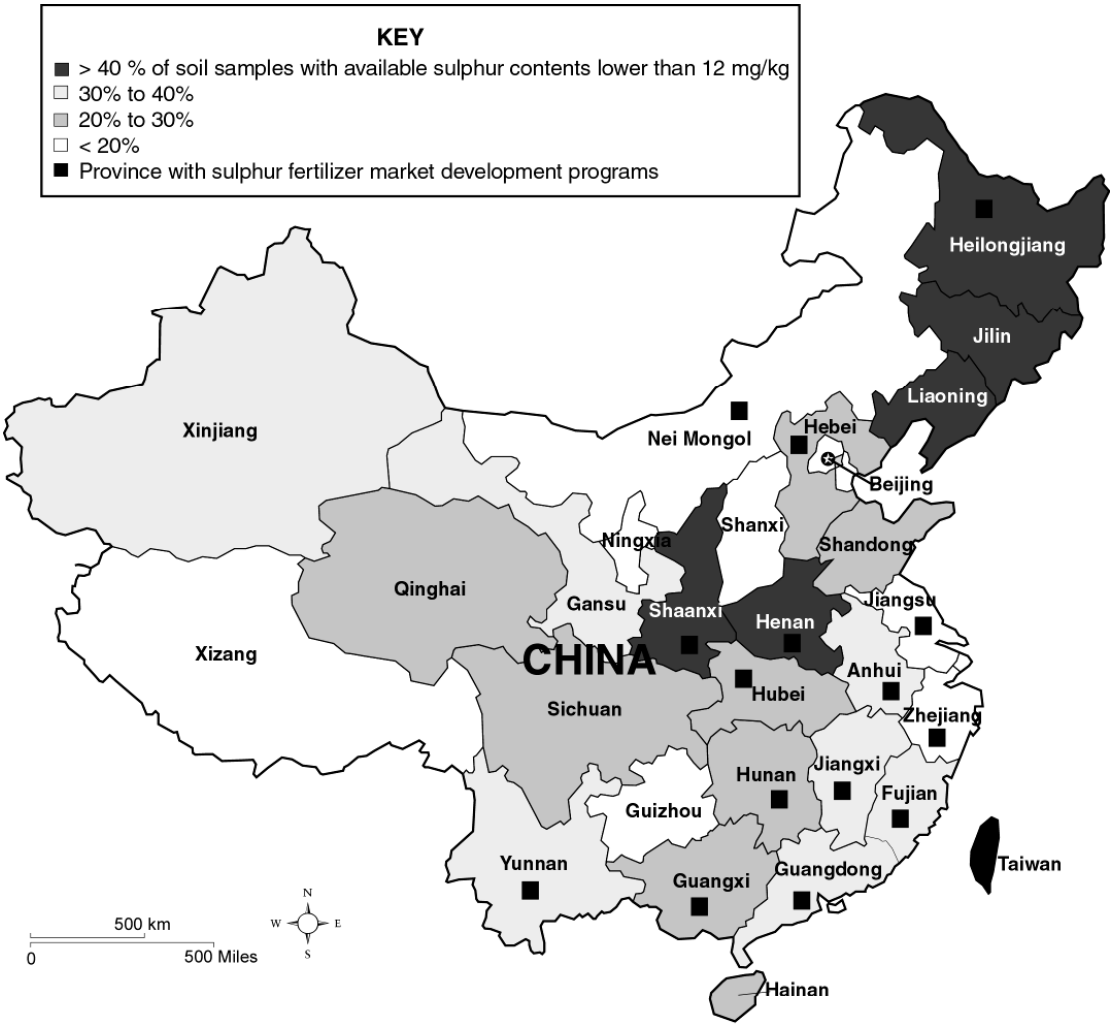

Figure 6:
Soil sulfur deficiency distribution and the location of The Sulphur Institute and PRISM Sulphur Corporation's research projects within China.

To increase agricultural production, efficiency and farmer's income, the Chinese government has been adjusting agricultural production structures by increasing cash crop production, such as oil, sugar, vegetables, tea and fruits; and by encouraging production of high quality products (Li Tianshen, 2003). Most cash crops and high crop quality production have higher demand for sulfur and balanced fertilizer technology for realization of their high quality and market values. Therefore, due to the well-demonstrated important role of sulfur in Chinese agricultural production, we encourage the Chinese government to recognize sulfur as an essential fertilizer nutrient like nitrogen, phosphorus, and potassium; to adopt favorable policies associated with sulfur fertilizer production, distribution and use; and to allow farmers to capitalize on the economic benefit with a relatively small input. With the development of effective sulfur fertilizer policy and strategy, it is expected that sulfur fertilizer use in China will increase significantly over the coming decade and make a greater contribution to increasing Chinese agricultural production through balanced fertilization, including sulfur.

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Sulfur and baking-quality of bread making wheat

Ingo Hagel[1,2]

Abstract

It is well known in biological science that all factors applied to living organisms (light, water, warmth, fertilizers etc.) show an optimum, when their input is increased. Healthy organisms and sustainable systems are, on the long run, only achieved when care is taken not to destroy this equilibrium of factors producing an optimum. With regard to the baking quality of wheat breeders and cereal scientists obviously failed to achieve this aim by breeding their cultivars on the background of ample S depositions in the ecosystems. They (involuntarily) selected plants showing definite characteristics of S deficiency (higher proportions of HMW-glutenin, stronger gluten and dough) even under conditions of ample S supply. I suppose they also selected plants with a high warmth susceptibility as this also delivers firm protein structure. When this environmental pollution was stopped and S supplies returned to natural conditions, even with a non S craving plant like wheat, problems arose with the gluten structure as doughs turned out so strong that the baking volume decreased. So one may ask, particularly with regard to S, if the plant constitutions of our modern wheat cultivars are still harmonious and in balance. And as a consequence ot that also the nutritional quality of these cultivars is rather questionable.

Key words: wheat, sulfur, baking quality, gluten, maximum resistance, temperature influence, nutritional quality

Introduction

When wheat is milled into flour and the dough is baked into bread, the developing carbon dioxide gas bubbles that develop through fermentation (yeast or sour dough) are prevented from escaping the dough by its protein or gluten matrix. By keeping the gas bubbles in place, nice bread with attractive baking volume arises, not only delighting the bakers by lowering their flour-input costs, but also appealing to the human senses. Yet baking quality was not always as outstanding as it is today. For example, in Germany until shortly before the outbreak of the Second World War, many cultivars existed with low performance (Klemt, 1934) and very soft glutens. Some of them were like glue from a tube, so one could write one's name with it on the work surface (Kosmin,1934). And even at the beginning of the 60's German grown wheat had to be blended with 25-28% Canadian or American high quality wheat (Bolling, 1989).

So it is understandable, that especially after the Second World War cereal chemists provided innumerable contributions on wheat and its quality. Breeders successfully selected wheat cultivars with ever firmer and elastic glutens, a process that is still in progress. So the question may arise if this development only shifted the protein quality of the staple food wheat from one extreme to the other. One has to keep in mind that the word "quality" with regard to wheat almost exclusively means "technological quality", and this in fact means "baking quality", not "nutritional quality". The mediation of all life processes are closely linked to proteins. As an increasing number of people nowadays suffer from wheat incompatibility, one may ask whether we have lost sight of the nutritional needs of human beings through the changing of wheat protein for merely technological reasons.

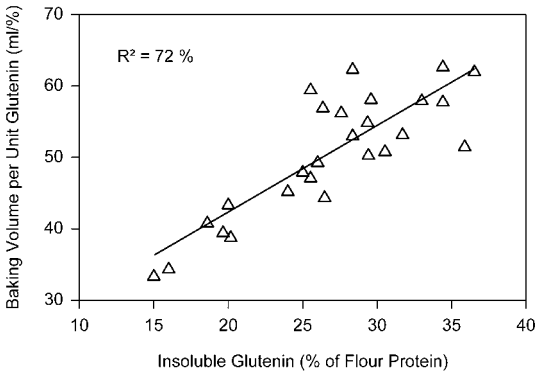

Figure 1:
Relation between the amount of acetic-acid-insoluble glutenin and the baking volume per percent-unit of protein in the flour (Orth und Bushuk, 1972, from Bushuk, 1989)

Today we will focus on the question whether the firm protein structure and excellent baking quality of modern wheat varieties comes from some sort of a S deficiency syndrome induced involuntarily by breeding (Hagel 2000a, 2002).

[1] Dr. Ingo Hagel, Federal Agricultural Research Center, Institute of Plant Nutrition and Soil Science, Bundesallee 50, D-38116 Braunschweig Germany; [2] Umkreis-Institut, Martinstrasse 73, D-64285 Darmstadt, Germany

Sulfur and wheat proteins

The crude protein of wheat can be separated into several fractions (according to their solubility in different solvents), which also contribute quite differently to baking quality. The salt soluble albumins and globulins are concentrated in the periphery of the grain, directly under the bran (Hagel, 2000b). Therefore their content depends very much on thousand-kernel weight and flour quality (whole grain flour or flours with a lower ash content). With regard to a flour featuring a low ash content of 0.55% they account for approximately 11 – 22% of protein, depending on the total protein content of the grain and cultivar (Wieser and Seilmeier 1998; Wieser et al., 1980a). So the vast majority of the wheat proteins in such flour are gluten proteins, comprising the gliadins and the glutenins. Type and proportion of these two protein fractions greatly influence the structure of gluten, the rheological performance of the dough and therewith the technological quality of the wheat i.e. the baking volume. While gliadins contribute to viscosity and extensibility, glutenins are regarded as the main factor for elasticity and firmness (Wieser et al., 1994). Additional gliadin leads to softer and more extensible glutens (Kim et al., 1988). On the other hand, according to the basic results of Orth and Bushuk (1972, Figure 1), the strengthening effect of glutenin to gluten and dough (Seilmeier et al., 1992; Wieser et al., 2000; Antes and Wieser, 2000; Wieser and Kieffer, 2001) (and thus leading to higher baking volume) has been consistently corroborated (Field et al., 1983; Gupta et al., 1993; Kieffer et al., 1998).

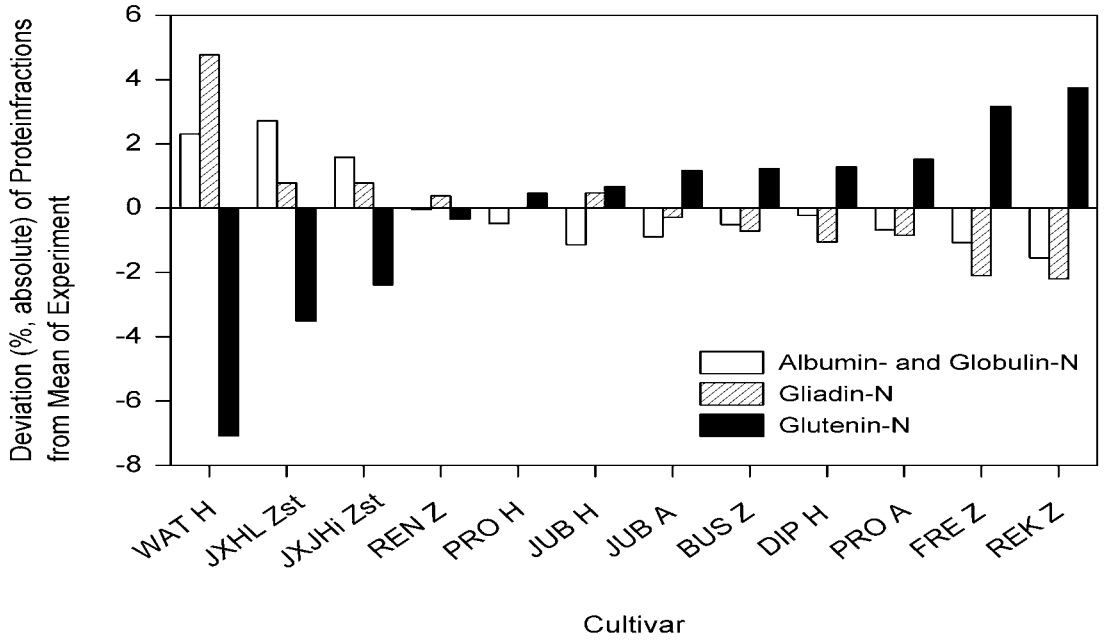

Figure 2:
Deviations (%, absolute) of protein fractions from the mean (regressions of the protein fractions versus N content) of all cultivars of wheat (whole grain, harvest 1994; Hagel et al., 1998a).

So it is understandable that in the course of the last 60 years the development from old to modern wheat cultivars has led to a drastic shift in the proportions of protein fractions (Hagel et al., 1998, Figure 2): The very old wheat type Weisser Ammertaler (WAT), a cross of the older cultivar Jubilar with an old Hessischer Landweizen (JXHL) as well as a cross of Jubilar with another Jubilar-cross (JXJHi) showed glutenin proportions far below the average of all other variants of this trial, but with higher gliadin proportions and thus leading to extremely soft glutens (gluten indices of 42-56%; Hagel et al., 1998a). On the other hand, particularly the modern cultivars Fregatt and Rektor had very high proportions of glutenin above the mean, but also the modern cultivars Bussard and the older cultivars Diplomat, Jubilar and Progress showed glutenin proportions well above the mean and lower gliadin, which led to very firm glutens (gluten indices of 84-99% (Hagel et al., 1998)). Parenthetically, from Figure 2 it can be seen that not only gliadin was replaced by glutenin, but also albumins and globulins, being the protein fractions with the highest S contents (see below and Table 1).

Table 1:
Contents (Mol-%) of cysteine, methionine and lysine of protein fractions of wheat (cultivars: KOLIBRI and REKTOR), (Wieser et al., 1980 & 1991).

	Cysteine	Methionine	Cysteine + Methionine	Lysine
Albumins	3.3	1.6	4.9	3.1
Globulina	3.2 - 3.7	2.0 - 2.1	5.8	4.1
Gliadins (total)	1.8 - 2.2	1.1 - 1.4	2.9 - 3.6	0.8
ω5-Gliadins	0	0	0	0.4 - 0.5
ω1,2-Gliadins	0	0.0 - 0.3	0.0 - 0.3	0.3 – 0.6
Glutenins (total)	1.4	1.3	2.7	2.1
HMW-Glutenins	0.6 - 1.3	0.1 - 0.3	0.7 - 1.6	0.7 – 1.1
LMW-Glutenins	1.9 - 2.6	1.2 - 1.6	3.1 - 4.2	0.2 – 0.6

Glutenins can be separated into (high-molecular-weight) HMW-glutenins (M_r = 80.000-120.000) and (low-molecular-weight) LMW-glutenins (M_r = 30.000 -52.000 (Wieser, 2000)). HMW-glutenins are highly responsible for inducing firmer protein structure i.e. higher resistances of the glutens (Wieser et al., 1994; Seilmeier et al. 1992; Schropp and Wieser, 2001) and therefore play a key-role in gluten structure (Wieser and Zimmermann, 2000). The LMW-glutenin does not (or to a much lesser extent) contribute to the firmness (resistance) of the gluten (Antes and Wieser, 2000; Wieser and Kieffer, 2001). So HMW-glutenin appeared to be such an interesting research topic for cereal chemists that Shewry et al. (1992) stated that the 1980s could well be considered as the "decade of the HMW subunit". The ratio of HMW:LMW-glutenin of wheat cultivars of widely differing baking quality varied from 0.35-0.65 (according to data from Wieser et al., 1994, Wieser and Kieffer, 2001). These variations make it plausible that breeders consciously (by analyzing for HMW-glutenin) and involuntarily (by selecting wheat with firm and elastic glutens, high sedimentation values, high baking volumes etc.) developed their wheat cultivars not only by increasing the glutenin content (Figure 2) but also by increasing the HMW:LMW ratio, though for the latter assumption no data is available. Anyway, all these measures (including of course replacing albumins and globulins by gluten proteins (Figure 2)) led to an increase of proteins low (gliadins, glutenins) or very low (HMW-glutenins) in S compared to albumins and globulins. These salt soluble proteins are very rich not only in essential amino acids such as lysine but also in S containing cysteine and methionine (Table 1). Moreover, as mentioned above, these non-gluten-proteins are concentrated in the periphery of the grain and can make up to 37% of the total wheat protein of whole grain wheat (Hagel 2000 b, Figure 3).

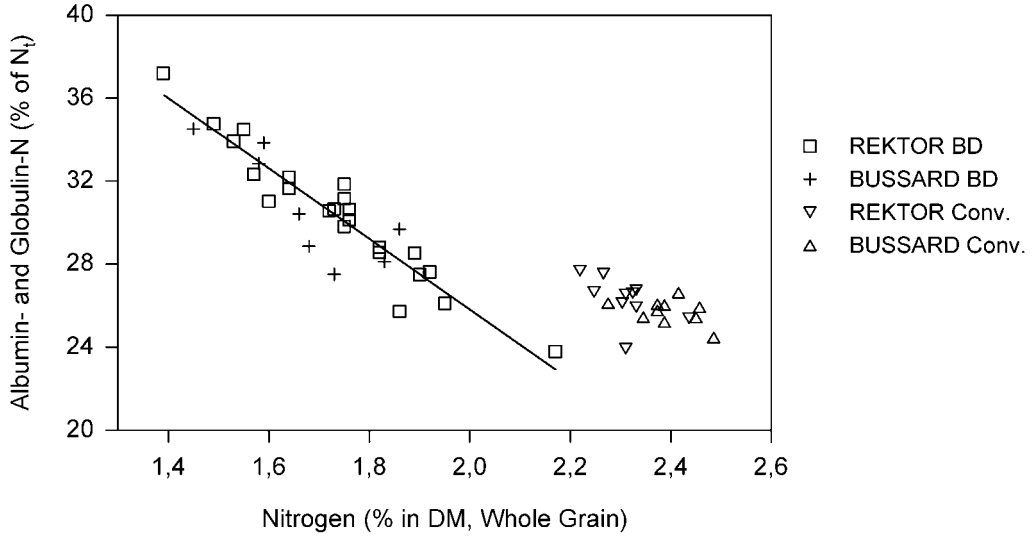

Figure 3:
Relations between nitrogen content and proportions of albumin- and globulin-nitrogen of total nitrogen content from wheat (cultivars: Rektor and Bussard, whole grain) from biodynamic (BD) and conventional (Conv.) agriculture, harvest 1996 (Hagel, 2000b).

As their content remains constant, their proportion of the total protein sharply declines with increasing protein content of the grain. So increasing the N content of the grain by N-fertilization, of which bakers are very fond of for technological reasons, increases only S low gluten proteins, not S rich albumins and globulins (Doekes and Wennekes, 1982; Wieser and Seilmeier, 1998). Consequently, for example, an increase of the protein content from approximately 1.4 to 2.2% N of the (biodynamic) wheat samples leads to a decline of the proportions of the S rich albumins and globulins from 37% to 24% of the total grain protein, respectively (Figure 3).

It becomes obvious that especially in conventional agriculture the aim of high contents of grain-N achieved by mineral fertilizers induces an imbalance between N and S, as S does not increase to the same degree as N (Hagel und Schnug, 1999; Hagel et al., 1998b). For instance, in Figure 4 the S contents fall below the diagonal of the graph. So many of the conventional wheat samples with high N content have come very near or have already crossed the line of an N:S ratio of 17:1, indicating S deficiency. Yet organically grown wheat with much lower N content is no guarantee for sufficient S supply (Hagel and Schnug, 1999). Figure 4 clearly demonstrates that the set of biodynamic samples from harvest 1996 must be differentiated into two different sub samples featuring N:S ratios < and > 14.5. Biodynamic samples from harvest 1995 also showed the same phenomenon of apparently different S supply including many samples with S deficiency (Hagel and Schnug, 1997).

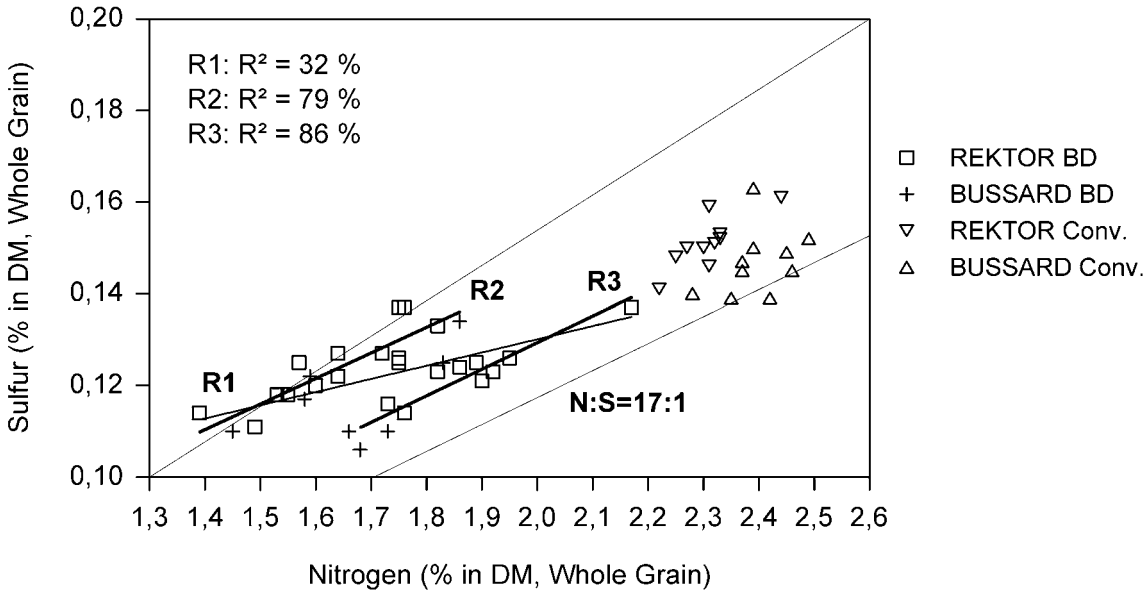

Figure 4:
Nitrogen and sulfur contents of wheat (cultivars Rektor and Bussard) from biodynamic (BD) und conventional (Conv.) agriculture (harvest 1996). Regression lines R2 and R3 differentiate the biodynamic samples into two sub-samples N:S ratio <and > 14.5:1 (Hagel et al., 1998b)

Sulfur and baking quality

With regard to gluten quality, S deficiency leads to much firmer and less extensible doughs (Moss et al., 1981; MacRitchie and Gupta 1993; Wrigley et al., 1984a). In Figure 5 the flour sufficiently supplied with S showed an extensogram with low energy (175 Brabender units at 50 mm extension). In contrast, the dough of the flour featuring S deficiency was much firmer, with a resistance of 365 Brabender units at 50 mm extension. Decreasing S contents lead to ever firmer doughs and low baking volumes, whereas S fertilization and increasing S content of the wheat grain induces less tough doughs and higher baking volumes (Figure 8; Moss et al., 1981).

Interestingly, the features of S deficient wheat described above (strong extensograms, stronger and tougher glutens and doughs) and shown in figure 5 were just what breeders and bakers were aiming at for decades on their quest for cultivars with high technological quality. Also biochemically, S deficient wheat shows characteristics of good baking quality wheat: less polypeptides with low M_rs (8,000-28,000, mainly albumins) and more polypeptides with high M_rs of 51,000-80,000 (Wrigley et al., 1984 a), higher content of HMW-glutenin (Castle and Randall, 1987), increasing amounts of

HMW-glutenin (Seilmeier et al., 2001), and increasing ratio of HMW:LMW glutenin (MacRitchie and Gupta, 1993; Seilmeier et al., 2001).

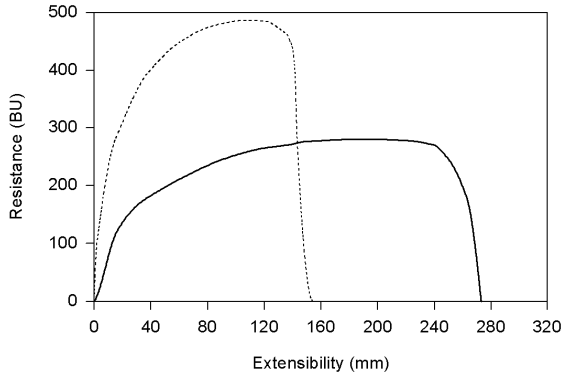

Figure 5:
Extensographs for flour (cultivar OLYMPIC) with normal and low content of sulfur. Control (—): 0.146% S, 1.82% N, N:S = 12.5:1. Flour with low sulfur content (---): 0.089% S, 1.72% N, N:S = 19.3:1 (Wrigley et al., 1984 a). BU = Brabender Units

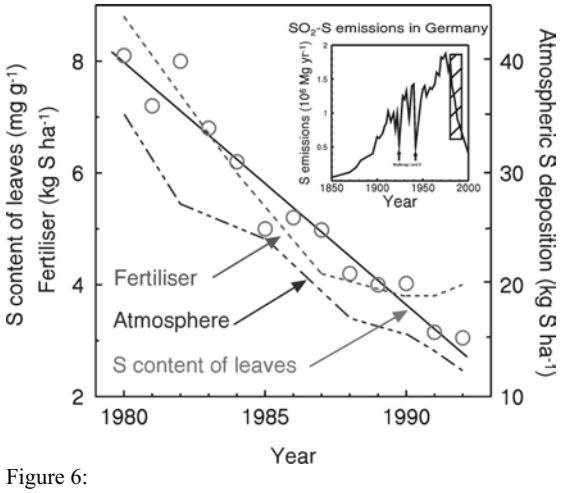

Figure 6:
Development of atmospheric SO_2- sulfur deposition, use of sulfur containing fertilizer and content of sulfur in leaves of rape (*Brassica napus*) in Northern Germany (Schnug and Haneklaus, 1994)

This development in wheat breading went "well" and led to cultivars with higher baking volumes until the moment when real S deficiency appeared. Due to the successful installation of desulfurization plants, a drastic reduction of the deposition of S in the ecosystems occurred. The application of S low mineral fertilizers also increased. By 1980 the average deposition of S in northern Germany was up to 35 kg/ha x year. This amount then decreased and in 1990 was 60% less (Schnug and Haneklaus, 1994; Figure 6). In the same period, the concentration of S in rape leaves decreased from 8 to 3 mg/g. At the beginning of the 80s no severe and relatively few cases of S deficiency (24% of all samples) could be observed in rape. At the end of the 80s the situation had changed dramatically: Only 1% of the rape samples were sufficiently supplied with S (Schnug and Haneklaus, 1994). In northern Germany an S application of 50 kg/ha is recommended (Schnug, 1991) for rape to avoid yield deficits through S deficiency.

Wheat is a crop which hungers after much less S than rape. But also with wheat S deficiency has become a problem leading to yield losses of up to 30% (Bloem et al., 1995). In contrast to rape, S deficiency in wheat cannot be compensated by foliar applications of SO_4-fertilizers (Schnug et al., 1993), because surplus S gets quickly translocated into the vacuoles, from which a re-translocation for the protoplasma of plant cells and their functions can only occur at a very moderate level (Bell et al., 1990; Cham 1990; Clarkson et al., 1993). If wheat insufficiently supplied with S shows a N:S ratio wider than 17:1, such flour leads to excessively tough and firm doughs and thus lower baking volumes (Wrigley et al., 1984b; Byers et al., 1987; Haneklaus et al., 1992; Bloem et al., 1995).

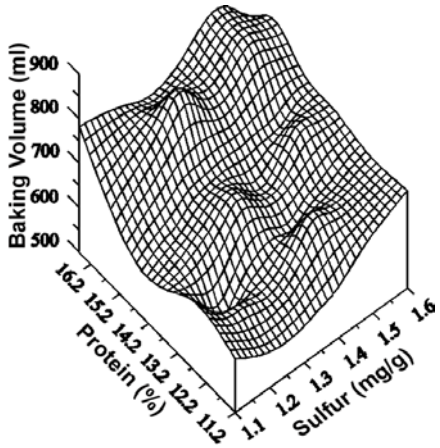

Figure 7:
Loaf volume of flour derived from German wheat varieties depending on sulfur and protein concentration in the grain (Haneklaus et al., 1992).

It is important to keep in mind that these reductions of baking volume occur not because of excessively soft glutens and doughs (as 30-50 years ago) but because of excessively firm ones: The pressure of the fermentation gases cannot sufficiently overcome the loafs' tough structure and thus produces lower baking volumes. Obviously, the breeding process selecting wheat types featuring the characteristics of S deficiency mentioned above has passed its optimum. When, in addition, a second S deficiency occurred as a changed ecologic-historical situation and decreased S depositions,

unforeseen problems arose in baking technology. S fertilization now induced higher baking volumes (as known previously through N fertilization; Figure 7; Haneklaus et al., 1992), not because of any strengthening impact to the dough structure, but, on the contrary, because of the softening effect of an increasing content of grain S on the resistance of the dough, thus leading to higher baking volume (Moss et al., 1981; Figure 8).

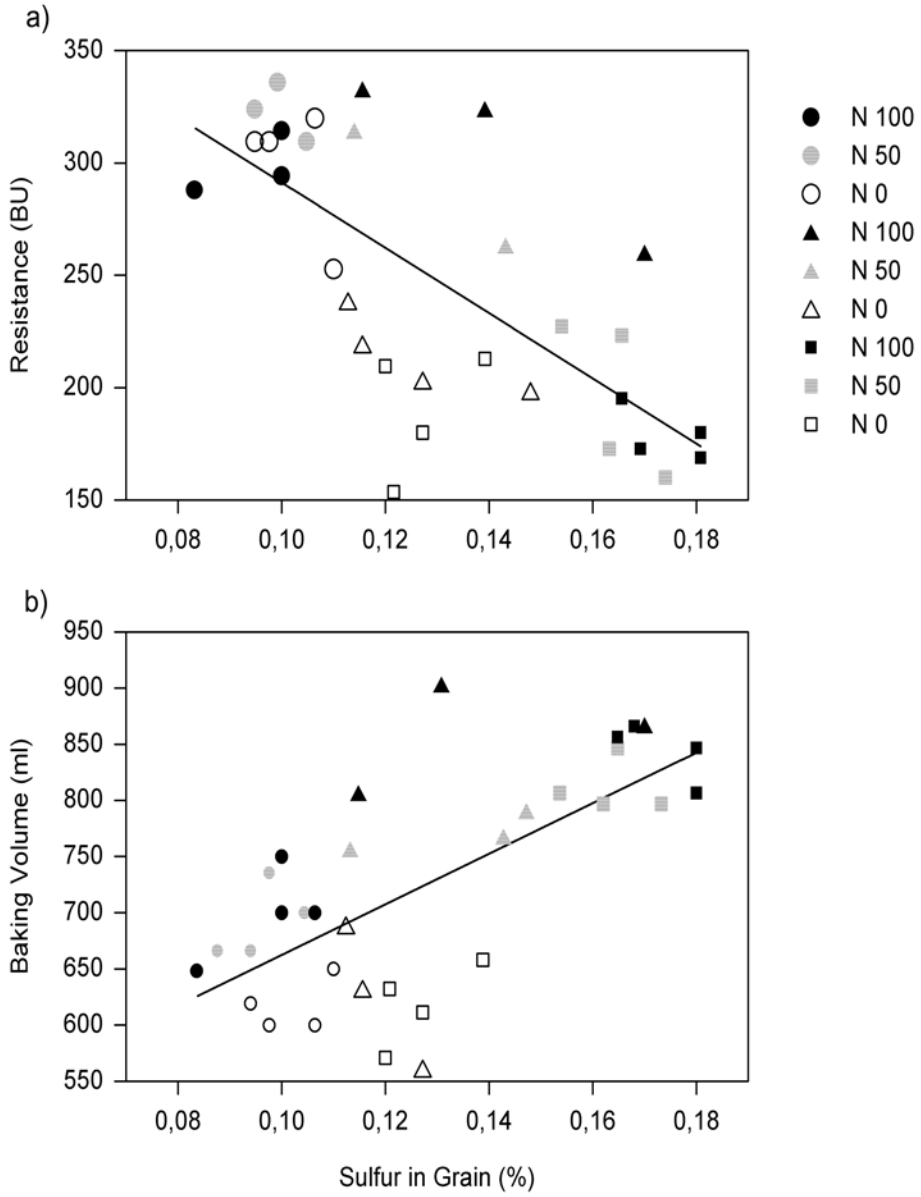

Figure 8:
Relations between content of sulfur and a) resistance of dough and b) baking volume. N0, 50 und 100 = nitrogen application in kg ha^{-1} (including different sulfur applications of 0-50 kg ha^{-1} (Moss et al., 1981). BU = Brabender Units.

Similar phenomena were observed with S fertilization trials on organic farms located in the coastal area of Northern Germany with very low rates of S deposition (Hagel, 2000c). The variability in the N content of the wheat samples shown in Figure 9 was only due to the field's variation, not to any N fertilization. One part of the samples received no S fertilizer, but in part (except the control) $MgCl_2$ in order to identify any effects in grain yield resulting from the magnesia in the S fertilizer ($MgSO_4$), but there were none. The N:S ratio of the control was 15.4 showing low S supply near to the limits. Increasing N content of these samples induced higher baking volumes only up to a certain optimum of

approximately 1.95% N. Higher N contents lowered the baking volume (Figure 9), probably because of too firm doughs, though no extensograms were performed. The other part of the samples received S applications of 20, 40 and 60 kg ha^{-1} (as elemental S and $MgS0_4$). N:S ratios were 14.1 (elemental S) and 13.9 ($MgSO_4$), which were significantly lower than the control. Here with increasing N content no depression in loaf volume occurred. Instead a linear relation between the parameters was to be observed (Figure 9).

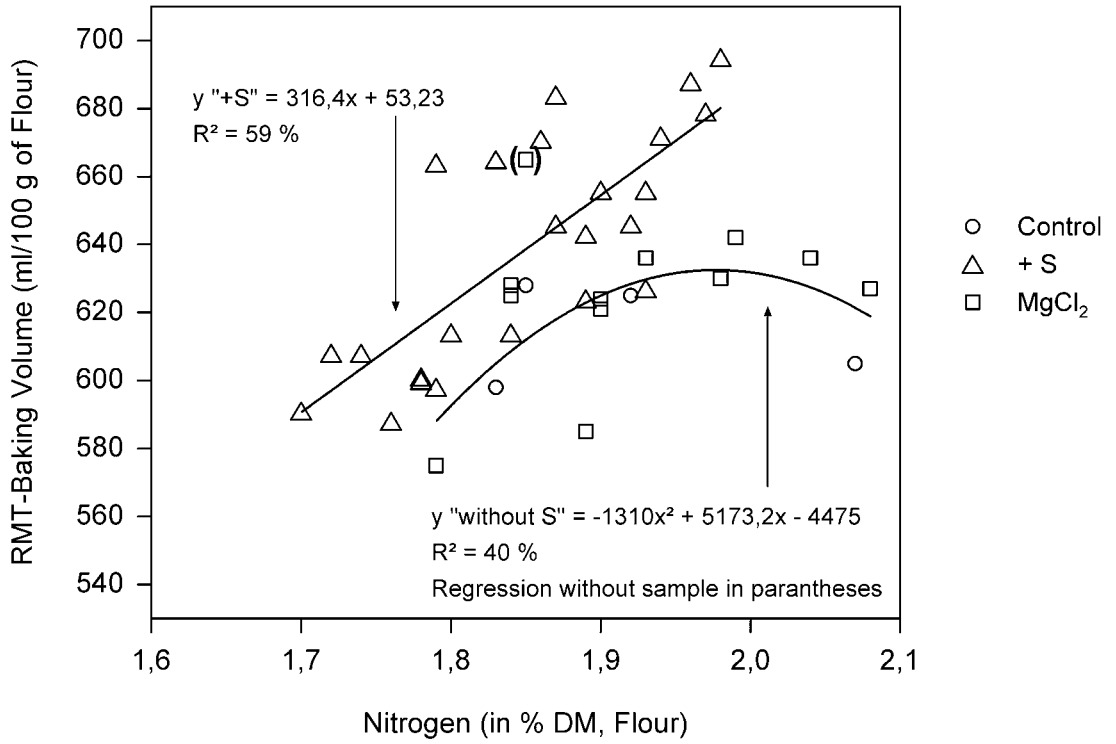

Figure 9:
Relationships between nitrogen content and baking volume (Rapid-Mix-Test = RMT) of wheat (cv. RENAN) of a sulfur fertilization trial on an organic farm (harvest 1998; location: Tröndel) (Hagel 2000 c). +S, sulfur fertilization: 20, 40 and 60 kg S ha^{-1} as elemental sulfur and $MgSO_4$-sulfur.

The effect of a S fertilization softening the protein matrix of wheat was not only demonstrated on locations where S was lacking but even on sites sufficiently supplied with this element. For this purpose up to 400 kg S/ha were applied to wheat grown on an organic farm (Hagel et al., 1999). Though the S content of the straw was increased by 50% by these quantities, the S content of the grain and the flour remained unaffected. Also the N content and the N:S ratio of the flour were not altered significantly (Table 2). But already 200 kg S ha^{-1} lowered the resistance of the gluten significantly (the impact of 100 kg S ha^{-1} only slightly differing from that) (Table 2; Figure 10). This effect was not influenced by a shift in the amount of protein fractions, especially HMW-glutenin (Table 2). Also different amounts of glutathione of the flour are probably not the reason, if the experiments as in this case are performed with flour sufficiently stored (Kieffer et al., 1998).

Warmth, baking quality and sulfur

We also have to deal with the impact of warmth with regard to the rheologie of wheat, because S and warmth are closely linked. S is exceptional for its many allotropic modifications induced simply by different temperatures as described in many textbooks (Mortimer, 1996; Cotton et al., 1999). E.g. S changes from rhombic crystals into monocline crystals upon mild heating. Further heating delivers a yellow readily flowing liquid, then a red highly viscous substance which is turned into a rubber like plastic material upon sudden cooling in water and so on. The spicy flavors of e.g. mustard, onion and garlic with their S containing glucosinolates are termed “hot” not by chance. Numerous therapeutic measures make use of these substances in nutrition and medicine (from spices to warmth stimulating baths). Looking at the phenomena, there are many relationships between S and warmth. So let us have a closer look to what hap-

pens with the baking quality of wheat grown at different temperatures.

It is well known that climate influences baking quality by altering yield and/or the protein content of the wheat (Svensson, 1974; McDonald et al., 1983). I will not focus on that now, but rather on different baking qualities induced by different temperatures, especially during the grain filling period of the wheat. Fajersson (1975) demonstrated different baking volumes of wheat (at comparable protein contents) from climatically different years (Figure 11).

Table 2:
Content of nitrogen and sulfur, N:S ratio of flour and resistance of gluten (measured in Newton) of wheat of a sulfur fertilisation-trial (0-400 kg S/ha). Multiple-Range-Test: $\alpha = 5$ %. Gliadin and Glutenin = RP-HPLC-analyses, (proportions (%) of the different subunits from total gliadin and glutenin (Hagel et al. 1999).

					Gliadin					Glutenin		
kg S/ha	% N	% S	N:S	Resistance	ω5	ω1,2	∑ω	α	γ	ωb	HMW	LMW
0	1.80	0.103	17.5	0.544a	3.9	4.5	8.4	49.4	42.2	3.7	21.9	74.4
50	1.77	0.103	17.2	0.523ab	3.6	4.2	7.8	47.9	44.3	3.7	20.6	75.7
100	1.86	0.107	17.4	0.444ab	3.7	4.4	8.1	47.8	44.1	3.8	21.6	74.6
200	1.83	0.100	18.3	0.441bc	3.6	4.2	7.8	48.0	44.2	3.4	21.4	75.2
400	1.95	0.105	18.6	0.370c	3.6	4.3	7.9	49.0	43.1	3.6	22.7	73.7

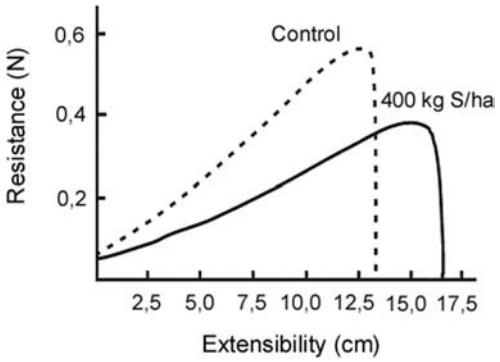

Figure 10:
Extensograms of wheat gluten of a sulfur fertilization experiment. Variants: 0 and 400 kg S ha^{-1} (Hagel et al., 1999).

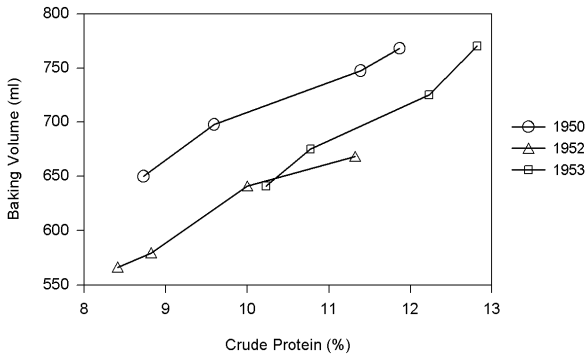

Figure 11:
Crude protein and baking volume of wheat from climatically differing harvest years (1950, 1952, 1953). Mean of five cultivars each (Fajersson, 1975).

In Sweden warm and dry climatic conditions (mean day temperatures of 20 °C) during grain filling periods of 1994 and 1995 led to high gluten strength with low bread volumes (Johansson and Svensson, 1999). Investigating the effects of weather parameters on some Swedish wheat cultivars Johansson and Svensson (1998) found that the temperature, specially during the grain filling period, was the most important weather parameter explaining only 34% of the variation in grain protein concentration, but 49% of the variation in mixogram index in spring wheat. Finney and Fryer (1958) found with hard red winter wheat samples from different states of the US and thus different climatic conditions, that increases in accumulated degrees of temperatures above 90°F (32°C) during last 15 days of the fruiting period led to loaf volumes much lower than expected with regard to the protein content (Figure 12). Although the authors did not investigate rheological parameters in detail, their descriptions of these samples with loaf volumes considerably below normal (subnormal mixing requirements and poor dough handling) characterizes excessively strong doughs exactly. Excluding these “irregular” samples increased the correlation coefficients between protein content and loaf volume from 0.76 to 0.97. The cultivar Chiefkan in particular was “highly susceptible to the damaging effects of high temperatures during fruiting”. Also Johansson and Svensson (1999) observed that the susceptibility of wheat cultivars with regard to warmth influences differed.

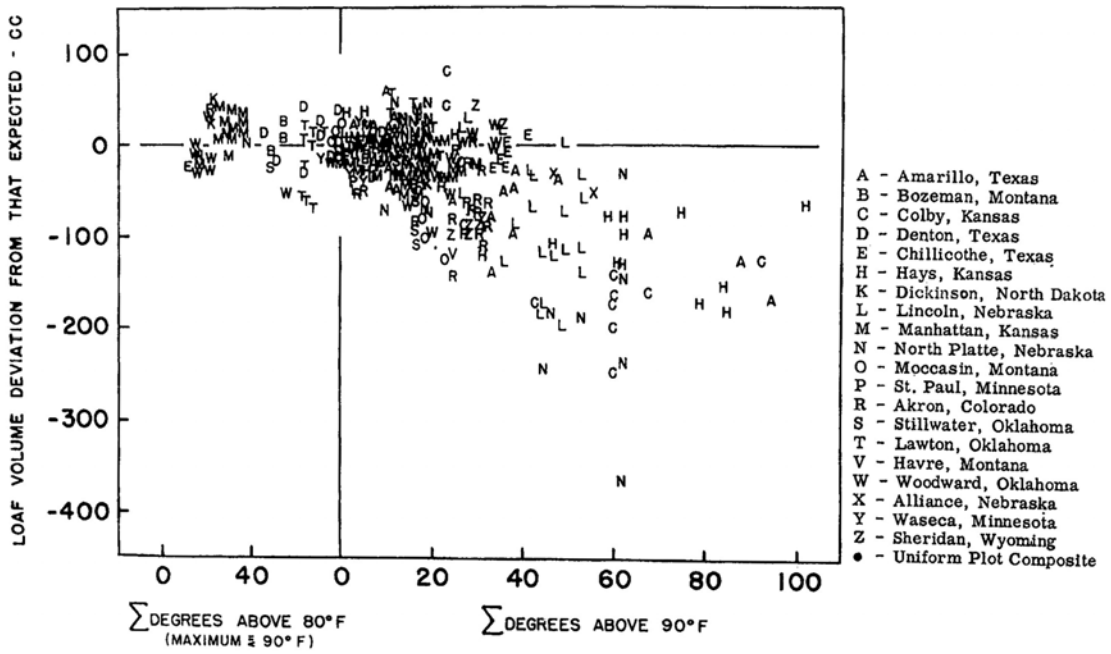

Figure 12:
Relations between loaf volume deviations from those expected and temperature during the last 15 days of the fruiting period for 391 hard red winter wheat samples. Letters indicate samples from different states of the US and 20 different experimental stations, 90° F = 32° C (Finney and Freyer 1958).

Jahn-Deesbach (1981) carried out pot experiments with wheat. With anthesis, they were transferred from outdoors into growth chambers. These variants only modestly supplied with N showed better farinograms (higher energy) under the influence of warm temperatures compared with cool conditions. In these experiments it nevertheless remained unclear, if rheological differences were only due to temperature or partly also to secondary effects on grain protein concentration. Later this handicap was tackled successfully in experiments by Schipper et al. (1986) and Schipper (1991). They grew wheat in field experiments (warmer or cooler sites during the grain filling period) and growth chambers and managed to achieve variants with comparable grain protein content. In both environments, warmer temperatures during grain filling period produced dough extensograms with lower extensibility, higher resistance and higher energy. Some examples of the many results are shown in Figure 13 and 14. Though samples grown at higher temperatures had somewhat higher glutenin:gliadin ratios, this could not explain the differences in extensograms (Schipper et al., 1986; Schipper, 1991). So possibly conformational changes in the protein structure may be the reason for these rheological differences.

Sosulski et al. (1963) conducted growth chamber experiments with wheat grown at different moisture and N levels. Different temperatures of 16.7, 21.1 and 23.9°C were applied from a very early growth stage (tillering). The results provide valuable information as they indicate different susceptibility of grain quality parameters to warmth: At comparable concentrations of grain protein sedimentation values were increased already at temperatures of 21.1°C (Figure 15a), while mixogram areas were not different from their pattern until a temperature of 23.9°C was attained (Figure 15b).

These results once more demonstrate that warmth is an important parameter influencing grain quality characteristics by strengthening protein structure and dough. Further evidence was also provided from wheat cultivars grown in glasshouses at different temperatures and under different N applications (Randall and Moss 1990). One half of the samples was moved at 30 (low N) and 34 days (high N) after anthesis to a “hot” glasshouse (23-26°C average daily temperature with a maximum temperature up to 36°C). The other half remained in the “cold” environment (18°C). Though grain N concentration of the wheat samples grown at different temperatures did not differ significantly, the maximum resistances of the doughs were significantly higher from wheat samples grown under the “hot” temperature regime, while extensibility was lowered (Table 3). Randall and Moss (1990) also point to the fact that indeed sulfur deficiency and higher temperatures have very much in common with regard to baking quality: “Sulfur deficiency

increases dough resistance and decreases extensibility, and in the present work, raising the temperature caused similar changes. However, the effect of temperature on dough resistance is unlikely to be mediated through effects on grain sulfur as sulfur concentration was largely unaffected by temperature treatment".

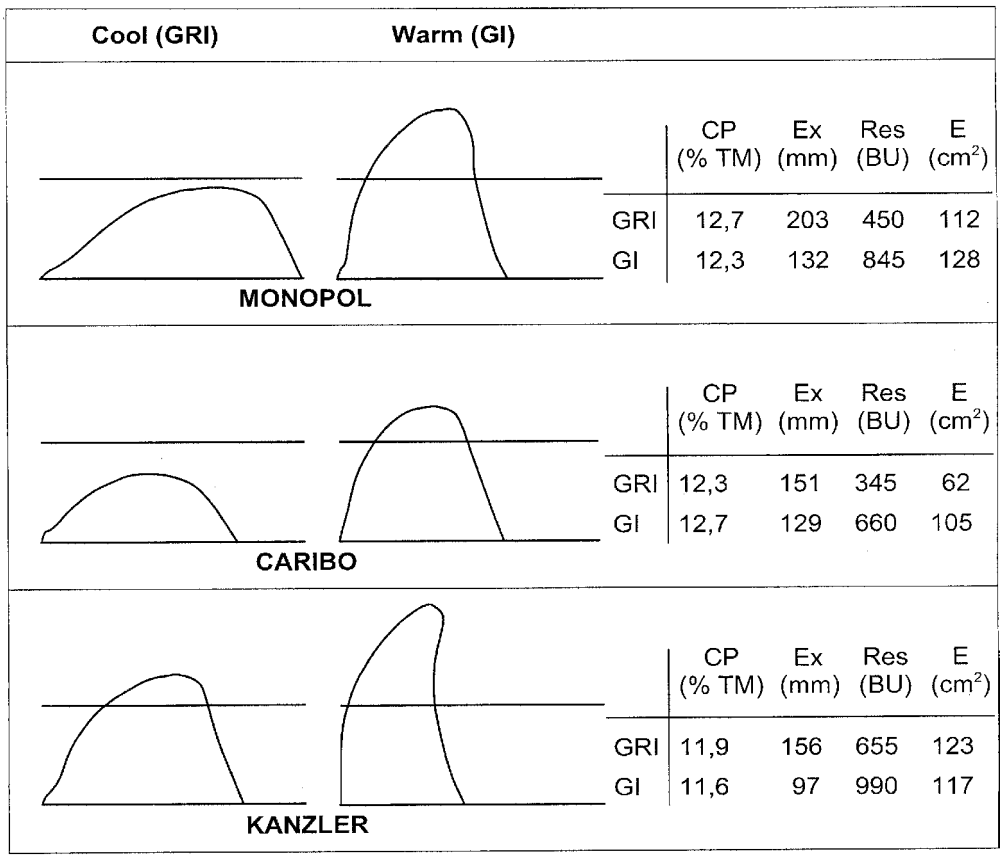

Figure 13:
Influence of different temperatures during grain filling period on wheat (cultivars: MONOPOL, CARIBO, KANZLER) of a clima-field-experiment (harvest: 1986) on the extensogram of doughs (Schipper 1991). Locations: GRI = Grimersum (cool climate); GI = Gießen (warm climate), CP = crude Protein; EX = extensibility; RES = resistance; E = energy

Table 3:
Effects of temperature on grain nitrogen and dough resistance and extensibility in three wheat cultivars, in two experiments with contrasting nitrogen levels (Randall and Moss 1990).

	OLYMPIC		HARTOG		SKUA	
Experiment	Cool	Hot	Cool	Hot	Cool	Hot
			Grain N (%)			
low N	1.51	1.72	1.78	1.88	1.63	1.72
high N	2.45	2.33	2.55	2.42	2.24	2.24
Differences: N: $P < 0.001$; temperature: n.s.; cultivar: $P < 0.01$						
			Maximum resistance (E.U.)			
low N	190	225	238	252	148	178
high N	290	383	290	345	190	215
Differences: N: $P < 0.001$; temperature: $P < 0.001$; cultivar: $P < 0.001$						
			Extensibility (cm)			
low N	16.9	16.3	20.7	18.0	16.8	15.9
high N	23.7	21.5	27.3	26.1	23.6	22.9
Differences: N: $P < 0.001$; temperature: $P < 0.001$; cultivar: $P < 0.001$						

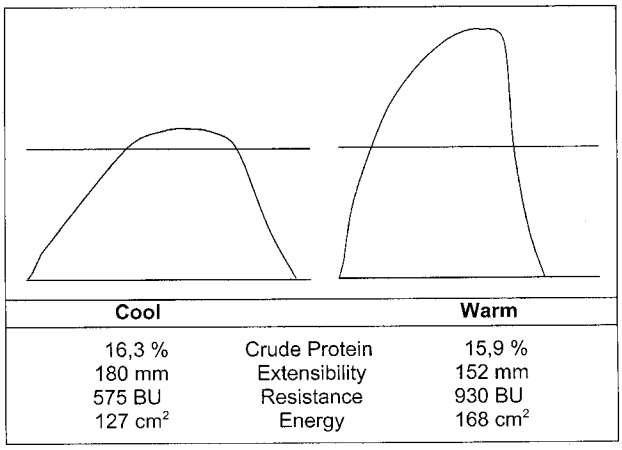

Figure 14:
Influence of different temperatures during grain filling period of wheat (cultivar: SCHIROKKO) from a pot experiment in a growth chamber in 1984 on the extensogram of dough (Schipper 1991), BU = Brabender units

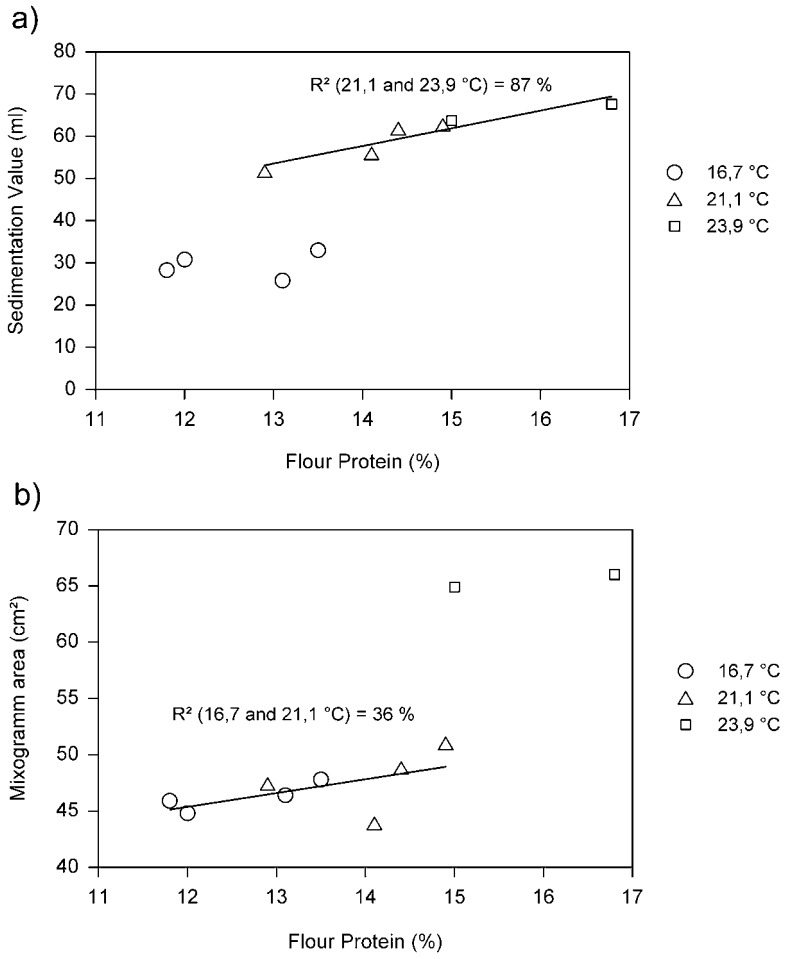

Figure 15:
a) Relationship between protein content and sedimentation value of wheat grown at different temperatures approx. 32 days after anthesis (Sosulski et al. 1963).
b) Relationship between protein content and mixogram area of wheat grown at different temperatures approx. 32 days after anthesis (Sosulski et al., 1963).

It becomes clear that gluten structure, rheological performance and the baking quality of wheat are not given, but are reactions of the plant as a living organism to certain impulses from the environment. Here S and warmth belong to the most prominent and important factors. If there is sufficient S as a substance from "below" (soil, groundwater, fertilizer) and insufficient warmth from above (cool weather, which can be regarded as little S as a process, not as a substance), the wheat plant will tend to lower proportions of HMW-glutenin and softer glutens and doughs. If on the other hand there is S deficiency from "below" and hot weather (much S from "above", which means S as a process, not as a substance) during grain filling period, the wheat plant will produce increased amounts of HMW-glutenin and tougher glutens and doughs. It is well known in biological science that all factors applied to living organisms (light, water, warmth, fertilizers etc.) show an optimum, when their input is increased. Healthy organisms and sustainable systems are, on the long run, only achieved when care is taken not to destroy this delicate equilibrium of factors producing an optimum. With regard to the baking quality of wheat breeders and cereal scientists obviously failed to achieve this aim by breeding their cultivars on the background of ample S depositions in the ecosystems. They (involuntarily) selected plants showing definite characteristics of S deficiency (higher proportions of HMW-glutenin, stronger gluten and dough) even under conditions of ample S supply. I suppose they also selected plants with a high warmth susceptibility as this also delivers firm protein structure. When this environmental pollution was stopped and S supplies returned to natural conditions, even with a non-S craving plant like wheat, problems arose with the gluten structure as doughs turned out so strong that the baking volume decreased. So one may ask, particularly with regard to the supply of S, if the plant constitutions of our modern wheat cultivars are still harmonious and in balance: On the one hand they were shifted merely for technological reasons into the realm of S deficiency characteristics, and on the other hand, in all probability, had attained an enormous warmth susceptibility. So it might well be that the nutritional quality of these cultivars is rather questionable.

The development in breeding towards ever tougher gluten and higher baking volumes is not yet complete: Both in organic and conventional agriculture the aim of lower grain protein shall be compensated through ever better technological quality. Besides milk protein wheat is often the reason for allergic reactions (Husemann and Wolff 1993). More and more people exhibit incompatibility for wheat. Several people are able to distinguish wheat (*Triticum aestivum*) from spelt (*Triticum spelta*) by observing their allergic observing their allergic symptoms (rash). They tolerate the spelt, which was not modified so intensively by breeders during the last decades. But wheat leads to skin reactions. After doubling the gluten content of baby food (< 2 years old) in Sweden there was a 300% higher incidence of celiac disease. After reducing gluten content, a reduction occurred to the normal occurrence of this illness (Ivarsson et al., 2000). Gluten-sensitivity is not confined to the small intestine (celiac disease) but also causes an inflammation of the nervous system with chronic migraine. This could be cured in 9 from 10 cases by strictly eliminating wheat from the diet (Hadjivassiliou et al., 2002).

Again several questions may arise from these phenomena: Was this alarming situation always the same or are we experiencing a sneaking development that is only the top of the iceberg? Is merely a poor human immune system the reason for the increase of allergies or does food quality play an important role? Is wheat and its protein no longer a harmless staple food? Could a shift in wheat plant constitution towards S deficiency symptoms be the reason for all the problems?

More research should be done with regard to wheat breeding with rigorous reference to the human being as a whole and his / her nutritional needs.

Acknowledgements

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Relationship between sullfur deficiency in oilseed rape (*Brassica napus* L.) and its attractiveness for honeybees

Silvia Haneklaus[1], Anja Brauer[1], Elke Bloem[1] and Ewald Schnug[1]

Abstract

Oilseed rape showing macroscopic symptoms of sulfur deficiency influences the behavior of honeybees, something that has been observed repeatedly in production fields. The symptomatology of sulfur deficiency in cruciferous crops is characteristic during the whole vegetation period. The peculiarity of sulfur deficiency symptoms during flowering depends on the moment when sulfur becomes a limiting factor. An early appearance of sulfur deficiency is regularly related to the change of the petal color from bright to pale yellow, or even white petals. At the same time, the petals are modified in size and shape. By comparison, a late occurrence of sulfur deficiency usually results in the change of color mentioned earlier, while other morphological parameters are not affected. It was the aim of this paper firstly to provide a condensed description of sulfur deficiency symptoms in oilseed rape during the vegetation period, secondly to determine the influence of the sulfur supply on morphological characteristic of oilseed rape petals, and thirdly to present for the first time data about the attractiveness of flowering oilseed rape for honey bees in relation to the S supply.

Key words: flower color, honey plants, petal deformation, pollen

Introduction

Macroscopic sulfur (S) deficiency was first observed on production fields in 1981 (Schnug and Haneklaus 1994a), and more then 20 years later it is still the most widespread nutrient disorder in northern Europe (URL://www.pb.fal.de). The significance of the S supply for crop production, crop quality and plant health has been outlined for example by Schnug and Haneklaus (1998), Schnug (1997) and Haneklaus et al. (2004). Visual symptoms of S deficiency in cruciferous crops are very specific and can be addressed in the field throughout the whole vegetation period. Oilseed rape provides an important source of nectar and pollen for honeybees, which are attracted by the bright yellow color of the crop in bloom (Pierre et al. 1999). During flowering, characteristic changes of macroscopic S deficiency are to be seen in color and shape of the petals. It was observed repeatedly that S deficient oilseed rape is less attractive for honeybees. These findings were, however, subjective, while bias-free experimental studies have not been carried out so far.

Oilseed rape is one of the most important European melliferous crop for beekeepers as it is an important foraging plant in early summer. In Germany, oilseed rape is grown on an area of about $1.27 \cdot 10^6$ ha (Anon 2004). The main pollinators in oilseed rape are insects of the family *Apidea* (e.g. honey bees, wild bees and bumble bees) (Corbet, 1992; Williams, 1996) and the significance of honeybees as pollen vectors for seed set and yield has been described in the literature (Steffan-Dewenter, 2003). Although oilseed rape is self-pollinating (Saure, 2002), the cross-pollination rate, predominately by honeybees, was estimated to be about 20% (Downey et al. 1980). According to Olsson (1960) the cross-pollination rate may vary in relation to genotype and climatic conditions between 5 % and 95 %. By comparison, on fields where composite hybrid oilseed rape varieties are grown or male-sterile lines for breeding of restored hybrid cultivars, these plants have a high dependence on pollination by vectors (Steffan-Dewenter, 2003). Thus, determining the influence of the S supply of oilseed rape on its attractiveness for foraging honeybees is a fundamental contribution from both the agronomic and ecological point of view. It was the aim of this paper to provide a comprehensive description of macroscopic S deficiency symptoms in oilseed rape during the vegetation period with special attention being paid to visual symptoms during flowering, in order to quantify the influence of the S supply on morphological parameters of the flowers and last, but not least to show first results about the attractiveness of flowering oilseed rape for bees in relation to the S supply.

Materials and methods

Two field experiments with winter oilseed rape were conducted at Braunschweig (E 10° 27`, N 52° 18`). In the first experiment S was applied at rates of 50 kg S ha^{-1} in fall and 100 kg S ha^{-1} in spring to the cultivar *Bristol*. N was applied at a rate of 200 kg N ha^{-1}. The plot size was 40 m^2 and each treatment had

[1] Institute of Plant Nutrition and Soil Science, Federal Agricultural Research Centre (FAL), Bundesallee 50, 38116 Braunschweig, Germany

four replicates. For a detailed description of the experimental design see Salac (2004). Growth stages of oilseed rape were recorded according to the code number of the BBCH scale (Strauss et al. 1994).

Bee traps

A beehive was placed in front of the experimental field on 19 April 2004. At the start of flowering (BBCH 60), in each plot four white and four yellow dishes were positioned at a height corresponding with that of the crop plant. The bees were collected on two following days (20 April 2004 and 21 April 2004).In the second experiment, macroscopic symptoms of S deficiency on leaves and flowers were visible in the cultivar *Smart* in relation to mineral N fertilization (100 and 200 kg N ha^{-1}) and application of manure (0 and 4.8 t ha^{-1}). The plot size was 65 m^2 and each treatment had four replicates. For experimental design see Rogasik et al. (2004).

Plant sampling and analysis

In total, 10 individual flowers from 10 different plants with different degrees of visual S deficiency symptoms (extreme, severe, none) were collected on 4 May 2004 (BBCH 65) and 40 or 80 petals analyzed. The petals were carefully separated by using tweezers and directly fixed on object slides. For the determination of length and diameter of each petal, the object slides were scanned and the images interpreted afterwards automatically by employing the ArcView 3.2 software package (Esri, 1999).

For the determination of the amount of pollen produced the anthers of 10 flowers were placed in 1.5 ml Eppendorf tubes. The pollen was dissolved from the anthers by using dimethylether. Then the anther peduncles were removed, the ether vaporised and finally the amount of pollen weighed.

The differences in color of the oilseed rape petals were determined colorimetrically by employing the method of Miyamjima et al. (2000). From each level of S supply (extreme S deficiency, severe S deficiency, sufficient S supply) 100 petals from at least 25 different plants were collected and shock frozen in liquid nitrogen and freeze-dried before analysis.

Statistical analysis

The software package CoHort (Anon, 1990) was used for ANOVA (Tukey-Kramer test).

Results and discussion

Sulfur deficiency symptoms of oilseed rape during the vegetation period

Severe S deficiency symptoms were often described in the literature as being less specific and more difficult to identify than other nutrient deficiency symptoms (Bergmann, 1993). *Brassica* species such as oilseed rape, however, reveal characteristic macroscopic symptoms of S deficiency that can be found throughout the vegetation period. As a supplement to the description of S deficiency symptoms, illustrative digital colour images (WWW No.) can be retrieved from the World Wide Web (URL://www.fal.pb.de). Physiological changes in plant metabolism as a result of S deficiency are described for instance by Schnug (1988) and Schnug and Haneklaus (1994a).

Macroscopic S deficiency symptoms of oilseed rape before winter (BBCH 1-19)

Even before winter, during the early growth of oilseed rape, leaves may start to develop symptoms of S deficiency (WWW 2). Though the plants are still small, symptoms can cover the entire plant (WWW 3). Sulfur fertilization before or at sowing will ensure a sufficient S supply, particularly on light, sandy soils and promote the natural resistance of plants against fungal diseases (Haneklaus et al., 2004)

Macroscopic S deficiency symptoms of oilseed rape from the start of the main vegetation period until appearance of inflorescences above upper leaves (BBCH 30 - 59)

Plants suffering from severe S deficiency, show a characteristic marbling of the leaves. The chlorosis starts from the leaf's edge spreading over intercostal areas but the zones along the veins always remain green (Schnug, 1988) (WWW 4). Deficiency symptoms in younger, fully developed leaves of oilseed rape at the start of stem elongation begin to appear when the total S concentration drops below 3.5 mg g^{-1} S in double low varieties (Schnug and Haneklaus, 1994a, b).

Chlorosis very rarely turns into necrosis (Schnug, 1988, Ulrich et al., 1993) as it does with nitrogen and magnesium deficiency, which is an important criterion for differential diagnosis. Even under conditions of extreme S deficiency where an oilseed rape plant shows severe disorders it will not wilt (WWW 6).

A characteristic secondary symptom of severe S deficiency is the reddish purple color due to the enrichment of anthocyanins in the chlorotic parts of *Brassica* leaves (WWW 8). Under field conditions, the formation of anthocyanins starts 4 - 7 days after chlorosis. In particular those leaves not fully expanded produce spoon-like deformations when struck by S deficiency (WWW 9). The reason for this is a reduced cell growth rate in the chlorotic areas along the edge of the leaves, while normal cell growth continues in the green areas along the veins,

so that S deficient leaves appear to be more succulent. The grade of the deformation is stronger the less expanded the leaf is when the plant is struck by S deficiency (WWW 10). Marbling, deformations and anthocyanin accumulation can be detected up to the most recently developed small leaves inserted in forks of branches (WWW 11).

Macroscopic S deficiency symptoms of oilseed rape plants during flowering (BBCH 60 - 69)

During flowering S deficiency causes one of the most impressive symptoms of nutrient deficiency: the 'white blooming' of oilseed rape (WWW 12). The white color presumably develops from an overload of carbohydrates in the cells of the petals caused by disorders in the protein metabolism, which finally ends up in the formation of leucoanthocyanins (Schnug and Haneklaus, 1995). As with anthocyanins in leaves, the symptoms develop strongest during periods of high photosynthetic activity. Besides the remarkable modification in color, size and shape of oilseed rape the petals change, too. This apparently influences the attractiveness of oilseed rape for honeybees as according to initial personal observations this is seen as well as changes in the petal color, a weaker scent and a reduced number of bees. A verification of this appraisal would be of utmost significance for beekeepers and farmers alike in order to warrant a high yielding oilseed rape crop and honey harvest. In two field experiments the influence of the S supply on morphological parameters of oilseed rape flowers and the behavior of bees was investigated and the first results are presented below.

Macroscopic S deficiency symptoms of oilseed rape during ripening (BBCH 71 - 99)

The strongest yield component affected by S deficiency in oilseed rape is the number of seeds per pod, which decreases significantly (WWW 16) (Schnug, 1988). As described earlier for leaves, the branches and pods of S deficient plants are often red or purple colored due to the accumulation of anthocyanins. Extremely low numbers of seeds per pod, in same cases seedless 'rubber pods' are characteristic symptoms of extreme S deficiency.

Influence of the S supply on morphological parameters of oilseed rape flowers and the attractiveness for honey bees

Honeybees are attracted by scent, colour and form of the honey-bearing plants, but it is the scent which has the fastest and strongest impact (Menzel et al. 1993). Honey bees might assess the amount and concentration of nectar in each flower by employing different senses: directly by visual access to the nectar (Throp et al. 1975, Willmer et al. 1994), or by olfactory sensation (Heinrich 1979; Galen and Kevan, 1983), indirectly by an indicator of the reward for foraging such as colour (Gori, 1983; Weis, 1991), flower size (Galen and Neport, 1987; Eckhart 1991), or the particular floral structures (Bell et al., 1984; Gonzalez et al., 1995).

Influence of the S supply on volatiles from oilseed rape flowers

Volatiles released during flowering of plants facilitate flower recognition by the honeybee and thus increase their foraging efficiency. The chemical analysis of volatiles from various plant species revealed a multiplex composition of floral odors with

Table 1:
Influence of the S nutritional status on the shape of petals in field grown oilseed rape plants at main flowering (BBCH 65).

S Status	(n)	Mean diameter (D) (mm)	Mean length (L) (mm)	Mean D:L ratio
Extreme S deficiency	40	5.2	12.5	0.41
Severe S deficiency	80	6.0	13.5	0.45
Sufficient S supply	80	10.0	16.4	0.61
$LSD_{5\%}$		0.29	0.40	0.015

Table 2:
Influence of the S nutritional status on the absorbance at 440 nm of rapeseed petals at main flowering (BBCH 65).

S status	Sample (mg)	Absorbance at 440 nm	Absorbance g^{-1} dry matter
Extreme S deficiency	21.8	0.654	30.0
Severe S deficiency	28.5	0.952	35.6
Sufficient S supply	21.2	1.575	74.3

more than 700 different compounds that were found in 60 families of plants (Knudsen et al. 1993). The mechanisms by which honeybees process this complex chemical information and adapt their behavior accordingly are as yet unknown (Wadhams,1994).

A total of 34 different compounds were found in volatiles of oilseed rape (Tollsten and Bergström, 1988; Robertson et al., 1993; McEwan and Smith, 1998). The main volatiles from oilseed rape flowers were 3-hydroxy-2-butanone > 2,3-butanedione > dimethyl disulfide >> formaldehyde > 3-methyl-2-butanone > dimethyl trisulfide (Robertson et al., 1993). Omura et al. (1999) determined nitriles and isothiocyanates in large quantities in the floral volatiles of *Brassica rapa*. Honeybees use volatiles for discrimination whereby a conditioning threshold was determined for individual components (Pham-Delégue et al., 1993). Previous studies have shown that the S supply increases the glucosinolate in vegetative plant tissue, seeds and petals of oilseed rape (Schnug, 1988, 1993). Additionally, 2-phenylethyl isothiocyanate yielded limited conditioned responses in honey bees, but was an active component after being learned in a complex mixture of volatiles (Laloi et al., 2000). Thus a relationship between the S-containing compound, intensity of the scent and finally the attractiveness to honey bees seems possible.

Influence of the S supply on the size and shape of petals of oilseed rape

Severe S deficiency also causes deformations of leaves and petals (Schnug and Haneklaus, 1994a). If S deficiency strikes the plant early in the vegetation period, the size of the petals is reduced most severely and instead of a bright yellow color, the characteristic white flowering can be observed (see above). In comparison, if S deficiency occurs later in the vegetation period the reduction in size and changes in color are distinctly less. In cases where S deficiency sets in shortly before flowering, the petal size remains unaffected, while changes in color can still be seen.

Egg shaped petals are characteristic of extreme and severe S deficiency, which are a result of the reduction in diameter and length of the petals. The progression of deformations in relation to the S supply was assessed by establishing the relationship between the diameter of the petals and the quotient of diameter and length (Figure 1). Similar results were found by Schnug and Haneklaus (1994a). A classification of plants into three groups of S supply (extreme S deficiency, severe S deficiency and sufficient S supply) revealed that the petal diameter may be reduced by 50 % and petal length by 24 % as a result of enduring S deficiency (Table 1).

The size of flowers was an important criterion for bumble bees as with decreasing diameter, from 25 to 8 mm, the time for searching was drastically prolonged from 10.4 to 124.3 seconds (Spaethe et al., 2001).

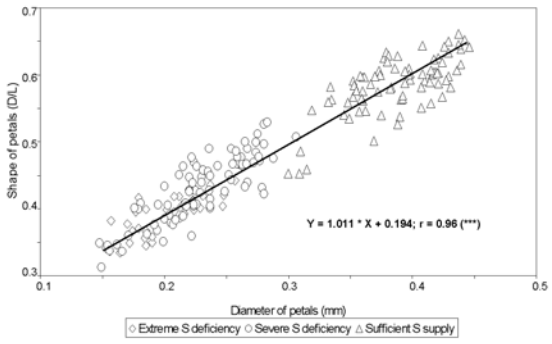

Figure 1:
Influence of severe S deficiency on deformation of petals by modification in diameter (D) and shape of petals expressed by the diameter:length ratio (D/L) from field growing oilseed rape plants at main flowering (BBCH 65).

Influence of the S supply on the petal color of oilseed rape

On S deficient sites, yellow and white petals exist side by side, thus excluding genetic influences and indicating nutritional effects. Changes in the colour of the oilseed rape petals are possibly related to increasing sugar concentrations in the plant tissue due to disorders in the protein metabolism. By pigment formation, plants prevent excessive accumulation of free sugars. One major pigment causing the yellow colour of rapeseed flowers is the flavonol quercetagatin and its isorhamnetin 3-glycoside (Harborne, 1967). Glycosylation of flavonols has a hypsochromic effect, which might lead to a shift of the absorption spectra to the UV range, which is invisible to the human eye. Another hypothesis to explain the change in color is that the synthesis of colorless anthocyanins is increased (Schnug and Haneklaus, 1995). The influence of the S nutritional status on the absorbance at 440 nm is shown in Table 2.

The differences in the absorbance were strongest between petals showing extreme S deficiency and those plants with a sufficient supply, but also verifiable for extreme and severe S deficiency (Table 2). The results are in agreement with those found by Schnug and Haneklaus (1995).

Influence of the S supply on the pollen content of oilseed rape

Oilseed rape offers ample pollen, which is of high relevance for the development of the honeybee population after winter (von der Ohe and von der

Ohe, 2002). Besides this, the pollen supply contributes to a satisfying and healthy development of the bee hive (von der Ohe and von der Ohe, 2002). Von der Ohe and von der Ohe (2002) showed that genotypical differences in the pollen content were not significant, while abiotic factors such as climatic conditions had a distinct impact. The determination of the pollen content revealed that S deficiency did not affect the supply (Table 3).

Table 3.
Influence of the S nutritional status on the pollen content of oilseed rape at main flowering (BBCH 65).

S status	Pollen content (g)
Extreme S deficiency	0.020
Severe S deficiency	0.022
Sufficient S supply	0.023

Ongoing studies investigating the influence of the S supply on the nectar content and quality of oilseed rape in relation to the S supply under greenhouse conditions revealed that both parameters were not influenced by the treatment. Thus it may be concluded that S deficient oilseed rape offers a nutriment, which is comparable to that of a sufficiently supplied plant in both the amount and quality of pollen and nectar, respectively. Differences in the attractiveness of S deficient oilseed rape therefore must be related exclusively to scent and morphological features.

Influence of the S supply on the attractiveness of flowering oilseed rape for honey bees

For studying the attractiveness of oilseed rape for foraging honey bees in relation to the S supply under field conditions the experimental design of the field experiments was not appropriate because of the missing spatial distance of at least 200 m (von der Ohe, 2004) between S deficient and plants with a sufficient S supply. This is essential for assessing behavioral differences related to this nutritional factor. The collection of honeybees in white and yellow dishes in plots with different S application rates must therefore only be treated as strictly indicative for the behaviour under natural conditions with white and yellow flowers (Figure 2).

Hill et al. (2001) found out that the foraging behavior of honeybees was related among other things to the colour of the flowers and that a white and yellow colour, together with blue yielded discriminative behavior in relation to reward volume and quality. It is also interesting in this context that some insects such as syrphid flies preferred yellow flowering wild radish plants to white flowering cross-wild F-1 hybrids, while bumble bees showed no such preference (Lee and Snow, 1998).

The dishes were only installed for two days in order to limit the losses of honeybees. Yellow dishes are attractants for honeybees which use yellow flowering plants for foraging (Saure, 2002). The results reveal that a significantly lower number of honeybees was attracted and finally collected in the white dishes than in the yellow ones. This result was consistent on both days. During the second day a significantly lower number of bees was gathered, which suggests a rapid messaging within the beehive.

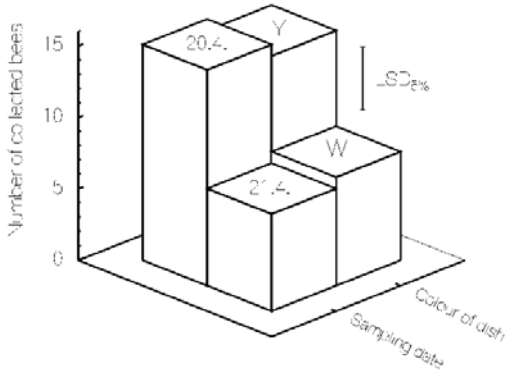

Figure 2:
Number of collected bees in relation to sampling date and dish colour (Y=Yellow; W=White) at main flowering (BBCH 65).

Conclusions

S deficiency results in significant morphological changes such as shape and color. Additionally, the scent might also be related to the S nutritional status of the plant. In contrast, pollen and nectar content and quantity are obviously not influenced by the S nutrition, so both factors can be excluded from being the causal reason for different attractiveness of S deficient and sufficiently supplied plants for honey bees. Bees proved to be more attracted to yellow than white dishes so that next to scent and shape this parameter seems to be relevant for foraging honeybees. Further research with free flying bees will be carried out in field experimentation in order to answer these open questions.

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Influence of drought and flooding on sulfur nutrition of deciduous trees at the whole plant level

Cornelia Herschbach

Abstract

In deciduous trees of the temperate zone sulfur nutrition is strongly influenced by environmental conditions. However, the effects observed during drought in oak (*Quercus robur*) and during flooding in poplar (*Populus tremula* x *P. alba*) do not consist to the 'demand-driven control' model of sulfur nutrition. Moreover, the observed changes could either be originate from general stress reactions in the case of drought stress or from adaptation mechanism in the case of flooding. During drought stress the sulfate loaded into the xylem was diminished in mycorrhizal-oak roots and so was sulfur transport in the phloem, probably by diminished loading of sulfur into the phloem. After the 'demand-driven control' model of sulfur nutrition these findings lead to assume an increment in reduced sulfur contents, mostly of glutathione in shoot and root tissues, which however was not observed. Obviously, the reduced water availability seems the reason for the decreased loading of sulfate into the xylem and the diminished sulfur transport in the phloem. If oak seedlings were simultaneously subjected to elevated pCO_2 these lead to an increased resistance against drought probably due to the changed pre-disposition.

Water logging, i.e. anoxic conditions in the rhizosphere, caused increasing cysteine contents in lateral roots of poplar. Since activity of APS reductase the key enzyme of the sulfate assimilation pathway disappeared below the detection limit and, the sink strength of the roots for sulfur from the shoot was decreased the enhanced cysteine must be formed by a process uncoupled from sulfate reduction under these conditions. H_2S produced in the rhizosphere by sulfate reducing bacteria could be taken up into root cells in analogy to hydrogen sulfide exposure of leaves. Increasing cysteine contents may than be the consequence of sulfide detoxification indicated by an enhanced *O*-acetylserine (thiol)lyase (OASTL) activity during flooding. Poplar is a flooding tolerant species, so the capability of sulfide detoxification may be a means of stress avoidance and/or stress tolerance during water logging.

Key words: drought stress, flooding, glutathione (GSH), phloem transport, Populus tremula x P. alba, Quercus robur, stress concept, sulfur, sulfide detoxification

[1] Albert-Ludwigs-University Freiburg, Institute of Forest Botany and Tree Physiology, Chair of Tree Physiology, Georges-Köhler-Allee 053/054, 79110 Freiburg, Germany

Introduction

Plants are living in a changing environment. The environment changes in the course of the day, during the year, from season to season and between years as a consequence of climate variation and climate change. Especially long-living organism like trees that live more than a century have to cope with a high variability's of environmental factors during their lifetime. For example, water supply, temperature, and light intensity are subjected to global climate change. This, especially trees have to adapt physiological processes to the changing environment. These reactions physiological allow the plant to compensate stress caused by environmental changes and to modulate the rate of growth its senescence and the onset of reproductive growth.

Deciduous tree species from the temperate zone, such as oak, beech or poplar, show differences in they sensitivity to environmental factors like drought and flooding. Since most tree species, which are not domesticated they possesses a high genetic variability and have developed ecotypes, which are acclimated to stand specific environmental factors. Therefore, it is difficult to define stress reactions of trees. Different models have been published aimed to describe stress reactions (Beck and Lüttge, 1990; Tesche, 1995; Brunold, 1996; Larcher, 2001). The simplified model of Tesche (1995) shows an alarm reaction and adaptation within the normal variability of a plant (Figure 1) that ensures high vitality. However, plant may lose part of their variability by adaptation to the environment even within the range of the normal variability of growth. If the intensity and/or duration of stress exceeds the normal variation, the plant first shows an alarm reaction which depends on the stress factor. During mild stress conditions the plant can cope with the stress and will probably adapt to the changed environment. However, the fitness of the plant may than be reduced. If the plant is attacked by additional stress or the duration of stress proceeds the extend to which a plant is able to cope with this situation depends on its pre-disposition and on its capability to avoid or tolerate the consequences of the specific stress factor. If the plant is able to adapt to the stress situation it is called eustress and the plant may show an enhanced resistance. When the plant is unable to manage the in-

creased stress, reaches the boundaries to cope with it and finally dies this is called distress.

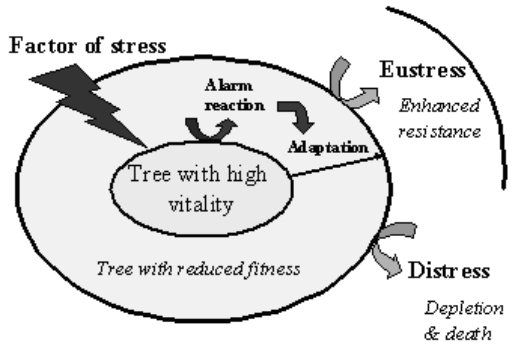

Figure 1:
An altered model of stress reactions after Tesche (1995). A plant with a high vitality is in the center of the model. If a factor of stress has affected the plant, an alarm reaction is induced followed by an adaptation mechanism. Additional stress such as prolonged duration of a stress factor or an additional factor leads to eustress or distress.

Stress reactions can be observed at different levels. Morphological changes are most obvious, but prior to morphological symptoms differences are measurable at the level of physiological processes and/or gene expression (Brunold et al., 1996; Larcher, 2001). For example: before the leaves wilt as a consequence of drought stress, a decreasing predown water potential is detectable (Schwanz et al., 1996). Also changes within other metabolic processes, i.e. photosynthesis, chlorophyll fluorescence, antioxidant levels can be detected before visible symptoms appear (Brunold et al., 1996; Larcher, 2001).

Global environmental conditions have changed rapidly over the last century. As a consequence of human activities atmospheric pCO_2 has increase from 290 to 350 ppm and is expected to double in this century (Hasselman, 1997; Houghton, 1997). Since atmospheric pCO_2 contributes to the greenhouse effect, it is also assumed that mean global surface temperature will rise by about 1-3.5 (www.ipcc.ch/present/graphics.htm). As consequence, precipitation and evaporation patterns will change and forests and other ecosystems will be exposed to drought and flooding events (Rennenberg et al., 2004).

Sulfur is an essential macro-nutrient for growth and development of plants. As essential part of the plants primary metabolism the use of sulfur in growth and development is strongly affected by the environmental changes indicated above. Within cells the amino acids cysteine (Cys) and methionine (Met) are essential constituents of proteins and, therefore, for growth and development. Plants can reduce sulfate and assimilate it into Cys and Met (Saito, 1999; Droux et al., 2000; Leustek et al., 2000) in both, photosynthetically active tissues as well as in heterotrophic tissues in roots or stems (c.f. Herschbach and Rennenberg, 2001b; Herschbach 2003). The contribution of sulfate reduction in heterotrophic organs to the whole plants' needs for reduced sulfur, however, is still unknown. For reduction the relatively inert sulfate has first to be activated by ATP sulfurylase which forms adenosine 5'-phosphosulfate (APS) and pyrophosphate (PP_i, Brunold, 1990). In higher plants APS is directly reduced to free sulfite via APS reductase (Gutierrez-Marcos et al., 1996; Setya et al., 1996; Suter et al., 2000). From studies with *Arabidopsis thaliana* root cultures it appears that this reaction controls the flux through the whole sulfate reduction pathway (Vauclare et al., 2002). In the next step the sulfite produced is reduced to sulfide without a release of intermediates by a sulfite reductase. O-acetylserine(thiol)lyase (OASTL) catalyses the final reaction of assimilatory sulfate reduction forming Cys (Giovanelli, 1990). In this reaction sulfide is transferred to O-acetylserine (OAS) which itself is synthesized by serine acetyltransferase (Giovanelli, 1990). The availability of OAS can limit the rate of Cys formation (Neuschwander et al., 1991; Saito et al., 1994) and, therefore, provides a regulatory link between nitrogen, carbon and sulfur metabolism (Brunold et al., 2003; Kopriva and Rennenberg, 2004). O-acetylserine(thiol)lyase and serine acetyltransferase co-operates in a multienzyme complex in which protein-protein interactions are based on the OAS concentration (Hell et al., 2002; Hell, 2003). Cys can further be used for Met synthesis (Giovanelli, 1990; Droux et al., 2000), protein formation, or glutathione production. Synthesis of glutathione (GSH) occurs in the cytosol as well as in the stroma of the plastids (Bergmann and Rennenberg, 1993). In both compartments GSH is produced by the consecutive action of γ-Glu-Cys synthetase (γ-ECS), synthesizing γ-Glu-Cys (γ-EC) from Glu and Cys, and glutathione synthetase (GSH-S), adding Gly to the C-terminal end of γ-EC (Bergmann and Rennenberg, 1993). GSH functions as a storage and transport form of reduced sulfur (Rennenberg 1984), is involved in the regulation of sulfur nutrition (Hawkesford, 2000; Herschbach and Rennenberg, 2001a,b; Kopriva and Rennenberg, 2004), and is an essential component of the plants' defense system for abiotic and biotic stress (Foyer and Rennenberg, 2000; Tausz et al., 2004).

To all plants sulfur is available in the soil in its oxidized form as sulfate. Sulfate is distributed within the plant via a range of sulfate transporters which are expressed in different tissues and compartments of the cell and are differently sensitive to sulfur deficiency (Buchner et al., 2004). Since SO_4^{2-}

reduction is thought to mainly occurred in leaves (Brunold, 1990), the surplus of reduced-sulfur must be transported out of leaves and, subsequently, loaded into the phloem for transport into the sink organs of the plant (Herschbach and Rennenberg, 2001a,b). Organs assumed to be sinks for reduced sulfur include young leaves, developing seeds and heterotrophic stem and root tissues. Still this view is based on carbon metabolism it is surprising, that mature leaves are no source-organs to supply young, developing leaves of oak (Schulte et al., 1998) and poplar (Hartmann et al., 2000) with reduced-sulfur. Moreover, young poplar leaves (Hartmann et al., 2000) and poplar roots (Herschbach, 2003) are able to reduced their own sulfate. Nevertheless, sinks must communicate with sources and *vice versa* to signal the demand for S in order to regulate sulfate uptake by the roots but also the whole-plant supply of reduced sulfur. According to the 'demand-driven control' model of S nutrition this signal is GSH which regulates sulfate uptake as well as sulfate reduction (Rennenberg, 1995; Lappartient and Touraine, 1996).

The presented review summarizes published literature on the influence of drought and flooding on the sulfur nutrition of deciduous trees. The observed effects are discussed with respect to the 'demand-driven control' model of sulfur nutrition and with respect to the stress concept of Tesche (1995).

Influence of drought on the sulfur nutrition of oak (*Quercus robur*)

Effects of drought in combination with elevated pCO_2 were analyzed under controlled and natural growth conditions (see Wullschleger et al., 2002). These investigations clearly showed that stress responses to drought dependent on the pre-disposition of the plant, e.g., whether the tree was exposed to elevated pCO_2 or not. For the consequences of drought for sulfur nutrition detailed data are only available for the drought tolerant *Quercus robur*. Pre-dawn water potential was diminished after 21-days withholding water supply in mycorrhizal and non-mycorrhizal oak seedlings independent both at ambient and elevated pCO_2 (Table 1, Schulte et al., 1998, Schwanz and Polle, 2001). However, at elevated pCO_2 the reduction was less pronounced. These findings corresponds to the observation that water-use efficiency increased under elevated pCO_2 (Saxe et al., 1998). After re-watering, pre-dawn water potential recovered in *Quercus robur* within a few days (Schwanz and Polle, 2001). Whereas photosynthesis was diminished due to drought stress, no changes were found in chlorophyll, carotenoids and soluble proteins (Table 1, Schwanz et al., 1996). Moreover, in this study it was demonstrated that trees subjected to water stress showed down regulation of enzymes involved in the anti-oxidative system. The activity of these enzymes increased if oak seedlings were simultaneously subjected to elevated rather than ambient pCO_2. The authors concluded that under elevated pCO_2 leaf tissues of the oak seedlings had a higher metabolic flexibility to cope with oxidative stress. After the stress concept of Tesche (1995) elevated pCO_2 enhanced stress resistance against drought in oak seedling.

This was also evident when the sulfur nutrition was investigated (Table 2). After the drought period of 21-days transport of sulfur from mature leaves into bark or root tissues was turned off if oak seedlings were cultivated at ambient pCO_2. In contrast, sulfur transport was still observed in seedlings grown at elevated pCO_2 (Schulte et al., 1998). The extent of drought tolerance, however, was dependent on mycorrhization of the oak seedlings. At elevated pCO_2 drought diminished ^{35}S-sulfur export out of leaves of non-mycorrhizal oak seedlings whereas this was not observed in mycorrhizal seedlings. Although S transport into lateral roots was diminished in both, mycorrhizal and in non-mycorrhizal roots, the GSH content of the roots remained unchanged (Schulte, 1998). After the 'demand-driven control' model sulfate uptake and sulfate transport into the xylem should remain unchanged under these conditions (Rennenberg, 1995; Lappartient and Touraine, 1996; Herschbach and Rennenberg, 2001a). Still mycorrhizal oak seedlings showed decreasing rates of sulfate loaded into the xylem during drought stress (Seegmüller, 1998). Obviously, the effects of drought on sulfur nutrition are not consistent with the 'demand-driven control' model. In conclusion, this example supports the assumption that the predisposition of the plant is very important for the extent of drought stress. The diminished sulfur transport in the phloem and the reduced rate of sulfate loaded into the xylem was the result of water deficiency and not a consequence of a changed sulfur status.

Influence of flooding on the sulfur nutrition of poplar (*Populus tremula* x *P. alba*)

A long-term strategy of adaptation to flooding is the formation of aerenchyma to prevent anoxia in roots. Short-term effects of flooding result in a shift from respiration to glucose fermentation, predominantly ethanol fermentation by using reserved carbohydrates (reviewed in Armstrong et al., 1994; Drew 1997). This is accompanied by diminished synthesis of housekeeping proteins and an induction of anaerobic stress proteins (Christopher and Good, 1996). Toxic ethanol contents could be prevent by ethanol transport into the xylem and transport to the

shoot with the transpiration stream (reviewed in Armstrong et al., 1994; Drew, 1997). The most important consequence is the diminished ATP availability and, the decreased energy charge within the roots (Sieber and Brandle, 1991; De Simone et al., 2002). This could influence transport processes including nutrient uptake, xylem loading, phloem unloading and consequently growth and development. Therefore, more glucose originating from the phloem-mediated carbon transport from the leaves or from storage tissues is required to keep up growth, development and nutrient uptake.

Table 1:
Effects of drought on biometric and physiological parameters. Oak (*Quercus robur*) seedlings grown from acorns were cultivated under controlled growth conditions either in a greenhouse or in environmental growth chambers. Water supply was withdrawn for 3- to 4-weeks.

Atmospheric pCO_2	Ambient	Elevated
Predawn water potential[1]	=	↓
Photosynthesis[1]	↓	↓
Chlorophyll[1]	=	=
Carotinoids[1]	=	=
Soluble protein[1]	=	=
SOD activity in leaves[1,2]	↓	↑
Catalase activity in leaves[2]	↓	↓
Ascorbate peroxidase activity in leaves[1]	=	↑
Redox state (GSSG)[2]	↑	↑
Redox state (ascorbate)[2]	↓	↓

[1]Schwanz et al., 1996, [2]Schwanz and Polle, 2001

In the flooding tolerant poplar ethanol is produced from anaerobic glucose fermentation in flooded roots and the bulk is loaded into the xylem and transported with the transpiration stream to the leaves where the ethanol introduced into the leaves carbohydrate metabolism by oxidation to acetaldehyde and acetic acid (Kreuzwieser et al., 1999). Although pigment contents were slightly reduced and carbon assimilation was diminished to 70% of control poplar trees after 14 days of flooding (Kreuzwieser et al., 2002), soluble carbohydrates increased in leaves and phloem exudates (Herschbach et al., 2004). Also in *Fraxinus excelsior* a floodplain tree, glucose increased in phloem exudates due to flooding (Bartles, 2001). It appears that an increased transport of carbohydrates to the roots, probably from reserve mobilization, could meet the higher demand of carbohydrates for glucose fermentation in the roots to maintain a high energy charge. However, this was not observed with oak (Bartels, 2001), which is a moderate flooding tolerant tree species. Rather, soluble carbohydrates in phloem exudates of oak increased only during the 14-days period of recovery from flooding. To maintain metabolic processes during flooding synthesis of anaerobic proteins such as enzymes of the fermentation pathway, glycolysis and enzymes to prevent post-anoxic stress are induced in tolerant species (Christopher and Good, 1996). Although it may be assumed that protein contents changed due to this induction after flooding, non-uniform results were found for poplar (Kreuzwieser et al., 2002; Herschbach et al., 2004) and, protein contents of flooded oak roots remained unaffected after long-term flooding (Kreuzwieser et al., 2002). Soluble nitrogen compounds did not change in poplar roots, though several amino compounds decreased in flooded oak roots (Kreuzwieser et al., 2002). Both, the increased content of TSNN and of soluble carbohydrates in phloem exudates of flooded oak trees may indicate an inhibition of phloem unloading of amino compounds in the roots, since therein amino compounds decreased and soluble carbohydrates were unaffected (Bartels, 2001).

Whereas the nitrogen metabolism remained unaffected during anoxic conditions in poplar (Kreuzwieser et al., 1999, 2002) flooding clearly affected the sulfur metabolism (Herschbach et al., 2004). Even after 7 days of flooding the key enzyme of the

Table 2:
Effects of drought on oak (*Quercus robur*) seedlings with different pre-dispositions due to mycorrhization and atmospheric pCO_2. Oak seedlings were grown from acorns in environmental growth chambers under long-day conditions. To accomplish drought stress, water supply was withdrawn for 21-days. n.d., not detectable.

	Non-mycorrhizal		Mycorrhizal	
Atmospheric pCO_2	ambient	elevated	ambient	elevated
Pre-dawn leaf water potential[1]	↓	↓	↓	↓
Total plant biomass[1]	=	=	=	↓
Root biomass[1]	=	↓	=	=
GSH content in leaves[2]	=	=	=	=
^{35}S-sulfur export out of mature leaves[1]	n.d.	↓	n.d.	=
Proportion of ^{35}S-sulfur remained in the shoot[1]	n.d.	↑	n.d.	=
Proportion of ^{35}S-sulfur imported into lateral roots[1]	n.d.	↓	n.d.	↓
GSH content in lateral roots[2]	=	=	=	=

[1]Schulte et al. 1998; [2]Schulte 1998

Table 3:
Effects of flooding on sulfur nutrition of poplar (*Populus tremula* x *P. alba*). Results from Herschbach et al. (2004) are summarized. n.d., not determined.

	7 days of flooding	14 days of flooding	After 7 days of recovery from 15 days of flooding
APS reductase activity in leaves	↓	↓	↓
OASTL activity in leaves	↓	↓	↓
Cys content in leaves	=	=	=
GSH content in leaves	=	=	=
^{35}S-sulfur export out of mature leaves	=	n.d.	n.d.
GSH content in phloem exudates	↑	=	=
Cys content in phloem exudates	=	=	=
APS reductase activity in roots	↓	↓	=
OASTL activity in roots	=	↑	↑
Cys content in roots	↑	↑	↑
GSH content in roots	=	=	↑
Proportion of ^{35}S-sulfur imported into lateral roots	↓	n.d.	n.d.

sulfate assimilation pathway, the APS reductase, completely disappeared (Herschbach et al., 2004). This may be an indication that energy-consuming enzymes of anabolic pathways are eliminated in flooded roots to save energy. Indeed, the incorporation rate of ^{35}S-sulfate into insoluble cellular compounds was diminished and, consequently, the biomass increment was reduced (Herschbach et al., 2004). Based on the 'demand-driven control model' of sulfur nutrition enhanced amounts of GSH would be expected in flooded roots when the GSH dependent APS reductase activity is down-regulated (Lappartient and Touraine, 1996; Vauclare et al., 2002). Nevertheless, the GSH content in the roots of flooded poplars remained unaffected. Though unlikely (Bick et al., 1998, 2001; Kopriva and Koprivova 2004), it cannot be excluded that Cys acts as a feedback signal to prevent APS reductase expression during anoxia. Indeed, in poplar roots Cys increased under this conditions (Table 3, Herschbach et al., 2004). However, where does the sulfide incorporated into Cys comes from, if APS reductase activity is not detectable? Protein breakdown seems not the reason for the increased Cys content in lateral poplar roots, because the content of soluble protein increased (Kreuzwieser et al., 2002) or remained unchanged (Herschbach et al., 2004) during flooding. Export of sulfur out of mature leaves was not effected by flooding, but the proportion of sulfur transported into lateral roots decreased (Table 3, Herschbach et al., 2004). Therefore, the increased Cys content does not originate from enhanced sulfur transport to the roots and additionally, Cys must be synthesized uncoupled from sulfate assimilation. High amounts of H_2S are produced under anoxic conditions in the rhizosphere due to sulfate reducing bacteria (Dassonville and Renault, 2002). This sulfide could be taken up into root cells in analogy to hydrogene sulfide fumigation of leaves (Rennenberg and Polle, 1994). Since sulfide is phytotoxic because it inactivates metalloenzymes by forming disulfides, it must be detoxified. One strategy may be the metabolization to non-toxic compounds, such as thiols as described by Fürtig et al., (1996) for *Phragmites australis*. In this case, a greater activity of O-acetlyserine(thiol)lyase (OASTL), the enzyme which forms Cys from O-acetylserine (OAS) is expected and was really detected after 14 days of flooding in poplar roots (Table 3, Herschbach et al., 2004) and in roots of several herbaceous plants after feeding sulfide (Pearson and Havill, 1988). After 4 days in hydroponic culture poplar roots fed with sulfide showed also increased OASTL activity. This clearly indicates that sulfide can be detoxified in roots under anoxic conditions and can be used for Cys synthesis uncoupled from sulfate assimilation. These results demonstrate that changes in the sulfur state of plants must not necessarily correlate to the 'demand-driven control' model of sulfur nutrition. Moreover, increasing Cys contents under anoxic conditions from flooding could be a strategy in stress tolerance and stress adaptation (see Fig 1).

Conclusions

The influences observed within the sulfur metabolism during drought and flooding do not support the 'demand-driven control' model of sulfur nutrition as a sole possibility to explain regulation of sulfur nu-

trition. Moreover, the observed effects could be best explained when stress reactions are considered. During drought the reduction in water availability general reduced xylem transport as well as mass flow of phloem sap. These changes are also relevant for the sulfur nutrition and may explain the decreased loading of sulfate into the xylem and the diminished sulfur loading into the phloem and/or transport in the phloem. Water logging, i.e. anoxic conditions of the pedosphere induced changes in the rhizosphere. Probably, the decreased energy charge of the roots may the reason for the diminished sulfate reduction in flooded roots. And, sulfate reducing bacteria are induce during flooding which enhanced production of sulfide. Since sulfide has toxic properties the increased sulfide assimilation capacity may be a mechanism of stress tolerance and stress avoidance. These summary demonstrates that physiological changes due to environmental changes must be considered when changes within the sulfur metabolism are discussed.

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Chemical behavior of soil sulfur in the rhizosphere and its ecological significance

Zhengyi Hu[1], Silvia Hanekalus[2], Zhihong Cao[1] and Ewald Schnug[2]

Abstract

Sulfur naturally occurs in valences of –2 to +6. Various organic sulfur compounds can be found in soils. The rhizosphere is a key zone with view to the mechanisms of soil nutrient dynamics. This contribution summarizes the current knowledge about the chemical behavior of sulfur in the rhizosphere and its ecological impact and highlights future research needs.

Key words: arylsulfatase, elemental sulfur, soils, sulfur fertilization, rhizosphere

Introduction

Human activity highly influences the sulfur (S) cycle through anthropogenic emission from fossil fuel burning. Global SO_2 emissions from anthropogenic sources increased about 20-folds in 1985 compared to 1850 (Brimblecombe et al., 1989). This increase was strongest between 1940 and 1970 in Europe and North America, but then with the introduction of clean air acts coming into force the rend was reversed (Brimblecombe et al., 1989). However, SO_2 emissions are still increasing in Asia. Here the S emissions increased from 33.7 Tg in 1990 to 39.2 Tg in 1997, and peak values of 40-50 Tg are expected for the year 2020. China contributes with 66% of the total S emissions (David, et al., 2000).

Atmospheric S loads are closely linked to soil quality and an imbalanced S nutrition of plants (Hu, 2002a; McGrath et al., 1995; Schnug, et al., 1998). Atmospheric S depositions vary regionally in China and follow industrial activities (Wang et al., 2000). So, the hat total S deposition was 95 kg S/ha at the Experimental Station of Red Soil Ecology, Yingtan, Chinese Academy of Sciences in 1998/1999 (Hu et al., 2002b), and the soil pH value decreased by 0.6 units since 1992 (Xu et al., 2004). The excess of S may have a negative effect on the soil-plant system, for example on flooded paddy soils. Here, S will be reduced to H_2S, which obstructs plant growth (Hu et al., 2002a). In contrast, atmospheric S depositions are not sufficiently high in order to satisfy the demand in remote areas of China (Hu et al., 2002a). Yield responses to S fertilization of more than 20 different agricultural crops ranged from 4% to 81% (Cao et al., 1996).

The rhizosphere is a key zone with view to the mechanisms of soil nutrient dynamics (Darrah et al., 1993). Physico-chemical processes at the soil-root interface differ considerably from those in the non-rhizosphere soil. The effect of plant growth on soil nutrients in the rhizosphere was studied intensively for P (Gahoonia et al., 1992; Zoyza et al., 1997), N, K, Ca and Mg (Moritsuka et al., 2000). Only limited data is, however, available for the effect of plant growth on the chemical behavior of S in the rhizosphere, which is nevertheless required in order to assess agronomic and ecological impacts in relation to the local S cycle. This paper summarizes the present knowledge about the chemical behavior of soil S in the rhizosphere.

Chemical behavior of soil S in the rhizosphere

Oxidization of S^0 in the rhizosphere and non-rhizosphere

Elemental S (S^0) is used as a fertilizer to satisfy the S demand of cop plants. This reduced S needs to be oxidized to SO_4^{-2} before it becomes plant available. Oxidation of S^0 in soils is primarily a microbial process (Wainwright, 1984). The activity of *thiobacilli* is highly important for the oxidation of elemental S (McCaskill and Blair, 1987). Heterotrophic micro-organisms are other S^0 oxidizers in soils (Wainwright, 1984). Elemental S is oxidized by *thiobacilli* to sulfuric acid. The application of S^0 together with inoculation decreased soil pH rapidly from about 7.3 to 3.2 after 12 weeks of incubation. Adding *thiobacilli* together with S^0 to the rhizosphere yields a significantly faster oxidation than application of S^0 on its own (Fan et al., 2002). Grayston et al. (1991) isolated 273 bacterial phylas and 70 fungal species from the rhizosphere of canola (*Brassica napus*). From these 273 bacterial isolates, 245 (89.7%) oxidized S^0 to thisosulfate or tetrathionate, and 133 (48.7%) oxidized S^0 to SO_4^{-2}. All 70 fungal isolates oxidized S^0 to SO_4^{-2}. Bacterial isolates showed the highest S^0 oxidization rate (Table 1).

The rhizosphere is a key zone with view to the mechanism of soil nutrient dynamics. Physico-chemical processes in the soil-root interface differ considerably from those in the non-rhizosphere

[1]State Key Laboratory of Soil and Sustainable Agriculture, Institute of Soil Science, Chinese Academy of Sciences, Nanjing, 210008, P. R. China.
[2]Institute of Plant Nutrition and Soil Science, Federal Agricultural Research Centre (FAL), Bundesallee 50, D-38116 Braunschweig, Germany

soil. A rhizobag culture experiment demonstrated that the oxidation of S^0 in the rhizosphere and non-rhizosphere varied in dependence on soil moisture content and soil type (unpublished data). The oxidation rate of S^0 was generally lower under waterlogged (1 cm water depth) than aerobic conditions (80% water holding capacity; Figure 2). On a paddy soil originating from lime rock, the oxidation rate of S^0 was higher in the rhizosphere of rice than in non-rhizosphere under waterlogged and aerobic conditions (Figure 2). However, these differences were not observed on the paddy soil originating from granite. The reason could possibly be different contents of plant available S and micobiological species.

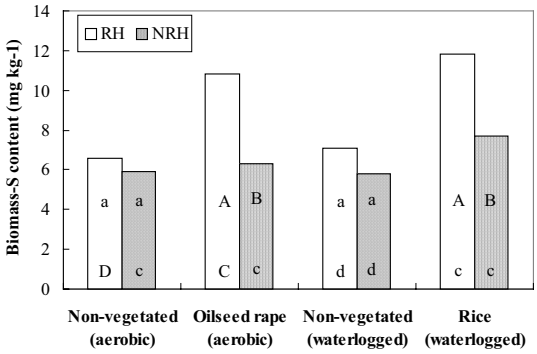

Figure 1:
Concentration of microbial biomass-S (MB-S) in the rhizosphere (RH) and non-rhizosphere (NRH). Different letters (a,b) and (A, B) indicate significant differences between RH and NRH at $p<0.05$ and $p<0.01$ level (student T-test).Different letters (c, d) and (C, D) indicate significant differences of MB-S in RH and NRH relative to no-vegetated soils at $p<0.05$, $p<0.01$ level(student T-test), respectively; source: Hu et al. (2003).

Soil microbial biomass S in the rhizosphere and non-rhizosphere

Soil microbial biomass is defined as the living part of soil organic matter (Chapmam, et al., 1987). The microbial biomass S in agricultural soils varied between 4.4% and 4.9% in non-vegetated soils and 5.2 and 8.8% in vegetated soils (Saggar et al., 1981; Chapmam, et al., 1987; Wu, et al., 1994). Despite its small size, the microbial bio-mass is a highly active fraction that acts as the driving force behind mineralization-immobilization and oxidation-reduction processes. A rhizobag culture experiment demonstrated that the S content of microbial mass was 6.3 mg S kg^{-1} in the non-rhizosphere soil and 11.8 mg S kg^{-1} in the rhizosphere soil of rice (Hu et al., 2003). The S content of microbial bio-mass was up to 72% higher in the rhizosphere of rice than in the non-rhizosphere (Figure 1). In non-vegetated soil samples the S content of microbial bio-mass was generally and significantly lower than in the vegetated treatments (Hu et al., 2003), because cropping increases the biological activity (Castellano et al., 1990).

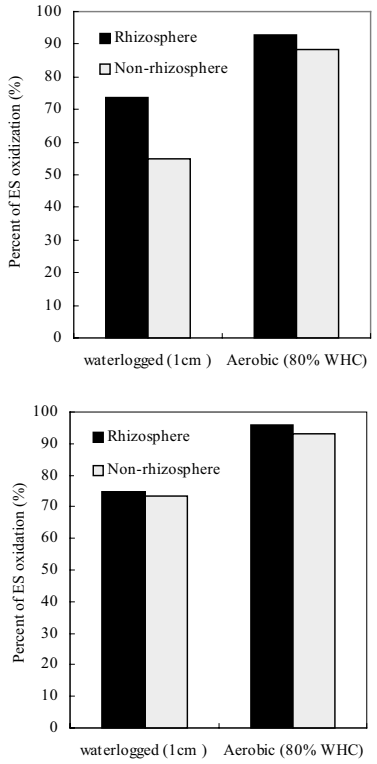

Figure 2:
Oxidization of elemental S (ES) in the rhizosphere of rice in dependence on water management and soil type (upper: paddy soil originated from lime rock; lower: paddy soil originated from granite; unpublished data).

Variations in the chemical behavior of S in the non-rhizosphere and rhizosphere

In a rhizobag experiment it was demonstrated that the distribution of S fractions in the rhizosphere and the non-rhizosphere soil varied in dependence on the crop type (Table 2). More total and inorganic SO_4^{2-}-S was found in the rhizosphere of oilseed rape and rice (Table 2), which supposedly relies on mass flow to the roots (Barber, et al., 1995). More organic S was found in the rhizosphere of oilseed rape, while inverse results were obtained for rice (Table 2). A possible explanation is that the turnover of organic matter was hampered under anaerobic conditions (Williams et al., 1967). Stanko-Golden (1991)

Table 1:
Number of S^0-oxidizing bacterial isolates from the rhizosphere and rhizoplane of canola grown in a growth chamber (source: Grayston et al., 1991).

Soil	Area of isolation	Total isolates	Number of isolates producing		
			$S_2O_3^{2-}/S_4O_6^{2-}$	SO_4^{2-}	$S_2O_3^{2-}/S_4O_6^{2-}$ and SO_4^{2-}
Haverhill	Rhizosphere	56	49 (87.5%)*	25 (44.6%)	25 (44.6%)
	Rhizoplane	43	42 (97.7%)	30 (69.8%)	30 (69.8%)
Carrot River	Rhizosphere	31	26 (83.9%)	15 (48.4%)	14 (45.2%)
	Rhizoplane	40	38 (95.0%)	20 (50.0%)	20 (50.0%)
Asquith	Rhizosphere	19	18 (94.7%)	7 (36.8%)	7 (36.8%)
	Rhizoplane	32	29 (90.6%)	13 (40.6%)	10 (31.2%)
Laird	Rhizosphere	15	11 (73.3%)	6 (40.0%)	5 (33.3%)
	Rhizoplane	37	32 (86.5%)	17 (45.9%)	13 (35.1%)
Total bacteria		273	245 (89.7%)	133 (48.7%)	124 (45.4%)

*Sulfur oxidizers as percentage of total isolates.

Table 2:
Contents of different S Fractions (mg S kg^{-1}) in the rhizosphere (RH), non-rhizosphere (NRH), and the RH to NRH ratio (mean; source: Hu et al., 2003).

Water management	Non-vegetated /cropping	RH/NRH	Total S	S fractions						
				Organic S fractions				Inorganic S fractions		
				Total organic	Ester bonded	Carbon bonded	Residual	Total inorganic	Soluble SO_4^{2-}	Adsorbed SO_4^{2-}
Aerobic condition	Non-vegetated	RH	141.9a	99.3a	20.4a	11.7a	67.2a	42.6a	33.0a	12.0a
		NRH	133.9a	91.4a	20.1a	13.8a	57.2a	42.5a	28.8a	13.7a
	Oilseed rape	RH	122.3a	99.6a	30.0b	14.6a	53.8A	44.0a	34.0a	10.0a
		NRH	120.4a	87.6a	44.5a	17.3a	25.8B	32.8b	23.0b	9.8a
		Ratio	1.02	1.13	0.67	0.84	2.08	1.34	1.48	1.02
Water-logged condition	Non-vegetated	RH	145.3a	96.0a	24.0a	15.7a	56.3a	49.3a	43.0 a	6.3 a
		NRH	133.0a	87.8a	27.0a	14.4a	46.4b	45.2a	40.4 a	4.8 b
	Rice	RH	155.2a	29.6B	6.0B	11.0a	12.6B	125.6A	110.3A	15.3A
		NRH	131.2a	91.2A	33.6A	10.7a	46.9A	40.0B	34.7B	5.3B
		Ratio	1.18	0.33	0.18	1.03	0.27	3.14	3.18	2.89

*Values followed by different letters (a, b), and (A, B) indicate significant differences between RH and NRH at $p < 0.05$, and $p < 0.01$ level (student T-test), respectively.

Table 3:
Concentrations (mean value ± SD, n=4) of different S fractions (mg S kg^{-1}) in the rhizosphere (RH) and non-rhizosphere (NRH) in dependence on soil and crop type (source: Hu et al., 2002c).

Soils	Treatment	RH/ NRH	Total S	Sulfur fractions							
				Total S in 0.01 M $CaCl_2$	Adsorbed SO_4^{2-}	Ester bonded	Carbon bonded	Residual	Total S in 0.01 M $Ca(H_2PO_4)_2$	SO_4^{2-} in 0.01 M $Ca(H_2PO_4)_2$	HI-reducible S
Haplic Acrisol	Fallow	RH	202±2	13.9 ± 1.3	13.8 ± 1.9	76.0 ± 5.1	19.7 ± 1.4	79.0 ± 8.6	39 ± 2	28 ± 2	104 ± 8
		NRH	182±14	14.8 ± 0.4	13.3 ± 0.9	75.3 ± 2.5	18.1 ± 2.1	69.4 ± 9.7	38 ± 1	28 ± 1	103 ±12
	Wheat	RH	193 ± 17	15.2 ± 1.8	10.8 ± 2.0	48.0 ± 3.1	19.2 ± 3.0	99.0 ± 7.8	36 ± 5	26 ± 3	74 ± 4
		NRH	175 ± 7	9.8 ± 0.7	14.0 ± 1.1	56.0 ± 4.1	22.3 ± 3.3	72.3 ± 9.3	33 ± 2	24 ± 1	80 ± 6
	Oilseed rape	RH	179 ± 5	8.8 ± 1.3	5.5 ± 1.4	58.6 ± 5.8	18.7 ± 2.0	87.4 ± 6.9	23 ± 2	14 ± 0	73 ± 6
		NRH	170 ± 9	5.5 ± 0.5	9.8 ± 1.3	60.8 ± 5.7	20.7 ± 2.4	72.9 ± 7.2	22 ± 1	15 ± 1	76 ± 6
	Radish	RH	194 ± 9	8.7 ± 1.4	5.4 ± 1.0	75.3 ± 1.5	19.5 ± 1.4	85.5 ± 9.9	24 ± 2	14 ± 1	89 ± 9
		NRH	174 ± 10	5.7 ± 0.9	9.0 ± 1.7	86.9 ± 1.7	21.2 ± 1.4	51.4 ± 6.3	22 ± 1	15 ± 2	102 ± 1
Hortic Anthrosol	Fallow	RH	141 ± 6	19.2 ± 2.7	4.9 ± 0.7	40.1 ± 3.1	1.3 ± 0.1	75.4 ± 4.1	32 ± 4	24 ± 3	64 ± 2
		NRH	130 ± 9	20.3 ± 3.1	3.9 ± 1.5	32.4 ± 2.8	0.1 ± 0.1	73.2 ± 8.3	38 ± 3	24 ± 3	57 ± 2
	Wheat	RH	131 ± 10	19.8 ± 2.0	5.0 ± 1.8	34.2 ± 2.9	1.6 ± 0.2	70.1 ± 7.6	33 ± 1	25 ± 2	59 ± 3
		NRH	131± 2	22.1 ± 4.0	1.8 ± 2.7	43.4 ± 2.5	<LLD	63.3 ± 3.7	38 ± 2	24 ± 3	67 ± 2
	Oilseed rape	RH	122 ± 5	13.2 ± 1.6	-0.4 ± 1.0	36.4 ± 1.2	1.8 ± 1.2	71.3 ± 4.5	20 ± 2	13 ± 1	49 ± 4
		NRH	130 ± 3	16.4 ± 4.0	1.3 ± 2.2	40.2 ± 1.9	0.1 ± 0.1	71.7 ± 2.7	30 ± 3	18 ± 3	58 ± 2
	Radish	RH	137 ± 5	13.8 ± 0.7	0.11 ± 0.6	40.7 ± 1.3	1.4 ± 0.2	80.7 ± 5.9	26 ± 4	14 ± 1	55 ± 1
		NRH	115 ± 6	10.8 ± 4.6	0.60 ± 3.3	51.1 ± 5.2	0.2 ± 0.1	52.5 ± 6.9	18 ± 2	11 ± 1	63 ± 4

note: <LLD < Lower Limit of Detection; RH rhizosphere; NRH non-rhizosphere (bare soil)

reported that soil moisture was positively related with organic S. With view to rice the soil moisture is of minor relevance, because there are oxidizing conditions in the rhizosphere due to aeration tissues from the top to the roots which promote the activity of microbes and sulfatase (Han et al., 1982, Freney et al., 1966).

More ester-bonded S was found in the non-rhizosphere of oilseed rape and rice (Table 2). Hu et al. (2002c) observed similar results for oilseed rape, wheat and radish (Table 3). The reason could be a higher arylsulfatase activity in the rhizosphere as it is this enzyme, which catalyzes the decomposition of sulfate esters (Fitzgerald, 1978). Han et al. (1982) found, however, that the arylsulfatase activity was higher in the rhizosphere than in the non-rhizosphere of rice (Table 4). Additionally, the activity of microorganisms is higher in the rhizosphere as they use root exudates as an energy source (Yan et al., 1993). Thus, the rhizosphere soils had a higher organic C content than the non-rhizosphere soils (Hu et al., 2003).

Carbon-bonded S is not related to plant S uptake (Lee et al., 1979), though S may be mineralized from all organic S fractions (Li et al., 2001). Amino acids, such as cysteine and methionine are the major components of carbon-bonded S (Tabatabai et al., 1982; Freney et al., 1986). S-containing amino acids do not accumulate in free forms, because they are rapidly degraded in aerobic soils (Fitzgerald et al, 1978). Paul and Schmidt (1961) reported that the cysteine and methionine content was slightly higher in the rhizosphere than in the non-rhizosphere soil. Other experiments revealed no significant differences existed between the two compartments (Hu et al., 2002c; Table 2, 3). These results indicate that carbon-bonded S is of minor importance for the S nutrition of crops than for instance ester sulfate.

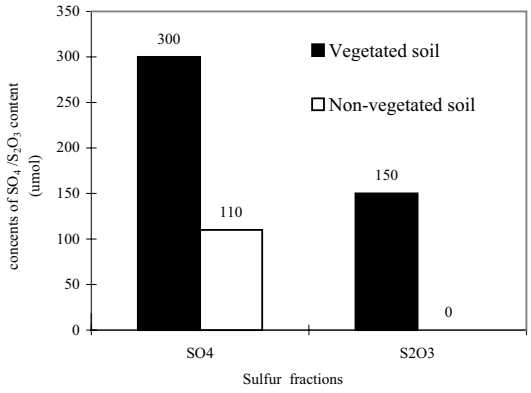

Figure 3:
SO_4^{2-} and $S_2O_3^{2-}$ content in the non-rhizosphere and rhizosphere of rice (Wind , 1995)

The amount of residual-S was significantly higher in the rhizosphere than in the non-rhizosphere of oilseed rape while opposite results were found for rice (Table 2). Other crops such as wheat and radish also showed higher levels of residual S in the rhizosphere (Table 3). Rice had a higher ability to utilize residual S from the soils which could be related to its aeration tissues.

In all treatments with plants, the content of soluble SO_4^{2-}-S and adsorbed SO_4^{2-}-S was higher in the rhizosphere than in the non-rhizosphere (Table 2). This can not be attributed generally to a higher mineralization in the rhizosphere, because the organic S content was higher in the rhizosphere of oilseed rape (Table 2). Enhanced mass flow of SO_4^{2-}-S to the rhizosphere after mineralization of organic S in the non-rhizosphere is supposedly the reason for this effect. Wind (1995) found that the concentrations of SO_4^{2-}, $S_2O_3^{2-}$ at the rhizosphere of rice were related to rice planting. The same author found more SO_4^{2-} in the rhizosphere (300 μmol kg^{-1}) of rice than in the non-vegetated (110 μmol kg^{-1}) treatment.

Ecological effects of soil S transformations in the rhizosphere

Grayston et al. (1991) selected eighteen isolated bacteria, which showed an increased efficacy of in vitro S oxidization for inoculating seeds, together with applications of elemental S. Results indicated that inoculation with 14 phyla increased canola leaf size, and root and pod dry weight at maturity was promoted by seven phyla. The shoot material had higher iron, sulfur, and magnesium contents after inocultion by two of the eighteen bacterial isolates (Table 5). In case of three isolates the treatment had a detrimental effect on the growth of the fungal pathogens, *Rhizoctonia solani* AG2-1, *R. solani* AG4, and *Leptosphaeria maculans* "Leroy". Besides a direct fungicidal effect of elemental S, the initiation of S induced resistance mechanisms through an enhanced oxidation of S^0 may explain the latter effect (Haneklaus et al., 2004).

Sulfur in nature occurs in valences from -2 to +6 (Hu et al., 2002a). Many types of organic S compounds were found (Morra et al., 1997, Hu et al., 2002a). Internal cycling reactions are responsible for maintaining a biologically available S supply through mineralization of organic substrates and redox transformation of inorganic species (Hu et al., 2002a). Speciation of S in natural organic matter could provide a clear understanding not only of bio-geochemical transformations of S, but also of the role of organic S in the complexation of toxic trace metals (Xia et al., 1998). Here, S-containing functional groups in humic substances may play an important role in complex formation with trace

Table 4:
Comparison of arylsulfatase activity in non-rhizosphere and rhizosphere soil of the different rice varieties grown on Pila clay loam and Maahas clay (source: Han et al., 1982).

Treatment	Weeks after transplanting				
	0	2	4	6	8
Pila clay loam soil*					
Non-rhizosphere soil	36	9.3	12.9	16.0	11.3
Rhizosphere soil of different rice varieties					
IR-8	36	25.5	31.3	45.1	54.6
IR-667	36	13.0	18.9	21.5	22.6
C-4	36	18.2	37.2	26.5	42.2
Maahas clay soil*					
Non-rhizosphere soil	7	5.8	5.4	4.4	5.8
Rhizosphere soil of different rice varieties					
IR-8	7	9.5	10.8	9.1	12.1
IR-667	7	8.2	8.7	9.1	11.2
C-4	7	10.4	10.4	8.1	10.4

* Cite from original text

Table 5:
Sulfur, iron, and magnesium content of canola shoots and pods after seed inoculation with sulfur-oxidizing rhizosphere (source: Grayston et al., 1991).

Treatment	Plant tissue	Mg (mg)	S (mg)	Fe (μg)
Control	Shoots	9.3 ± 1.6	21.6 ± 2.8	439 ± 64
Isolate No 13	Shoots	11.1 ± 1.1	23.2 ± 2.4	657 ± 155*
Isolate No. 14	Shoots	11.9 ± 1.2*	28.1 ± 1.2*	727 ± 118*

Note: Plants grown in 2 kg of soil amended with prilled S^0 fertilizer (50μg g^{-1}) in growth chamber. The control was inoculated with an autoclaved culture of isolate 10. Means of five replicates ± SD. *Significant increase above control ($p<0.05$).

metals such as Cd, Co, Ni, Pb, Zn, As, and Hg (Xia et al., 1998).

Conclusions

Only few studies about the chemical behavior of soil S in the rhizosphere were carried out (Hu, et al., 2002c, 2003, Wind , 1995; Grayston et al., 1991; Han, et al., 1982) so that information about factors influencing S transformation processes in the rhizosphere is still limited. In this context, the soil water regime, plant species, soil type, soil characteristics are parameters, which need to be paid more attention to.

A number of wetland plants, such as rice, have been shown to oxidize the rhizosphere, a process which may serve to protect against the entry of reduced phytotoxins, such as Mn^{2+}, Fe^{2+}, and S^{2-} (Armstrong et al., 1978). Iron plaque is commonly formed on the roots of aquatic plant species, such as *Oryza sativa,* and is mainly caused by the oxidation of ferrous to ferric compounds and the precipitation iron oxide on the root surface (Armstrong, 1967; Chen, et al., 1978). Results of Liu et al., (2004a) showed that P starvation can disturb formation of iron plaque onto the roots of rice plants grown under solution culture, but there is little information on the role of S in iron plaque development though S plays an important role for adjusting soil redox processes. Some reports have shown that iron plaque may be a barrier to the uptake of heavy metals, such as Cu, Ni, Mn, As, Cd (Taylor and Crowder, 1983; Greipsson, 1994; Liu et al., 2004a, b). Effect of chemical behaviors of soil S in the rhizosphere and iron plaque induced by S transformation is therefore of particular interest.

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Measuring fluxes of reduced sulfur gases

Beate Huber[1] and Werner Haunold[2]

Abstract

This paper gives an overview about techniques for measuring fluxes of reduced sulfur gases used in ecological sciences. Measuring fluxes reduced of sulfur gases (H_2S, COS, CH_3SH, DMS, CS_2) in atmospheric concentrations needs extensive measurement equipment. Because the concentrations of reduced sulfur gases in the atmosphere are very low - in the range of parts per trillion (pptv) - it is necessary to concentrate the gases with a cryogenic sampling system. For analyzing reduced gases a gas chromatographic system with a flame photometric detector is used. Fluxes of reduced sulfur gases between soil, plants and atmosphere are usually determined with dynamic chamber systems. Flux estimations on ecosystem scale require micrometeorological methods.

Keywords: reduced sulfur gases; analysis of atmospheric trace gases; cryogenic trapping; flux measurements; dynamic chambers; micro-meteorological methods

Introduction

Reduced sulfur is present in the atmosphere in several gaseous species, COS (carbonyl sulfide), DMS (dimethylsulfide), H_2S (hydrogen sulfide), CS_2 (carbon disulfide) and CH_3SH (methyl mercaptan, methanethiol). Reduced volatile sulfur compounds, which are released to the oxygen-rich atmosphere, are chemically oxidized during their lifetime and end up finally as sulfur dioxide (SO_2), sulfuric acid, particulate sulfate and methane sulfonate (Andreae and Jaeschke, 1992). These compounds are again removed from the atmosphere and re-enter the biosphere by dry and wet deposition (Andreae and Jaeschke, 1992).

In the atmosphere sulfate aerosols play an important role, because they act as cloud condensation nuclei, increase albedo of clouds and influence in this way the global radiation budget (Crutzen, 1976; Charlson et al., 1987; Andreae, 1992). Atmospheric sulfur originates from anthropogenic and numerous natural sources. One of the major uncertainties in global sulfur budget is the exchange between atmosphere, soils and vegetation (Rennenberg, 1991; Kesselmeier, 1991). Especially the lack of knowledge in diurnal and seasonal flux variations is critical for estimations on regional and global scale. In the last decade budgeting global sulfur cycles showed progress (Chin and Davis, 1993), but is not yet completed (Watts, 2000).

Gas chromatographic analysis of reduced sulfur gases

Measuring reduced sulfur gases is carried out in two main steps: (1) sulfur gases are cryogenically trapped from atmospheric samples and (2) they are analyzed by a gas chromatograph (GC) with a flame photometric detector (FPD) (Haunold et al., 1992; Hofmann et al., 1992a).

Cryogenic collecting

The air samples are concentrated by pumping atmospheric air through cryogenic collectors, which are cooled in liquid argon (- 186°C). The reduced sulfur gases with melting points between - 86°C and - 138°C freeze in the collectors, while N_2 and O_2 pass the traps. Haunold et al. (1992) use 20-cm U-shaped borosilicate glass tubes with 10 mm outer diameter and 6 mm inner diameter. A 5 cm plug of silanized quartz wool is inserted at the collector outlet to increase sampling efficiency. The air samples are collected with sampling rates between 100-200 ml per min. Under atmospheric conditions usually a volume of 5 l air is sampled in 30 min. The use of liquid argon (- 186°C) instead of cheaper liquid nitrogen (- 196°C) has the advantage that O_2 is just not trapped. Before use the glass collectors have to be conditioned. Each trap is kept under vacuum conditions for a few minutes and flushed with purified nitrogen to remove water vapor and residual air. During the sampling procedure humidity from ambient air is also trapped, which causes dramatic H_2S losses, when humidity is liberated together with H_2S. Haunold et al. (1992) developed a two-step desorption procedure (cold desorption and warm desorption) to retain co-trapped water in the traps when H_2S is liberated (as described below). Hofmann et al. (1992a) use similar cryogenic sampling equipment. But before trapping the gases with liquid argon Hofmann et al. (1992a) remove humidity from air samples in a Nafion dryer. Additionally Hofmann et al. (1992a) let pass the air through a cotton-wadding filter as an oxidant scavenger to avoid especially DMS losses. Atmospheric concen-

[1] GSF Research Center for Environment and Health, Institute for Soil Ecology, Ingolstädter Landstrasse 1, D-86764 Neuherberg, Germany
[2] Zentrum für Umweltforschung, Johann-Wolfgang-Goethe-Universität Frankfurt am Main, Georg-Voigt-Strasse 14, D-60325 Frankfurt am Main, Germany

trations of oxidants, like O_3, show daily patterns. Hofmann et al. (1992a) could show that during an afternoon with high ozone concentrations by sampling without a cotton wadding no DMS was found, whereas in samples collected with a cotton wadding DMS was present.

Gas chromatographic system

Haunold et al. (1992) developed a light-weight (5 kg) portable gas chromatograph, suitable for field operation. It is equipped with a packed column (Carbopack BHT 100, Supelco, Bellefonte, Pennsylvania, USA) and a commercially available flame photometric detector (FPD 84XO/8500, Perkin-Elmer, Norwalk, Connecticut, USA). The specialty of this gas chromatograph is the very small oven in comparison to commercially available GC systems. Temperature control of the analytical column is achieved by Peltier elements, which heat and cool a circular metal block (only 12 cm in diameter and 1 cm in height) containing the chromatographic column. The operating conditions of the column oven range between - 20°C and 120°C, with heating /cooling rates of 30°C min^{-1}. Nitrogen is used as carrier gas.

Analysis of atmospheric samples

After trapping the sulfur gases the cooled sampling tubes are integrated into the carrier gas stream of the GC. As mentioned above Haunold et al. (1992) developed a two-step desorption procedure to retain co-trapped water in the traps. In the first desorption step the sample loop is brought from - 186°C to - 79°C in a bath of dry ice and ethanol (Figure 1). At this temperature, CO_2 and the low boiling sulfur gases H_2S and COS are volatilized completely and transported into a capillary cold trap by the carrier gas (liquid argon, - 186°C) were they are focused again. This so called "cold desorption" step needs 5 min time. After the sampling trap is closed, the focus trap is transferred to warm water (+ 30°C) and the first analytical run starts. This "cold desorption" step is important, because it has to be avoided that H_2S is coming into contact with traces of liquid water. This would cause dramatic H_2S losses. For analyzing DMS and CS_2 a second desorption step in warm water (+ 30°C, "warm desorption") and a second analytical run is necessary to set free these higher boiling sulfur compounds. Hofmann et al. (1992a), who eliminated water before cryogenic collecting with the Nafion drier, are desorbing gases together in one "warm desorption" step.

Calibration, detection limits and sampling efficiency

For calibration Haunold et al. (1992) is using gaseous standards. Permeation sources of the sulfur compounds are commercially available (Vici Metronics, Santa Clara, California, USA). Haunold et al. (1992) constructed a permeation oven, which kept the standards at a constant temperature of 30°C in special glass bottles that are flushed with nitrogen. Between 0.025 and 10 ml of the standard gas samples are injected to the gas chromatograph using gas tight syringes.

Detection limits depend on the sensitivity of the detector and on the collected air volume. The reported detection limit is 10 pg sulfur per sample (Haunold et al., 1992). Usually air volumes of 2 l to 5 l are cryogenic collected. Haunold et al. (1992) and Hofmann et al. (1992a) describe for their similar systems detection limits under 10 pptv depending on the different sulfur compounds.

The collection efficiency of the cryogenic sampling process has been tested by sampling and analyzing gas from dilute calibration gas mixtures (pptv range) with two sampling loops in series. At sampling rates between 100 ml and 200 ml 94 % to 96 % of the reduced sulfur gases were found in the fist trap (Haunold et al., 1992).

Determining fluxes of reduced sulfur gases

Methods to study the trace gas exchange between biosphere and atmosphere developed from diverse scientific disciplines, like atmospheric chemistry, micrometeorology, ecology, botany and more. The different disciplines developed multiple approaches depending on different research topics and considered scales. For determining the fluxes of reduced sulfur gases on small scales between soil, plants and the atmosphere dynamic chambers are used, for studying fluxes on ecological scale micrometeorological methods like the gradient method are applied.

Dynamic chambers

The most frequently used technique is the dynamic chamber technique. This technique is relatively low in cost, simple to operate, and can be used in laboratory (Livingston and Hutchinson, 1995). Dynamic chambers are enclosures for soil, plants or soil and plants, they are flushed with an air stream of a certain flow rates (chamber air is exchanged about once in 10 to 15 min). Often fans are used to support the air mixture inside the chamber. For flux estimations an air sample at chamber inlet and an air sample at chamber outlet is collected simultaneously. Flux is calculated from the concentration difference between inlet and outlet, taking into account flow rate through the chamber and soil or plant surface area.

Not only the construction but also materials used for chambers are very important, because reduced

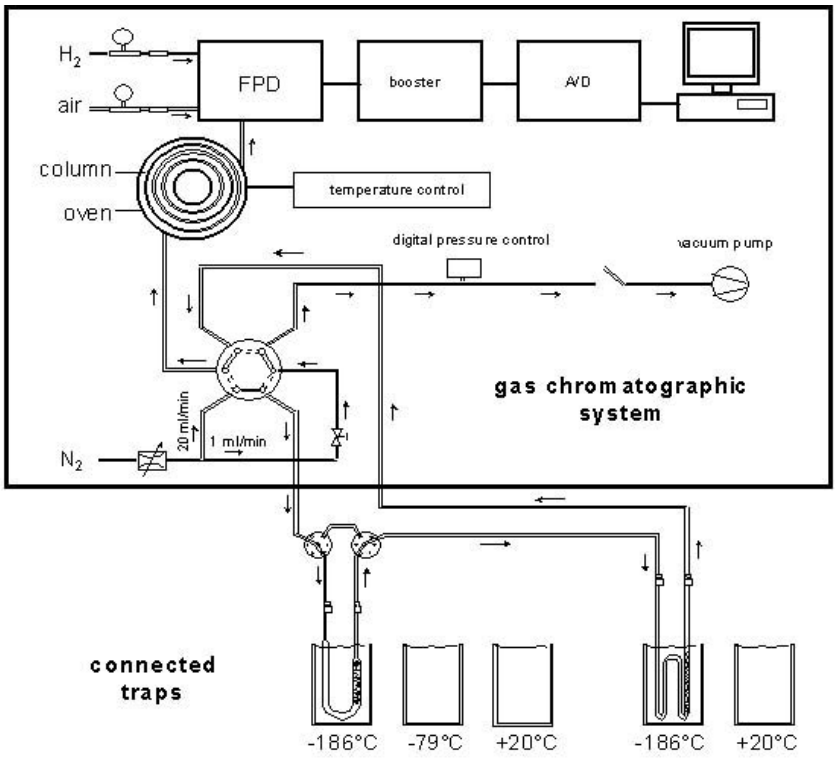

Figure 1:
Gas chromatographic system with connected traps for cold and warm desorption (Haunold et al., 1992; Huber, 1994).

sulfur gases, especially H_2S, are reactive. As a thumb all "smelling" materials should be avoided, as we as all materials that react on surface or are porous. Only inert materials should be used, like Teflon, stainless steel and glass. A further important feature especially for plant chambers is a good light transmittance of the used materials.

Table 1:
Comparison of laboratory and field experiments with spruce trees. In the lab the dynamic chamber was flushed with H_2S-free air from a bottle. In the field experiment the chamber was flushed with ambient air with various H_2S concentrations. <N means concentration under detection limit; positive H_2S flux means emission; negative H_2S flux means deposition and n is number of measurements (Huber, 1994).

Dynamic chamber flushed with:	H_2S concentration chamber inlet (pptv)	H_2S flux (nmol m^{-2} h^{-1})	n
"H_2S free" air (laboratory conditions)	<N	+0.26 to +2.1	53
Ambient air (field conditions)	<N to 228	+4.39 to -23.5	31

Furthermore the quality of air, which is used to flush the dynamic chambers is very important (Table 1). An experiment with spruce trees in the lab where the dynamic chamber was flushed with air, which contained no H_2S showed clearly emission of H_2S, depending to light/dark phases (Rennenberg et al., 1990; Huber, 1994). An experiment with spruce in the Bavarian forest, where the chamber was flushed with ambient air, which contains H_2S in varying concentrations, showed in most cases H_2S deposition (Huber, 1994). When sulfur free air is used to flush dynamic chambers the gradient between plant and atmosphere is artificially high and emissions are to observe, which are not to found when ambient air with varying ambient sulfur gas concentrations is used. More recent budget papers even ignore results of chambers flushed with "sulfur free" air (Watts, 2000).

Micrometeorological methods

Trace gases are both emitted and absorbed by soils and plants. The atmosphere near earth's surface is almost always turbulent, and the trace gases are rapidly diffused to or from the surface. Diffusion by turbulence is many orders of magnitude larger than molecular diffusion (Lenschow, 1995). This turbulent exchange processes can be measured in several

ways. Direct measurement of trace gas fluxes requires fast-response concurrent measurement of vertical air velocity and trace gas species. More sophisticated micrometeorological methods such as eddy correlation await the development of sufficient sensitive and fast sulfur detectors. The most common derived technique is the so-called gradient method (Lenschow, 1995), measuring sulfur gas concentrations parallel in different heights. Additionally a set of micrometeorological data (such as wind direction, wind speed, temperature, barometric pressure) for calculations of vertical fluxes is needed. The measurement equipment is fixed at micrometeorological towers. The lower part of the atmosphere, the so-called atmospheric boundary layer is divided in several sub-layers: a surface layer, a mixed layer and an entrainment zone. The height of atmospheric boundary layer is varying from a few of tens of meters, as it is typically over land at night, to several kilometers when surface is heated by the sun on a clear summer day (Lenschow, 1995). So the choice of measurement heights is of high importance for later gradient interpretation. Also the position of the measuring tower in a field or ecosystem is of great importance. It has to be ensured that the position of measuring tower is representative for the ecosystem, that landscape structures like hills or forests are not disturbing and the main wind direction has been taken into account.

Only few papers cover the measurement of sulfur gases with micrometeorological methods. Hofmann et al. (1992b) determined reduced sulfur compounds over wheat during a growing season in the Danube valley, Bavaria, with the gradient method: The choice of measurement heights was obviously of high importance and gradient interpretations were only possible, if the gradient was measured well above the canopy in the free atmosphere. Bartell et al. (1993) report about micrometeorological measurements of sulfur gas fluxes over a wet meadow close to Garmisch-Partenkirchen, Bavaria. Three micrometeorological methods were compared: eddy correlation, profile method and budget method. Both papers observed a non-monotonous behavior of COS profiles, but also a strong sink for COS over a plant canopy.

The finding that vegetation acts as a COS sink is also confirmed by results from dynamic chamber experiments (Kesselmeier and Merck, 1993). Responsible for COS deposition in plants is the enzyme carbonic anhydrase (Protoschill-Krebs and Kesselmeier, 1991).

Summarizing reduced sulfur gases can be analyzed after cryogenic sampling by gas chromatographic separation and flame photometric detection. Detection limits for these systems are in the range of 10 parts per trillion. Dynamic chambers are mostly used for determination of fluxes between soils, plants and atmosphere on small scale. They are relatively easy to handle and relatively low in cost. Very important are the use of inert materials for chamber construction and the use of air with sulfur gases in ambient concentrations to flush the chambers. For flux estimations on ecosystem scale micrometeorological methods are used. The micrometeorological methods are more costly and need much more experience for selecting measuring site, measuring heights and interpretation.

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The global sulfur cycle and China's contribution to atmospheric sulfur loads

Jürgen Kesselmeier

Abstract

Sulfur is playing a crucial role in biological processes and is exchanged with the environment. Several biogenic volatile and reactive sulfur compounds are released into the atmosphere where they are oxidized and join the fate of the anthropogenically produced sulfur gases. Some compounds may reach the stratosphere. Sulfur emissions from biogenic and anthropogenic sources together account for roughly 100 to 180 TG a^{-1}. However, S-gases, such as carbonyl sulfide (COS), are mainly deposited and consumed by the biosphere. The exchange of sulfur between the biosphere and the atmosphere and the fate within the troposphere/stratosphere are summarized for oceans and terrestrial surfaces. Regarding the role of sulfur within the atmosphere, Chinese emissions are shortly discussed in view of the current declining anthropogenic release of SO_2 in China.

Keywords: sulfur, biosphere, atmosphere, ocean, land surface, hydrogen sulfide, methyl mercaptan, carbonyl sulfide, carbon disulfide, dimethyl sulfide, dimethyl disulfide, sulfur dioxide, aerosol, clouds

Introduction

Sulfur as an essential nutrient for living organisms can be found everywhere in our environment. Sources and role of anthropogenic sulfur gases contributing to atmospheric pollution are well described in the literature (Lefohn et al., 1999). The negative effects on lakes and forest ecosystems as well as on humans have caused immense efforts to cut down the release of sulfur form anthropogenic sources, mainly combustion processes. Air pollution prevention within the last decades resulted in a significant decrease of SO_2 emission and thus S deposition in industrialized countries from 100 to 10 kg ha^{-1} a^{-1}). This decrease led to a significant recover of natural ecosystems, but caused a substantial loss of sulfur for agriculture. Especially cruciferous plants with a high sulfur demand reacted with substantial profit cuts. Hence, the sulfur deficiency in cultivated plants had to be compensated by increased sulfur fertilization. Furthermore, we have to keep in mind that sulfur is not only contributing to air pollution (acid rain) and nutrient availability. Sulfur compounds play a crucial role in the atmosphere (Andreae and Crutzen, 1997, Charlson et al., 1992; Chin and Davis, 1995; Kesselmeier et al., 1997) and have substantial biogenic sources in addition to the anthropogenic ones. An overview about sources and estimated emission ranges according to Andreae and Jaeschke (1992) is given in Figure 1. By oxidation to sulfate sulfur compounds are involved in aerosol particle and cloud production. This way they contribute to the regulation of the radiative budget of the earth. According to the latest IPCC report (2001), the direct radiative forcing of sulfate particles contributes substantially to a cooling of the earth. Estimates of the indirect effect, i.e. cloud production and its role in absorbing and reflecting radiation, are highly uncertain but may even be of higher importance.

Sulfur exchange over oceans and continents

For a sufficient understanding of the sulfur cycle, a more detailed picture and a closer look into sulfur speciation is needed. We may discern several sulfur compounds being emitted from different sources. Anthropogenic sources mainly emit sulfur dioxide which is oxidized to sulfate. Biogenic sources emit substantial amounts of other S species, such as hydrogen sulfide (H_2S), methyl mercaptan (CH_3SH), carbonyl sulfide (OCS, often called COS), carbon disulfide (CS_2), dimethyl sulfide (CH_3SCH_3, DMS) and dimethyl disulfide (CH_3SSCH_3, DMDS). These compounds are summarized as reduced volatile sulfur compounds. Regarding the distribution of anthropogenic and biogenic sources an interesting gradient is found between the two hemispheres (Bates et al., 1992) as shown in Figure 2. Anthropogenic sources are significantly higher in Northern latitude whereas the southern hemisphere is better described by biogenic sources, a feature which can be attributed to the larger marine areas in the southern hemisphere.

Table 1 summarizes the atmospheric lifetimes of several sulfur species. As shown, the S species may be sorted into two groups, the first containing the reactive compounds with lifetimes in the range of hours and days, and the second group containing only COS with a lifetime of years, though 25 years is at the upper edge of all estimates. Of special interest are DMS and COS. Besides sulfate containing sea spray, marine DMS is the main component of global sulfur emission, whereas COS is the most stable compound in the atmosphere and

[1] Max Planck Institute for Chemistry, Biogeochemistry Department, Joh.-J.-Becher-Weg 27, 55128 Mainz, Germany

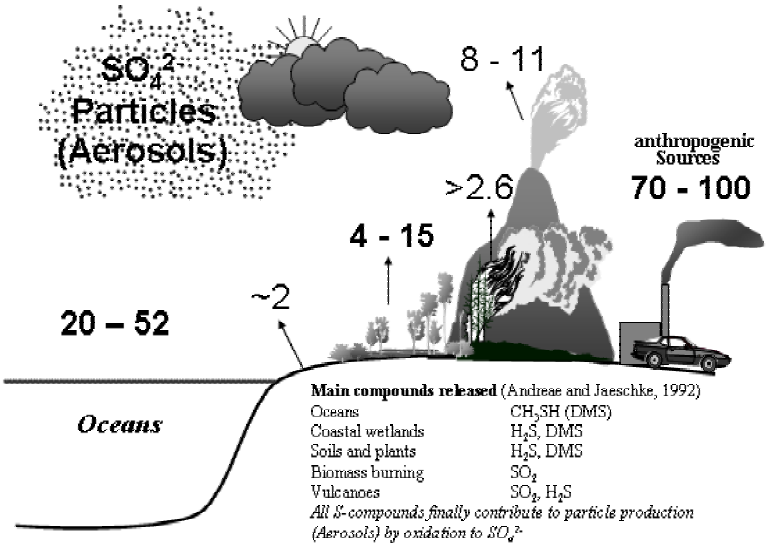

Figure 1:
Estimated ranges of global emissions of volatile sulfur compounds (Tg a^{-1}) according to Andreae and Jaeschke (1992).

is taken up by vegetation and soils, the two compartments representing the dominant sinks for this sulfur species.

Based on the atmospheric lifetimes we may discuss the cycling and the role of these compounds over the marine and terrestrial environments as shown in the Figures 3 and 4. The oceans are the dominant source of biogenic volatile sulfur compounds. DMS is the most important sulfur species released by abiotic cleavage of dimethyl sulfoproprionate (DMSP), which is produced by several algae and released into the seawater upon cell destruction (Malin and Kirst, 1997). COS, the second important marine sulfur species is produced by photochemical degradation processes of organo-sulfur compounds (Ferek and Andreae, 1984). The other sulfur species are of minor importance in terms of marine emissions. COS with its long tropospheric lifetime may be transported into the stratosphere where it underlies photochemical photolysis and oxidation delivering sulfate particles as nutrients for the stratospheric sulfate layer (Junge-Layer) around our globe. DMS and other reactive sulfur trace gases enter oxidation processes in the troposphere producing sulfate particles, which contribute to particle production, cloud condensation nuclei (CCN) and cloud production. Both, particles as well as clouds influence the radiation budget as indicated above. In case of the marine DMS source, the so-called CLAW hypothesis (Charlson Lovelock, Andreae and Warren, 1987) caused intensive discussions during the last decade. According to this hypothesis, DMS emission from algae controls a feedback mechanism with a coupling between DMS release, cloud albedo, radiation budget, temperature algal growth and DMS release. Over 700 papers have been published dealing with this subject, which seems to be neither proved nor weakened.

Table 1:
Tropospheric lifetimes of tropospheric sulfur gases according to Warneck (2000)

DMS	0.1 days
CH_3SH	0.4 days
DMDS	2.2 days
H_2S	3 days
CS_2	7.2 days
SO_2	1-40 days
COS	25 years

The sulfur cycle above terrestrial surfaces exhibits the same principal processes and mechanisms as described for the marine site. Several reduced sulfur species are produced within the soil and released into the atmosphere, where they underlie the same fate as found over the oceans. However, there are some special terrestrial features. Soils and terrestrial vegetation are dominant sinks for COS (Chin and Davis, 1993; Kesselmeier and Merk, 1993; Kesselmeier et al., 1999; Kuhn and Kesselmeier, 2000; Kettle et al., 2002). This uptake is quite well understood and is mainly based on the activity of an enzyme, the carbonic anhydrase, which is found in all biological organisms (Protoschill-Krebs et al., 1992 & 1995 & 1996). The enzymatic process could recently be modeled by Schenk et al. (2004). In addition to its COS sink quality, terrestrial vegetation also emits sulfur compounds into the atmosphere. Reports on the emissions of all reduced species can be found in the literature. Of special

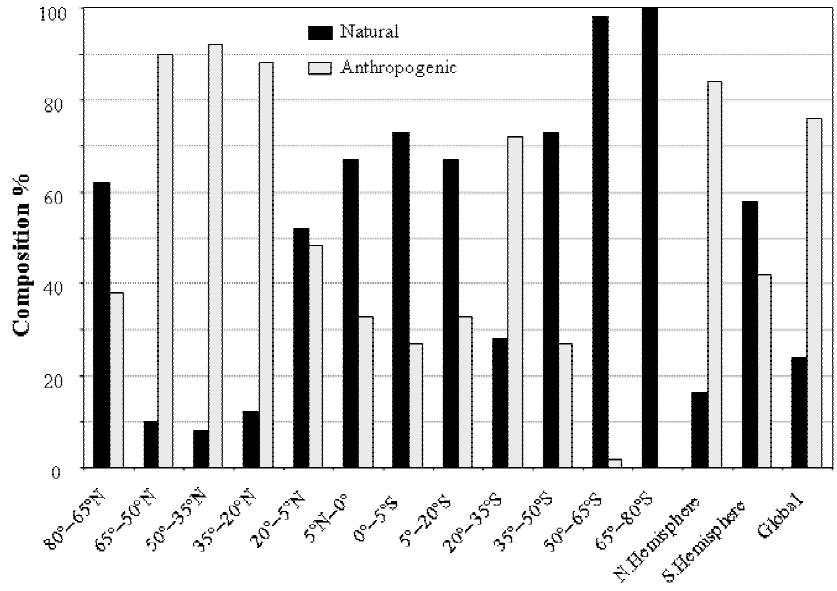

Figure 2:
Distribution of global sulfur emission sources between the hemispheres according to Bates et al. (1992). Note the decrease of anthropogenic sources towards the southern hemisphere.

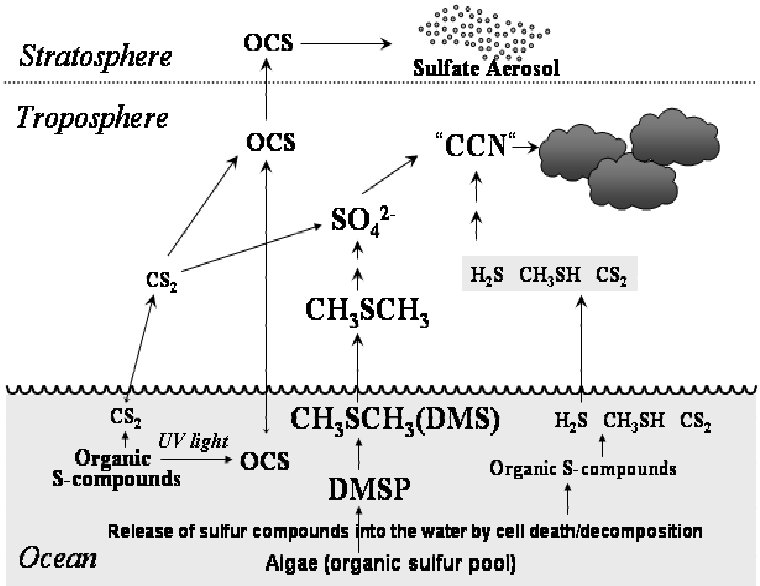

Figure 3:
Sulfur cycle within and above the ocean.

interest is the release of DMS by higher plants, among them tropical rain forest trees (Andreae and Jaeschke, 1993; Kesselmeier et al., 1993). In contrast to the DMS production in the oceans this DMS release is based on biological degradation and can also be found in case of decomposing leaf litter

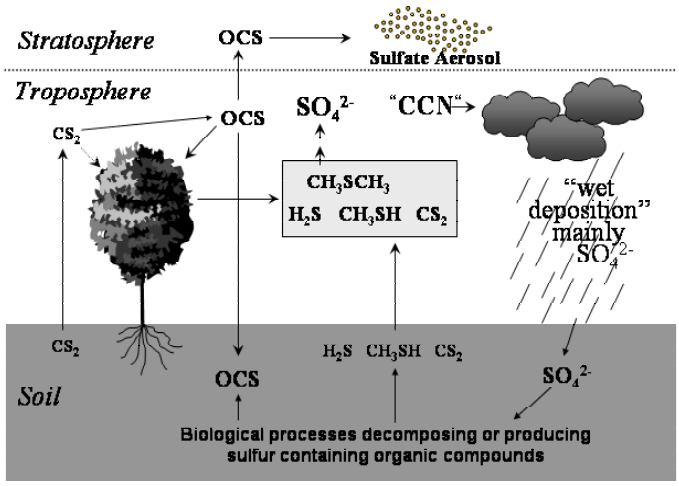

Figure 4:
Sulfur cycle within and above terrestrial surfaces.

(Kesselmeier and Hubert, 2002). This DMS release from terrestrial sources with its potential impact on atmospheric chemistry and physics needs further investigation.

All above indicated processes enclosing sources and sinks are summarized in budget estimates as shown in Table 2. Though these numbers seem to show a reasonable balance of sources and sinks, it has to be noted that great uncertainties exist for H_2S and CS_2. Furthermore, COS deposition needs further investigations, especially in case of the consumption by different soil types. DMS emission by trees and forest urgently needs more investigations for a global extrapolation. Other poorly understood ecotypes are fresh water wetlands where data are sparse.

Table 2:
Balance of sources and sinks, biological as well as chemical, for reduced sulfur compounds according to Watts (2000)

	SOURCE	SINKS
COS	1.31 ± 0.25	1.66 ± 0.79
*H_2S	7.72 ± 1.25	8.50 ± 2.80
*CS_2	0.66 ± 0.19	1.01 ± 0.45
DMS	24.45 ± 5.30	no estimate

*Note great uncertainties for H_2S and CS_2

Atmospheric concentrations of sulfur compounds in China

Reduced sulfur compounds are also of anthropogenic origin. Yujing et al. (2002) measured vertical distribution profiles of COS at three levels on the meteorological tower of the Institute of Atmospheric Physics, Chinese Academy of Sciences in Beijing during 23–24 November 2001. The authors found roughly 600 to 1700 ppt at the 8 m level, 400 to 1500 ppt at 160 m and 400-1300 ppt at 300 m. Within these data sets they observed clear concentration gradients with highest concentrations at the lowest level, clearly indicating COS sources at the ground. Furthermore, in early November they observed fluctuations between 1000 and 7000 ppt for COS, 100-1200 for CS_2 and 100-500 for H_2S. Such high concentrations at the surface point to anthropogenic sources, mainly traffic. It is remarkable that these values for reduced sulfur compounds were comparable to concentrations of SO_2 in polluted areas. Figure 5 gives an overview of SO_2 atmospheric concentrations in several cities in China for 1990-1995 compared with some other polluted areas in the world. For orientation, the World Health Organization (WHO) annual mean guidelines for air quality standards are 50 micrograms per cubic meter for sulfur dioxide. Though theses atmospheric data can be highly sensitive to local conditions they may be considered a general indication of air quality. As a result of several emission reductions since 1987 and the almost complete shut down of old industrial installations in the eastern part of Germany after the reunion in 1989, Germany was able to reduce the emission of SO_2 during the last two decades by 90 % down to values of a few µg m^{-3} (see also Wallasch 2003).

The above reported data show that the anthropogenic sulfur load in China is high. The Special Report on Emission Scenarios (SRES, IPCC

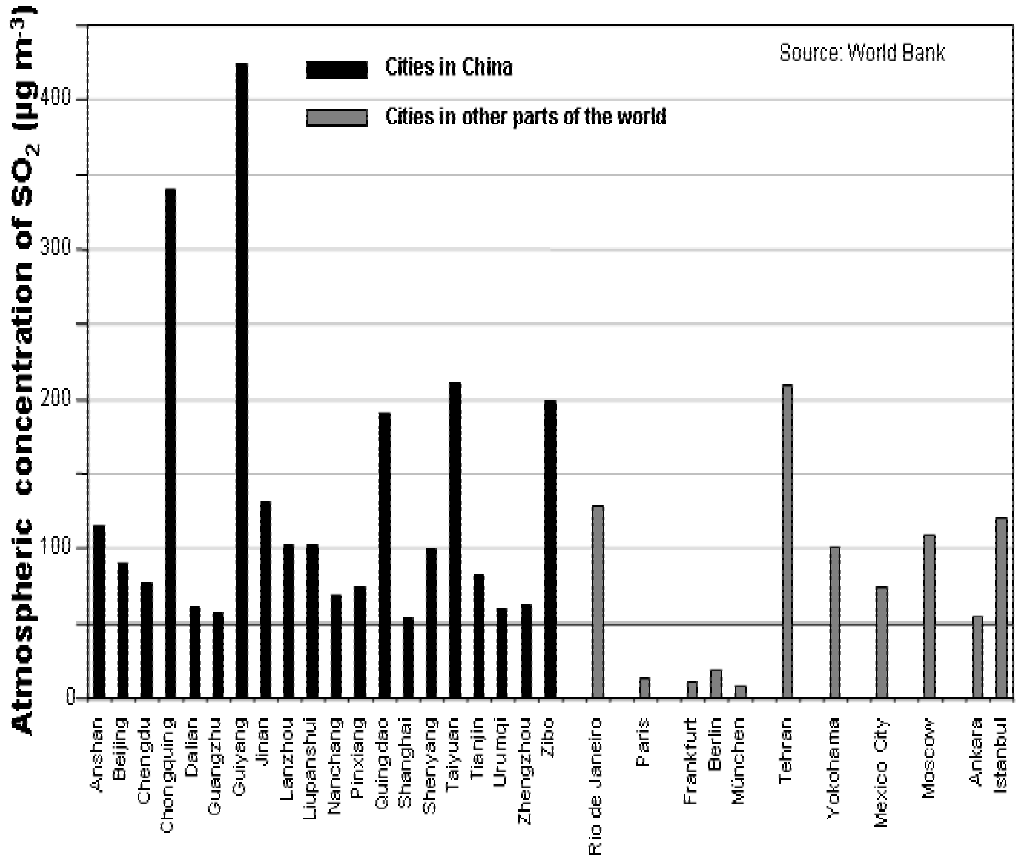

Figure 5:
Cities with reported levels of atmospheric pollutants in relation to WHO guidelines (bold line, 50 µg m^{-3}) in 1990-1995.

2001) estimated an increase of annual SO_2 emissions in Asia from 50-70 Tg a^{-1} (1990 data) to 80-110 Tg a^{-1} by 2020. However, recently published data show that this trend to increasing values obviously has been stopped (Carmichael et al., 2002). In contrast to the predictions of the IPCC report (2001) the emission load was constantly decreasing from the year 1995 to 2000 (Streets et al., 2000a, b) and the authors estimate a decrease to lower values of 40-45 Tg a^{-1} by 2020. The change of the trend is clearly caused by a decline of SO_2 emissions from 1995 to 2000 in China (2/3 of Asian SO_2!) due to a reduction in industrial coal use, slow-down of the Chinese economy and a closure of small and inefficient power plants. This relationship is highly significant, as atmospheric SO_2 pollution is nearly exclusively caused by coal burning power plants, as very recently observed during the "2003 North American electrical blackout" (Marufu et al., 2004).

Conclusions

Current anthropogenic release of SO_2 in China is declining. If this process continues, there will be huge health benefits for the society. However, it has to be accepted that the sulfur demand for agricultural purposes will grow and, consequently, the role of natural sources and cycles need to be better understood. Furthermore, as a consequence of the decrease of direct and indirect cooling effects

due to the decrease of sulfate aerosol particles, we may observe an increase of the global warming. This effect underlines the general necessity to recognize other air pollution processes and to fight a further increase of radiatively active gases.

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Sulfur-rich proteins and their agrobiotechnological potential for resistance to plant pathogens

Cordula Kruse[1], Ricarda Jost[2], Helke Hillebrand[3] and Rüdiger Hell[1]

Abstract

Thionins, defensins and a number of other related polypeptides form the group of small, sulfur-rich defense proteins. They are processed from larger preproteins and mostly localized in walls of epidermal cells of seeds and leaves. *In vitro* they display antimicrobial activity especially against fungi. Their genes can be constitutively expressed or induced by fungal pathogens, thereby supporting the host's defense against biotic stress. The effectiveness of thionins and defensins against phytopathogenic fungi has been demonstrated by overexpression in transgenic plants. The observed enhanced resistance against a number of agriculturally important pathogens such as *Alternaria ssp.* and *Fusarium ssp.* has prompted research on the biology of sulfur rich defense proteins and attempts to improve the resistance of crop plants.

Key words: glucosinolates, phytoalexins, thionins, pathogens, defensins, sulfur-rich proteins

Sulfur-containing defense compounds

Sulfur-containing compounds and their metabolism are well connected to plant stress resistance. Hardly any other element serves in so many different stress-related functions such as resistance against heat, cold, drought, flooding, heavy metals, organic xenobiotics and reactive oxygen species (Rennenberg and Brunold, 1994). The role of sulfur in biotic stress resistance is less investigated, but there is strong evidence that innate defense mechanisms against plant pathogens are based on sulfur compounds in several important cases. Whether sulfur nutrition and the sulfur status of a plant affect its ability to form protective sulfur compounds is currently under investigation. Secondary sulfur compounds that often are limited to special plant families are mostly involved in herbivore resistance. Prominent examples are the glucosinolates of the Brassicacea and alliins of the Liliaceae. Their pungent taste deterres feeding insects and other animals upon destruction of plant tissues, thereby releasing the stored compounds and the corresponding degrading enzymes, myrosinase and alliinase. In the end the breakdown products isothiocyanate and allicin exhibit toxicity to the enemy. Most of these compounds are preformed and stored until the plant is attacked. Only the indole glucosinolates appear to be inducible by defense pathways like the jasmonate signal transduction pathway (Bodnaryk, 1994).

Another inducible defense compound containing reduced sulfur is the phytoalexin camalexin that is only produced upon fungal and bacterial infection in Brassicaceaen plants. It is derived from indole-3-acetaldoxime and carries a thiazole ring. Its synthesis is triggered by the jasmonate and salicylate pathways. Camalexin shows toxicity towards both kinds of pathogens (Tsuji et al., 1992). A surprising discovery was the presence of elemental sulfur in plants (Cooper et al., 1996). The redox biochemistry and synthesis of S^0 in living plant tissue is still unclear, however, recent investigations provided evidence for the widespread occurrence of elemental sulfur as well as its function in defense against fungal and bacterial pathogens (Williams and Cooper, 2003). S^0 may exist preformed but can also be induced upon infection and then accumulates in vascular tissue, presumably to prevent the spread of infections along the plant transport routes. It has thus been suggested that plants since long possess 'man's oldest fungicide' (Williams and Cooper, 2004).

One of the best investigated sulfur-containing defense compounds in plants to date are sulfur-rich proteins. They can be grouped into several classes, including thionins, defensins, lipid-transfer proteins, snakins and others, according to their primary amino acid sequences and distribution (Garcia-Olmedo et al., 1998). They all share a relatively small size of 4 to 11 kDa, mostly polar amino acid composition, several disulfide bridges (2 to 6) and, as a consequence, a rather compact tertiary structure. They also share the notion that relatively little is known about their precise physiological functions and mechanisms of action. It can not be excluded that sulfur-rich proteins carry out a number of different functions *in vivo* in addition to defense (Florack and Stiekema, 1994). Among these protein families the thionins and defensins appear to be most important for plant defense against pathogens. They have been

[1] Heidelberg Institute of Plant Sciences (HIP), University of Heidelberg, 69120 Heidelberg, Germany
[2] Research School of Biological Sciences, The Australian National University, Canberra ACT 0200, Australia
[3] BASF Plant Science GmbH, Technology Management, 67117, Limburgerhof, Germany

intensively investigated with respect to signal transduction pathways and agrobiotechnology as judged from numerous patent applications (see patent homepages: http://www.uspto.gov/patft/index.html; http://ep.espacenet.com/espacenet/ep/en/e_net.htm).

Many thionins, thionin-like proteins and defensins have been isolated from seed and leaf material of a huge variety of different plants, including but not limited to members of the Brassicacea, Compositae and Leguminosae families. Within these groups *Raphanus*, *Brassica*, *Sinapis*, *Arabidopsis*, *Dahlia*, *Cnicus*, *Lathyrus* and *Clitoria* are prominent examples (Patent numbers: US 5.689.043; US 5.689.048; US 5.824.869; US 6.187.904; US6.605.698). Further proteins have been isolated and characterized from *Amaranthus*, *Capsicum*, *Briza* and related monocot and dicot species (US 5.691.199; US 6.521.590; US 20030096985) as well as from *Heuchera* and *Aesculus* (US 5.750.504), *Allium* (US 5.773.694) and *Impatiens* (US 6.150.588). The proteins described showed a wide range antifungal activity and some were also active against Gram-positive bacteria, yeasts, insects or nematodes. Antifungal activity was mostly measured by using *Fusarium culmorum* strain IMI 180420 as a test organism for *in vitro* bioassays (Broekaert et al., 1990). A great variety of suitable test strains used to assess the biocidal properties of such proteins are listed in patents US 5.942.663, US 5.919.018 and US 5.986.176. Efficient thionin or defensin genes can potentially form valuable traits in crop plants, either by using transgenic overexpression or by marker-assisted introgression into elite lines. This article will therefore focus on properties of thionins and defensins that are relevant for plant protection and summarize the approaches to improve plant resistance making use of these sulfur-rich proteins. More detailed overviews about biological aspects are available for thionins (Bohlmann and Apel, 1991; Garcia-Olmedo et al., 1998) and more recently for plant defensins (Thomma et al., 2002).

Genomic organization of gene families, expression and structure of the thionin and defensin proteins

By definition thionins and defensins belong to a group of polypeptides with 10% to 20% cysteine residues that exhibit toxic activity towards cells of bacteria, fungi and mammals. Thionins were discovered first as abundant component of wheat flour (cited in Apel et al., 1990). Their molecular organization was finally elucidated in context with the detection of leaf-specific thionins (Bohlmann and Apel, 1987). In earlier studies thionins were grouped into several classes, of which the gamma thionin class later turned out to be structurally related to mammalian and insect defensins and was thus renamed plant defensins. Their primary as well as three-dimensional structure is more conserved within this group as compared to other thionin classes. Remarkably, these proteins are not only ubiquitously throughout the plant kingdom but also widespread in other organisms, including mammals, insects and molluscs (Thomma et al., 2002). Plant defensins are 45 to 54 amino acids in length, carry a positive net charge at physiological pH and have 8 cysteine residues that form four disulfide bridges. The three-dimensional structure of several plant defensins has been determined. They all consist of a triple-stranded β-sheet and an α-helix in parallel orientation. This organization is largely conserved in defensins from other organisms and belongs to the superfamily of cysteine-stabilized α-helix / β-sheet proteins (Thomma et al., 2002). The overall structure is amphipathic, quite compact and stable (Almeida et al., 2002). Plant defensins (PDFs) are encoded by gene families of different sizes. *Arabidopsis thaliana* as the best characterized plant at the genetic level contains 13 defensin genes and 2 defensin-like genes. Earlier studies (Penninckx et al., 1996; Epple et al., 1997a) divided the defensins into two subgroups, PDF1 and PDF2. The first group contains seven defensins, of which five are very similar at the nucleotide level and identical at the amino acid level (PDF1.1, 1.2a, 1.2b, 1.2c, 1.3; Thomma et al., 2002), suggesting very recent genomic duplication events. The situation in crop plants is much less investigated, but EST databases for rape, rice and barley indicate the presence of gene families. Expression analysis of the defensin gene family in Arabidopsis revealed differential expression patterns. Most genes are expressed constitutively in one or more organs. Specifically, PDF1.1 is expressed in seeds and siliques, PDF2.1 in seeds, siliques and roots, PDF2.2 in all organs except seeds and stems, and PDF2.3 is present in all organs except roots. In addition, pathogen infection induces PDF1.2 in several developmental stages via the jasmonate and ethylene signaling pathways (Thomma and Broekaert, 1998; da Silva Conceicao and Broekaert, 1999). Mutants with defects in these pathways are susceptible to *Botrytis cinerea* due to the lack of expression of inducible defensins (Thomma et al., 1998). This finding strongly underlines the efficiency of these sulfur-rich proteins for pathogen defense. Furthermore, 11 of the Arabidopsis defensin genes carry a predicted signal peptide for secretion into the apoplast, hence are localized to the primary infection sites. In contrast, PDF1.4 and PDF2.4 appear to contain no signal sequence and may stay in the cytoplasm with so far unknown functions.

Thionins occur exclusively in the plant kingdom but are still much less conserved among each other

than the defensins. The size of the mature polypeptide chains is also 45 to 55 amino acids, but primary sequences show less homology. Signatures of the cysteine residues are highly conserved, although the number of disulfide bridges varies between 3 and 4 in thionins from evolutionary distant species. Three-dimensional structures have been determined for several thionins and revealed a conserved L-shaped structure formed by two parallel α-helices (long arm of L) and two β-sheets (short arm of the L; Bohlmann and Apel, 1991; Garcia-Olmedo et al., 1998). The overall structure is again amphipathic, but somewhat less compact and heat stable compared to the defensins. All of the Arabidopsis thionins and so far most of the thionins from other plant species possess amino-terminal domains with signatures for transport via the endoplasmatic reticulum to the apoplast. In addition, thionin presequences reveal a carboxy-terminal domain that is highly acidic. Interestingly, this domain harbors six cysteine residues and is also strongly conserved between thionins of different species, suggesting a conserved and essential function. It was assumed that the acidic residues could neutralize the basic amino acid residues of the central thionin domain in the pre-proprotein, but the precise function is unknown. N- and C-terminal domains are post-translationally processed, leaving mature thionins with a size of approximately 5 kDa (Apel et al., 1990). According to the Arabidopsis Sequence Initiative *Arabidopsis thaliana* contains 4 thionin-coding genes grouped into two subfamilies. Of these only two genes have been characterized (Epple et al., 1995), whereas genomic analyses suggested 50-100 copies in the barley genome (Bohlmann et al., 1988). Expression analysis showed that *THI2.1* is inducible by pathogens, wounding and chemicals via the jasmonate pathway, while *THI2.2* is constitutively expressed (Epple et al., 1995; Bohlmann et al., 1998). Knock-out mutants of thionins have not been reported, but constitutive overexpression of THI2.1 leads to enhanced resistance of Arabidopsis to *Fusarium oxysporum* infection (Epple et al., 1997b), pointing to the importance of thionins for pathogen defense. The inducibility of thionins and defensins helps to save valuable resources in the absence of pathogens. It is interesting to speculate whether reduced sulfur is available in sufficient amounts under less than optimal sulfur supply, thereby reducing the defense potential of an attacked plant.

Localization and mechanism of toxicity

Thionins and defensins were originally discovered as protein components of seeds: thionins were found in barley endosperm and defensins in the seed coat of radish (Florack and Stiekema, 1994; Thomma et al., 2002). It appears that especially the germinating seedling requires antimicrobial activities as protection against pathogens during this critical developmental stage. Later on both sulfur-rich protein types were found in mature leaves as well. In all cases these proteins were excreted into the cell wall, in many cases preferentially within the surface cell layers of the plant organ. This localization makes sense since the apoplast is the primary site of contact by a pathogen and would allow immediate interaction. Indeed, accumulation of apparently inducible leaf cell-wall thionins has been observed around the infection sites in case of barley and powdery mildew interaction (Ebrahim-Nesbat et al., 1989; Apel et al., 1990). As already mentioned the intracellular targeting is carried out by signal sequences. These may also be responsible for the occasionally observed vacuolar localization of some barley thionins (Reimann-Philipp et al., 1989) and missing as already mentioned from two of the Arabidopsis defensins with putatively cytosolic localization (Thomma et al., 2002).

The mechanism of toxicity of sulfur-rich proteins has long been debated, involving speculation about the role of the highly conserved amphipathic and compact structure provided by the disulfide bridges (Florack and Stiekema, 1994; Garcia-Olmedo et al., 1998). However, in several cases structurally closely related sulfur-rich proteins generated contrasting results in antimicrobial activity tests *in vitro* and *in vivo*, leaving the actual toxicity determinants in the respective proteins unclear (Thomma et al., 2002). Recently electrophysiological measurements using a β-purothionin from wheat flour revealed a possible general mechanism of toxicity based on *in vitro* assays with artificial lipid bilayer membranes and mammalian cell lines (Hughes et al., 2000). The authors observed the formation of cation-selective ion channels upon interaction of purothionin with plasmalemma components and concluded that this effect causes the dissipation of ion concentration gradients that are essential for cellular function. However, these assays were not carried out with authentic pathogenic fungi.

Membranes of the model fungi *Neurospora crassa* and *Saccharomyces cerevisiae* were shown to be permeabilized by defensins at low concentrations. Defensins from radish and *Dahlia merckii* were applied and their effect monitored using uptake of a fluorescent dye into fungal cells as reference (Thevissen et al., 1999). The authors suggest direct peptide-phospholipid interactions that can be suppressed by cations in the medium. It is concluded that cations alter the conformation of the binding site and that successful permeabilization is linked to the fungal growth inhibition. The mechanism of toxicity of defensins thus seems to be different from

that of thionins. The drawback of this and other studies (Thevissen et al., 1996; da Silva Conceicao and Broekaert, 1999) again consists in the lack of information on the reaction of membranes of phytopathogenic fungi.

Biotechnology approaches to enhance resistance in crop plants

Breeding and plant transformation both aim at the transfer of genes or effective alleles that confer improved resistance to economically relevant crop genotypes. One approach to this end is the identification and transfer of key components of resistance responses. Examples are the *R*-genes as specific receptors for the recognition of pathogens. Broad-spectrum disease resistance may be expected from overexpression of the *NPR1* and *PAD4* genes that seem to mediate responses for the salicylate signaling pathway (Rommens and Kishore, 2000). A second approach employs enhanced expression of downstream responses such as thionins and defensins. Some of these proteins show a direct and broad spectrum of antifungal activities *in vitro*. Ideally this property would copy those of insecticidal proteins from *Bacillus thuringiensis* showing toxicity against pathogenic fungi but being harmless against animal and human cells. A number of experiments have attempted to enhance pathogen resistance by overexpression of sulfur-rich proteins in plant models or crops. A list of successful approaches is given in Tab. 1. However, it should not be overlooked that, despite strong antimicrobial activities of the respective proteins in *in vitro* bioassays, similar experiments have also failed to confer resistance for mostly unknown reasons (De Bolle et al., 1996; citations in Florack and Stiekema, 1994; Broekaert et al., 1995; Epple et al., 1997). The earliest published example of transgenic expression of a sulfur-rich protein refers to an α-thionin from barley (Carmona et al., 1993). Expression in tobacco was driven by the constitutive Cauliflower Mosaic Virus 35S promoter and could be demonstrated by the presence of the α-thionin in tobacco protein extracts. Increased resistance against two pathovars of *Pseudomonas syringae* was observed, that clearly correlated with the amount of α-thionin present in the different transgenic tobacco lines.

The first overexpression of a plant defensin was carried out using a similar construct of 35S promoter and the antifungal protein 2 (AFP2) from radish (*Raphanus sativus*) and tobacco as heterologous host (Terras et al., 1995). The AFP2 protein was shown to have antifungal activity against *Alternaria brassicicola*, *Botrytis cinerea* and *Fusarium culmorum in vitro*. Transgenic T2 lines of tobacco were tested for disease resistance using a leaf lesion test and displayed 7- to 8-fold less lesions compared to control wild type and azygous plants upon infection with *Alternaria longipes*. The degree of resistance in the transgenic lines correlated closely with the protein level of AFP2, unequivocally demonstrating the function and suitability of plant defensins for fungal resistance.

A most promising demonstration of the suitability of defensin expression is the transformation of rice by *Agrobacterium tumefaciens* with a construct consisting of the 35S promoter and the Wasabi defensin from Japanese Radish (*Wasabia japonica;* Kanzaki et al., 2002). The Wasabi defensin was especially selected for its toxicity against rice blast disease, a worldwide fungal pathogen which causes severe damage and reduced yield. The Wasabi protein was present in transgenic rice lines, with the best lines reaching resistance levels comparable to a rice cultivar carrying the true blast resistance gene in leaf lesion tests. The resistance was stable over several generations, suggesting a durable and wide-spectrum resistance against various rice blast races in the field.

Despite several unsuccessful (and often unpublished) attempts these positive results have spurred the transformation of barley and wheat with antimicrobial proteins (Dahleen et al., 2001). Fusarium head blight (*Fusarium graminearum*) is one of the most devastating diseases for wheat, durum and barley. Only a limited number of genotypes of wheat and barley with only partial resistance have been found. The resistance trait that has been isolated apparently is under the control of multiple genes and functions independently of the gene-for-gene interactions that provide resistance against barley and wheat pathogens like powdery mildew (*Blumeria graminis*) and stem rust (*Puccinia graminis*). Fusarium head blight resistance is therefore a challenge for breeders, making insertion of individual genes into cereals an attractive alternative, although transformation of these recalcitrant species is still ineffective and cost intensive. Several approaches using barley and wheat thionins are under way, supported by the US Department of Agriculture (Dahleen et al., 2001, and references therein). These approaches are further complicated by the requirement of strong spike-specific promoters for the expression of thionins at the preferred infection site of *Fusarium culmorum* and *F. graminearum* that still need to be isolated.

An enhanced approach to use antifungal proteins against fungal pathogens is represented by fusions that consist of a defensin and a single chain antibody (Peschen et al., 2004). The single chain antibody was isolated by phage display and selected for surface determinants of *Fusarium ssp*.. A translational fusion of radish AFP2 and antibody CWP2 was expressed in Arabidopsis and yielded strongly en-

Table 1:
Reported successful approaches to express sulfur-rich proteins in transgenic host plants to enhance resistance against phytopathogenic fungi and bacteria.

Protein	Source	Plant transformed	Resistance tested	Reference
α-Thionin	Barley	Tobacco	*Pseudomonas syringae*	Carmona et al., 1993
RsAFP2 defensin	Radish	Tobacco	*Alternaria longipes*	Terras et al., 1995
Thi2.1 thionin	Arabidopsis	Arabidopsis	*Fusarium oxysporum*	Epple et al., 1997
Viscotoxin A3	Mistletoe	Arabidopsis	*Plasmodiophora brassicae*	Holtorf et al., 1998
Leaf thionin	Oat	Rice Tobacco	*Xanthomonas campestris* *Pseudomonas plantari* *Phytophtora infestans*	Ohashi et al., 2001
Wasabi defensin	Japanese radish	Rice	*Magnaporthe grisea* (blast fungus)	Kanzagi et al., 2002
α-Thionin, seed hordothionin	Barley, wheat	Wheat and barley	*Fusarium graminearum*	Dahleen et al., 2001
RsAFP2 + antibody	Radish	Arabidopsis	*Fusarium oxysporum*	Peschen et al., 2004

hanced resistance against *Fusarium oxysporum*. Interestingly, expression of the CWP2 antibody alone already increased resistance, pointing to a potentially new avenue of antifungal strategies. AFP2 expression alone also was effective, but the assumed targeting of the AFP2-CWP2 fusion protein to the invader and presumed concentration of the sulfur-rich protein at the infection site had an additive effect on resistance. The specificity of this recognition was demonstrated by the lack of resistance against the fungal pathogen *Sclerotinia sclerotinum,* which is not recognized by the CWP2 antibody.

Such experiments provide proof-of-function of the suitability and effectiveness of sulfur-rich proteins as targets of plant defense. Of course a number of constraints have to be overcome for successful application in biotechnology. One is the lack of knowledge about the molecular determinants of toxicity on both the sulfur-rich protein side as well as the fungal membrane side. If this problem was solved protein engineering would allow to screen for active proteins with broad specificity against fungi and possibly bacteria but reduced toxicity against mammalian cells. At this point thionins and defensins from natural sources can be expressed as recombinant proteins or isolated and selected for toxicity against microbes in bioassays. Another limitation is the small number of suitable promoters for targeted expression. The presence of thionins and defensins may be undesirable in edible parts of the plant or in the absence of corresponding pathogens. On the other hand the prominent infection sites such as germinating seeds and shooting spikes required localized, fast and strong presence of the defense proteins. Genomic and bioinformatic approaches are under way to identify expression patterns of interest to use the underlying promoters to drive defense gene expression in transgenic plants. Finally, most research is still carried out with model species for good reasons. Lack of genomic and expression information together with elaborate and inefficient transformation protocols still hamper progress with crop plants, but at the end of the day this is where the sulfur-rich defense proteins are required. Classical selection of resistant genotypes using sulfur-rich proteins as a target supported by marker-assisted breeding could be an alternative approach. However, the above listed requirements of biotechnology and consumer safety make this approach difficult. It will be very interesting to see how the wealth of knowledge on sulfur-rich defense proteins will be used in the future to improve crop resistance against important fungal pathogens

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Crop response to sulfur fertilizers and soil sulfur status in some provinces of China

Shutian Li[1], Bao Lin[1] and Wei Zhou[1]

Abstract

Field trials and demonstrations were conducted to investigate sulfur fertilizer effects on crops in some provinces of China, and soil sulfur status in Heilongjiang, Henan, Shaanxi and Jiangxi provinces was also evaluated. Results showed that sulfur application could increase crop yield by 6.9%, 6.8% 9.4%, 11.8% and 8.1% on average, respectively, for corn, wheat, rice, soybean, and oilseed rape. The effect of ammonium sulfate or potassium sulfate on crop yield was better than gypsum or elemental sulfur. The rational application rate for elemental sulfur and sulfate sulfur sources was 60 kg ha^{-1} and 30 kg ha^{-1}, respectively. Sulfur application increased S uptake by both grain and straw. For cereal crops sulfur content and total uptake of straw was more than that of grain, but opposite result was obtained for soybean. According to the critical level of soil available sulfur in upland soil determined by calibration study, which was 20.0 mg S kg^{-1}, about 41.4%, 35.6%, 42.7% and 38.5% of tested soil samples was S deficient in Heilongjiang, Henan, Shaanxi and Jiangxi province, respectively.

Keywords: crop response, sulfur fertilizer, soil sulfur, critical value

Introduction

There has been three phases in balanced fertilization which was on the basis of organic fertilizer application in China, i.e. only nitrogen application in 1950's, combined use of nitrogen and phosphorus fertilizer in 1960's and integrated application of nitrogen, phosphorus, potassium and micronutrient fertilizer since the mid of 1970's. However, the secondary nutrients such as sulfur, calcium, magnesium has not been paid more attention. Sulfur is an essential nutrient for plant production and the amount of sulfur uptake by plant is similar to that of phosphorus. The potential occurrence of sulfur deficiency in Chinese agricultural soils has increased due to high amount of N, P and K applied, intensive and increased crop production, increased use of high analysis S-free fertilizers, and more recently anti-pollution measures. At present, it is necessary to supply S for balanced fertilization. From 1996 to 2002 we conducted field trials/demonstrations in some crops such as corn, wheat, rice, soybean and oilseed rape to study crop response to S fertilizers, and selected some provinces to investigated soil S status.

Materials and methods

From 1996 to 2002 field trials/demonstrations of sulfur fertilizers on main crops such as corn, wheat, rice, soybean and oilseed rape were conducted in Heilongjiang, Jilin, Henna, Shaanxi, Hubei and Jiangxi provinces in China. Many sulfur sources such as ammonium sulfate, potassium sulfate, gypsum, single superphosphate and elemental sulfur were tested. Application rate ranged from 30 to 120 kg S ha^{-1}. According to the results from field experiments critical value for soil available S were determined.

From 1997 to 2001 soil samples from upland soils were collected in Heilongjiang, Henan, Shanxi and Jiangxi provinces. Soil total S, available S and organic C were tested and evaluated.

Organic C were determined by Walkley-Black (Nelson and Sommers, 1996). Total soil S was determined by acid oxidation with HNO_3, $HClO_4$, H_3PO_4 and HCl (Page et al., 1982), followed by ICP-AES to determine sulfate in the digest. Soil available sulfur was determined turbidimetrically (Hesse, 1971) after extraction with 0.01 mol l^{-1} Ca $(H_2PO_4)_2$ by shaking for 1 hour under soil to solution ratio of 1 : 5. Total S in the plant materials was determined by the procedure of Lisle et al. (1994) in which 0.5 g of plant materials was digested using a wet oxidation technique involving an acid mixture of HNO_3, $HClO_4$ and HCl and sulfate in the digests was determined by ICP-AES.

Results and discussion

Crop responses to sulfur fertilizers

From 1996 to 2002 total of 99 field trials and demonstrations were conducted on corn, wheat, rice, soybean and oilseed rape in Helongjiang, Jilin, Henan, Shaanxi, Hubei and Jiangxi provinces. Results showed that in cereal crops sulfur application increased grain yield of corn, wheat and rice by 6.9%, 6.8% and 9.4% on average, respectively. In economic crops sulfur could

[1] Soil and Fertilizer Institute, Chinese Academy of Agricultural Sciences, Beijing 100081, China

increased yield of soybean and oilseed rape by 11.8% and 8.1%, respectively. The average yield increase and S efficiency for these crops are listed in Table 1.

Table 1:
Effects of sulfur application on different crop yield.

Crops	No. of trials	Average yield increase		S efficiency
		kg ha^{-1}	%	kg grain kg S^{-1}
Corn	31	456	6.9	10.1
Wheat	6	388	6.8	7.5
Rice	39	603	9.4	15.1
Soybean	13	260	11.8	6.7
Oilseed rape	10	140	8.1	2.9

There were some differences in crop responses to various sulfur sources. At the same application rate ammonium sulfate or potassium sulfate increased crop yield more than gypsum or elemental sulfur. For elemental sulfur application rate of 60 kg S ha^{-1} was better than lower rate, further increase S rate could not increase crop yield. For sulfate-S sources such as gypsum, potassium and ammonium sulfate the increase effect on crop yield with 30 kg S ha^{-1} application was similar to that of 45 kg S ha^{-1} application rate (Table 2). So, for elemental sulfur and sulfate sulfur sources the rational application rate was 60 kg ha^{-1} and 30 kg ha^{-1}, respectively.

Sulfur uptake by crops

Total S in straw and grain was determined after harvest. Results showed that sulfur application did not significantly increase S content in grain but increased S content in crop straw to some extend. However, sulfur application increased total S uptake by both grain and straw due to the increase of the yield. For cereal crops sulfur content and total uptake of straw was more than that of grain. But opposite result was obtained for soybean, i.e. sulfur content and total uptake of grain was much more than that of straw. Wheat needed more S than other crops (Table 3). This indicated the nutritional difference of S in various crops.

Determination of critical values for soil available S

According to field experiments the relationship between soil available S extracted by 0.01 mol l^{-1} $Ca(H_2PO_4)_2$ and the relative grain yield (yield without S/yield with S × 100%) showed that the critical level of soil available sulfur for upland and paddy soil was 20.0 mg $kg^{-1}$and 25.0 mg kg^{-1}, respectively, estimated by Cart-Nelson method (Figure 1). Zhang et al. (1997) also indicated that the critical value of soil available S extracted by 0.01 mol l^{-1} $Ca(H_2PO_4)_2$ was 20 mg kg^{-1} for upland crops such as oilseed rape, soybean and wheat. However, the critical value of soil available S obtained by many scientists in other countries was lower than 20 mg kg^{-1} (Donahue et al., 1983; Blair et al., 1993; Zhao et al., 1994). The reason is that in China planting intensities and crop yield are higher than that in other countries, and large amount of S was removed from agricultural field. Furthermore, large amount of N, P, and K applied in crop production need more S for nutrient balance.

Table 2:
Effect of sulfur sources and application rate on crop yield.

Sulfur source	Application rate kg ha^{-1}	No. of trails	Average yield increase %
Elemental sulfur	30	51	6.8
	45	68	8.4
	60	55	9.6
	90	11	9.6
	120	7	9.1
Gypsum	30	15	8.1
	45	15	8.3
Ammonium sulfate or potassium sulfate	30	14	9.6
	45	25	9.5

Soil sulfur status in some provinces

From 1997 to 2000 total of 191, 222, 307 and 104 soil samples were collected from upland soil in Heilongjiang, Henan, Shaanxi and Jiangxi province, respectively. Soil available S and total S (Table 4 and Table 5) were determined. According to the above critical level of soil available sulfur in upland soil 41.4%, 35.6%, 42.7% and 38.5% of collected soil samples was S deficient in Heilongjiang, Henan, Shaanxi and Jiangxi province, respectively (Table 4). S deficiency existed in each soil type and the content of available S was variable among soil samples. Statistic analysis showed that there was significant relationship between total soil S and organic C in four provinces (Table 6). But the correlation coefficient was higher in Heilongjiang and Jiangxi provinces than in Henan and Shaanxi provinces where soils are calcareous with higher pH

Table 3:
Average sulfur concentration in plant tissues and total S uptake by crops.

Crop	Sulfur rate	S concentration		S uptake		Total uptake
	kg S ha^{-1}	mg kg^{-1}		kg S ha^{-1}		kg S ha^{-1}
		Grain	Straw	Grain	Straw	Grain + Straw
Corn	0	1031	1241	5.6	6.3	11.9
	30	1036	1493	5.9	8.1	14.0
	60	1022	1406	6.1	8.1	14.2
Wheat	0	1322	2904	7.0	15.1	22.1
	30	1416	3075	7.8	16.7	24.5
	60	1301	3015	7.5	18.4	25.9
Soybean	0	3968	1178	6.5	2.3	8.7
	30	3568	1136	6.9	2.4	9.2
	60	3679	1333	7.9	3.2	11.1
Rice	0	653	1014	4.8	5.3	10.1
	45	734	1161	6.0	6.7	12.7

Table 4:
Soil available sulfur in four provinces of China.

Province	Sample No.	Range mg kg^{-1}	Mean mg kg^{-1}	C.V. %	Distribution frequency %			
					≤20 mg kg^{-1}	20.1~40 mg kg^{-1}	40.1~60 mg kg^{-1}	>60 mg kg^{-1}
Heilongjiang	191	7.1 - 106	29.1	62.5	41.4	38.1	14.7	5.8
Henan	222	6.1 - 278	32.6	91.4	35.6	42.8	13.5	8.1
Shaanxi	307	4.6 - 255	30.4	85.2	42.7	33.8	12.4	11.1
Jiangxi	104	6.6 - 165	31.2	90.7	38.5	45.1	7.7	8.7

Table 5:
Soil total sulfur in four provinces of China.

Province	Sample No.	Range mg kg^{-1}	Mean mg kg^{-1}	C.V. %	Distribution frequency %			
					≤200 mg kg^{-1}	201~400 mg kg^{-1}	401~600 mg kg^{-1}	>600 mg kg^{-1}
Heilongjiang	191	102 -1334	514	46.7	2.1	38.2	24.1	34.6
Henan	222	41 - 808	347	49.0	18.9	46.9	25.2	9.0
Shaanxi	307	33 - 1541	364	50.3	22.1	41.7	26.4	9.8
Jiangxi	104	117 - 895	511	35.8	4.8	25.0	33.7	36.5

Table 6:
Relationships between soil available S, total sulfur and organic C (r).

Province	Samples No.	Organic C *vs* total S	Organic C *vs* available S	Total S *vs* available S
Heilongjiang	191	0.452***	0.341***	0.497***
Henan	222	0.219***	NS	NS
Shaanxi	307	0.173**	0.244***	0.313***
Jiangxi	104	0.603***	NS	NS

** P<0.01, *** P<0.001, NS, not significant

and sulfur may co-precipitated or co-crystallized with calcium carbonate (Tisdale et al., 1985; Roberts and Bettany, 1985). However, in Heilongjiang and Jiangxi provinces soil is neutral or acidic, so no free or co-precipitated gypsum can exist and organic sulfur is the main source of total S.

Total soil sulfur was different in four provinces. On average, total S was higher in Heilongjiang and Jiangxi provinces than in Henan and Shaanxi provinces. In Heilongjiang and Jiangxi Provinces total S in more than 90% of soil samples was above 200 mg kg^{-1} and more than 30% above 600 mg kg^{-1}.

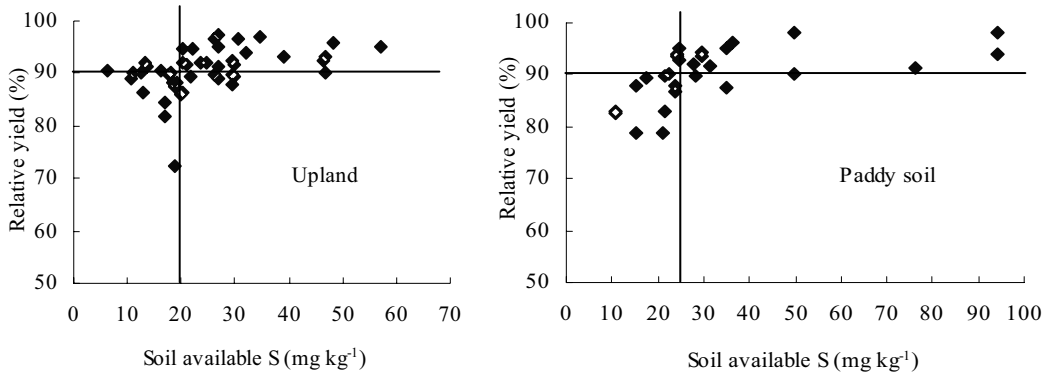

Figure 1:
Critical value determination for soil available sulfur.

But in Henan and Shaanxi provinces total S in most of soil samples was less than 400 mg kg^{-1} (Table 5).

Conclusions

Sulfur fertilizers could increase crop yield by 6.8% to 11.8%.Crop responses to ammonium sulfate and potassium sulfate were better than gypsum and elemental sulfur. The rational application rate of sulfur was 30 kg ha^{-1} and 60 kg ha^{-1} for sulfate-S fertilizers and elemental sulfur, respectively. The critical value for soil available sulfur was 20 mg kg^{-1} and 25 mg kg^{-1} for upland and paddy soils, respectively. About 41.4%, 35.6%, 42.7% and 38.5% of tested soil samples was S deficient in Heilongjiang, Henan, Shaanxi and Jiangxi province, respectively. Sulfur application in balanced fertilization strategies need to be considered in crop production.

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The sulfur cycle in the agro-ecosystems in southern China

Chongqun Liu[1] and Xiaohui Fan[1]

Abstract

The total sulfur content in soils of China ranges from 100 - 500 mg kg^{-1}. The organic sulfur in soils of southern China accounts for 86 - 94% of the total S. However, solely the inorganic sulfur (sulfate) can be directly taken up and utilized by plants. The mean total S content, organic S and available S in the cultivated soils of southern China is 299 mg S kg^{-1}, 267 mg S kg^{-1} and 34 mg S kg^{-1}, respectively. The S mineralized in the paddy soils of southern China is about 3.8 - 15.6% of the organic sulfur, with an average of 9.6%. The amount of the organic S mineralized is 15.0 - 33.1 mg S kg^{-1} soil. Inorganic sulfide in the soil and the elemental S in fertilizers are oxidized to sulfate by the S-oxidizing bacteria. The oxidation of S in the soil is associated with many factors such as temperature, moisture, the number of the S-oxidizing bacteria, and the particle size of elemental S. In most of the paddy soils in China, after flooding them, the concentration of soil H_2S was below 0.03 mg l^{-1}, and thus below the toxicity threshold. In southern China, the S input in the S balance comes from S fertilizers (25.8 kg ha^{-1}), wet deposition (13.4 kg ha^{-1}), and irrigation water (9.2 kg ha^{-1}), with a total input of 50.8 kg S; the main S output parameters are S-removal by harvest products (32.1 kg ha^{-1}), leaching (19.9 kg ha^{-1}), and runoff (7.2 kg ha^{-1}), with a total removal of 59.2 kg ha^{-1}. If one does not take into accounts the sulfur input from dry deposition, the input and the output of sulfur are nearly balanced. Nevertheless, the contribution of dry S deposition is presumably in the range of the wet deposition, however, these estimates as well as those for the gaseous S losses need verification by corresponding measurements.

Keywords: Agro-ecosytems, sulfur balance, sulfur cycle

Introduction

The S cycling in farmland can be described as transfer processes in the "soil-plant-atmosphere" system and the following major S pools and transformation processes can be identified: (1) Soil S can be divided into two main fractions, the organic and the inorganic, with organic S being the predominant part, which undergoes microbial decomposition and final formation of sulfate; (2) Sulfate, after being taken up by crop plants, is incorporated into organic S compounds. With animal and plant residues, or of animal excrements, organic S is supplied to the soil, and after being broken down by microorganisms, once again sulfate will be released; (3) With a dry and wet atmospheric S deposition significant amounts of S are applied to the soil in southern China; (4) S incorporated into the soil after applying organic manure and mineral S fertilizers; and (5) S losses by off-take with harvest products and leaching through percolating soil water (Figure 1).

Sulfur fractions and transformation processes in soils in southern China

The total S content in different soil types in China ranges approximately from 100 - 500 mg kg^{-1} (Liu, 1995). In the southern humid areas, S in soils mainly consists of the organic S. The organic S content amounts to 86 - 94% of the total S, whereas only 6 – 14% of the total S belongs to the inorganic S fraction. Inorganic S comprises of readily soluble S and adsorbed S (sulfate). According to statistics of 2,800 soil samples taken from 10 provinces in southern China (Liu, 1995), the mean content of total S, organic S and available S was 299 mg kg^{-1}, 267 mg kg^{-1} and 34 mg kg^{-1}, respectively (Table 1).

Table 1:
The mean sulfur content of different fractions in soils of southern China (Liu, 1995).

	Total S	Plant available S	Organic S	Organic S
		(mg kg^{-1})		(%)
Mean	299	34	267	89
Range	207-480	23-67	178-419	86-94

Each year about 1 - 3% of the soil organic is mineralized to sulfate; at the same time, about the same quantity of sulfate is fixed in the soil organic matter (Nriagu, 1978). After ten types of paddy soils from southern China were incubated for 10 weeks at 30° C and at 60% of the water holding capacity (WHC), 3.8 - 15.6% of the organic S was mineralized, with a mean value of 9.6%, (Zhu et al., 1982). Hu and Zu (2002) found a similar value with 10.6% (6.7 - 19.8%). These values correspond with about 15 to 33 mg S kg^{-1} soil (Zhou, 2004).

The oxidation-reduction reaction of soil S exerts strongly the S nutrition and soil pH. The oxidation of elemental S is an acidifying process. Elemental S, H_2S and FeS_2, are oxidized to SO_4^{2-} by S-oxidizing bacteria. The acid sulfate paddy soils in the coastal area of China contain larger amounts of sulfide. Under oxidizing conditions sulfuric acid is produced, and the

[1] Institute of Soil Science, Chinese Academy of Sciences, Nanjing 210008, China

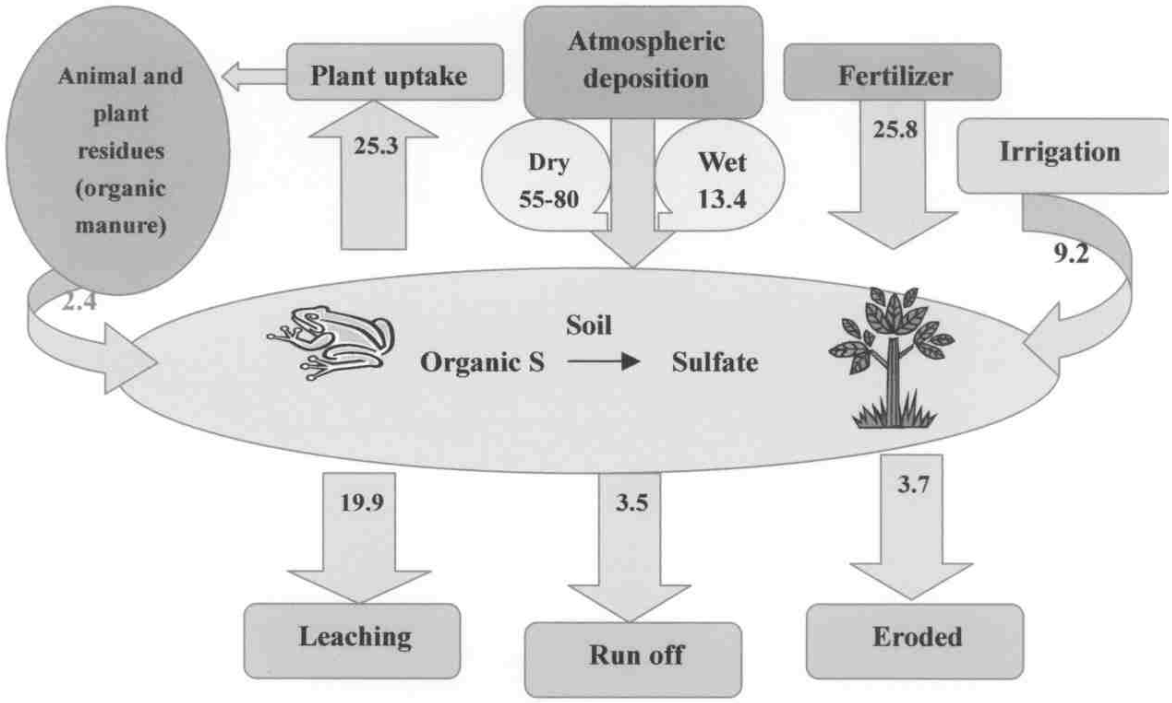

Figure 1:
Sulfur cycling (kg ha^{-1}) in arable land in southern China (Liu, 1995, 2000)

soil pH is reduced to a pH of 2 - 3. Elemental S is a commonly used fertilizer. Only after the elemental S oxidizes to sulfate by S-oxidizing bacteria in the soil, can the plant absorb it. According to the study of Li et al. (1998), the oxidation of S in the soil was markedly influenced by the temperature and moisture; phosphorus and organic substances may enforce the oxidation of elemental S, and with the reduction in particle size the rate of oxidation will increase. The S-oxidizing power of a soil is associated with the number of certain sulfur-oxidizing microbes in the soil.

Table 2:
Production of single superphosphate (SP) in China.

Year	Total P fertilizer (P_2O_5 $t10^4$)	SP (P_2O_5 $t10^4$)	% SP in total P fertilizer (%)	Sulfur in SP (S $t10^4$)
1980	231	165	71.3	141
1990	412	289	70.3	248
2000	663	364	54.9	312

$t10^4$ = ten thousand tons**

The reduction of S is caused by the sulfate-reducing bacteria, which are present in sewage water, sludge and bog land. In rice fields under a long continued submergence, sulfates are reduced and H_2S is formed; often insoluble FeS and ZnS is precipitated, and which may sometimes result in Zn-deficiency and Fe-deficiency. In addition, the formed H_2S is toxic to rice plants at higher doses. Yu and Liu (1964), however, stated that in most of the paddy soils in southern China, the soil pH was about 6.5 - 7.0 after flooding and the concentration of hydrogen sulfide was below 0.03 mg l^{-1}, which would be below the threshold level of direct toxic effects on plants.

Sulfur input by fertilization

In China, single superphosphate (SP) is used in large quantities as a mineral fertilizer, and during the period of the 1960s - 1990s, single SP always accounted for 70% of the total production of phosphate fertilizers. Although from 1990 to 2000 the relative production of single superphosphate was reduced to 55% of the total phosphate fertilizer output, its absolute output was still increasing year by year, from 2.90 million tons in 1990 to 3.60 million tons in 2000. If calculated according to the country's total cultivated area of land of 130 million hectares, on an average 25.8 kg S ha^{-1} per year are applied, which can satisfy

the needs of most of the farm crops in Southern China (Table 2).

In China, the commonly used organic manure includes farmyard manure, human feces and urine, green manure and crop straw. In recent years, straw has been used mostly as fuel, fodder and as industrial raw material, but very little is directly returned to the farmland. Meanwhile, the area for planting green manure crops is becoming increasingly small. Hence, human feces and urine as well as animal excrements have become the main source of organic manure. China Agriculture Yearbook 2000 shows that in China 1.2 billion tons of human and animal excrements were produced a year, which contain 356,000 t of S. On an average, about 9.5 t of human and animal excrements was applied to each hectare of cultivated land each year, corresponding to 2.4 kg S added to each hectare of land each year (Table 2).

Table 3:
Sulfur content of animal and human excreta in China.

	Number* (10^7)	Amount of Excreta $t \cdot 10^7$	S content ($t \cdot 10^4$)
Draft animal**	15	76	15.2
Sheep	29	4	2.6
Pig	45	22	6.7
Poultry	270	13	8.1
Human	120	60	3.0
Total	-	122	35.6
Average	-	9.4 t ha^{-1}	2.4 kg ha^{-1}

*Number of animals/humans
**Cattle, horse

Sulfur input by atmospheric depositions

S deposition in the southern provinces of China ranges between 14.4 and 39 kg ha^{-1} yr^{-1}, with an average of 27.3 kg ha^{-1} yr^{-1}. In the mountain and hilly districts of Southern China, the runoff volume accounts for 1/2 of the annual precipitation (Table 4).

The atmospheric dry S deposition may be directly absorbed by the vegetation, soil and water surface. Experiments have shown that even plants supplied with adequate soil sulfate are able to absorb 25 - 30% of their S from the atmosphere (Brady, 1984). Terman (1978) calculated that half of the plants' S demand could be supplied by absorbing SO_2 from the air. According to Wu's study by using ^{35}S (Wu et al., 1991), the atmospheric S taken up by soybean and corn accounted for 11.0 % and 23.6 %, respectively, of the total amount of S taken up by the plant. The SO_2 discharged by the atmosphere to the soil undergoes rapidly transformation processes.

According to Garland approximately 50% of the SO_2 is applied by dry deposition (Garland, 1978). Fowler (1978) estimated that in Britain the amount of SO_2 settled by dry deposition was 1.0×10^6 t yr^{-1}, whereas that settled by wet deposition was 0.6×10^6 t yr^{-1}. Estimating the dry deposition by using the Garland model, differences between calculated and measured values may be very high and thus often inadequate. However, in China there are no reliable data and thus 50% SO_2 input as dry deposition delivers only an approximate value.
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Table 4:
Average wet sulfur deposition in southern China (kg ha^{-1}).

Province	Wet S deposition	Range
Yunnan	14.4	5.0 - 23.2
Anhui	17.3	1.0 - 54.0
Jiangsu	23.5	8.0 - 40.0
Zhejiang	24.2	13.5 - 32.0
Jiangxi	26.7	14.0 - 40.0
Fujian	32.3	19.2 - 44.9
Guangdong	33.5	17.0 - 56.0
Guangxi	34.4	20.9 - 48.0
Hunan	39.0	37.5 - 41.9
Average	27.3	14.4 - 39.0

Sources: Liu (1984, 2000), Zhang and Gong (1987)

Table 5:
Mean sulfur content of irrigation water in southern China (mg l^{-1}).

Province	No. of samples	Even S concentration	Range
Jiangxi	76	1.94	0.71 - 7.64
Zhejiang	36	1.86	1.10 - 3.28
Hunan	36	1.69	0.90 - 2.83
Guangxi	38	2.32	1.96 - 3.93
Guangdong	74	2.94	0.81 - 6.85
Average	260	2.23	0.71 - 7.64

Sulfur in irrigation water

In general, irrigation water originates from rivers, reservoirs, wells, ponds, etc. According to statistics of 260 water samples taken from 5 southern provinces, the mean S content of irrigation water was 2.2 mg l^{-1}, ranging from 0.7 - 7.6 mg l^{-1} (Table 5). A comparison of the S content of irrigation water in 5 provinces in southern China, the mean S content of the reservoir water, the well water and the river water was 1.7, 1.8 and 1.91 mg l^{-1} respectively, and thus very similar. Water of ponds had a higher S concentration of 2.9 mg l^{-1} (Table 6).

As has been shown by the study of International Rice Institute (Wang et al., 1976), rice plants may take up 54 % of the S supplied by irrigation water. A concentration of at least 6 mg S l^{-1} in the irrigation

centration of at least 6 mg S l^{-1} in the irrigation water will satisfy the S demand of the plant (Wang et al., 1976). For rice crops in southern China irrigation water will supply about 9 kg ha^{-1} S if 7,500 m^3 of water with a concentration of 2.2 mg l^{-1} is applied and 54 % of the S utilized by the plant

Table 6:
Mean sulfur concentration in irrigation water of different origin (mg l^{-1})

Province	River	Reser-voir	Well	Pond
Jiangxi	2.60	1.74	1.87	1.01
Guangdong	2.17	0.72	1.76	5.96
Zhejiang	-	1.83	-	-
Hunan	1.36	-	-	2.05
Guangxi	1.13	-	-	2.94
Average	1.91	1.74	1.84	2.92

Table 7:
Sulfur concentration of grain and straw of various crops (%).

Crop	Grain	Straw	Grain/straw ratio*
Rice	0.093	0.12	1:0.9
Wheat	0.154	0.31	1:1.1
Corn	0.113	0.099	1:1.2
Oilseed	0.995	0.404	1:1.5
rape	0.259	0.078	1:1.6
Soybean	0.179	0.159	1:0.8
Peanut	0.204	0.077	1:2.2
Sesame			

*dry weight

Table 8:
Sulfur removal by different crop plants.

Crop	Yield (t ha^{-1})	S removal (kg ha^{-1})*
Sugarcane	57.6	17.3
Oilseed rape	1.5	24.0
Wheat	3.7	18.3
Rice	6.3	12.7
Corn	4.6	10.7
Peanut	3.0	9.2
Banana	19.8	7.5
Tobacco	1.8	7.1
Orange	6.9	6.9
Soybean	1.7	6.5
Sesame	1.0	3.7

*Removal (grain + straw)

Sulfur uptake by crops

Soil S is removed mainly by crop uptake and off take with harvest products, leaching and surface run-off. In southern China, farm crops have a S content between 0.1-1.0 %. Cereals have a rather low S content (0.1 - 0.3 %), while oil crops have a distinctly higher S content (0.2 - 0.9 %). Most of the straw is used as a fuel or industrial raw material and only rarely returned to the field so that the S off take with harvest products increased (Ye, 1995). As straw of cereal crops has about the same or even higher S concentrations than grain, significant S amounts are removed by the harvest products (Table 7).

The S removal is the product of S concentration and yield. The grain of the rice plant has for instance a low S content, but yield is regularly high, so that the S removal may be as high as 12.7 kg ha^{-1}. In southern China, the S removal is on an average 24.0 kg ha^{-1} for oilseed rape, 18.3 kg ha^{-1} for wheat and 17.3 kg ha^{-1} for sugarcane (Table 8).

The favorable growth conditions in southern China such as the ample heat and abundant rainfall, a long season for crop growth, and a high cropping index, make it feasible to plant 2 - 3 crops per year. The commonly practiced rotation systems in southern China are: early rice-late rice, wheat-rice, rapeseed-rice, peanuts-rice, and rapeseed-peanuts-rice. Among these systems, the rapeseed-peanut-rice rotation removes most S with 45.9 kg ha^{-1}, followed by the rotation of rapeseed - rice with 36.7 kg ha^{-1}, and the rotation of wheat-rice with 31.0 kg ha^{-1} (Table 9).

Sulfur losses by leaching

Sulfur leaching losses are closely related to soil properties, climatic conditions, farming practices and fertilizer application rates, which may be as high as 310 kg S ha^{-1} yr^{-1} (Freney et al., 1983). In southern China, the annual precipitation ranges from 1,200-2,000 mm, with a distinct division between the wet season and the dry season. In the wet season, there is a high rainfall, and a great deal of water is drained out of the field by leaching. In the dry season, however, the percolation water is dramatically reduced. In Yingtan, Jiangxi Province, during April to June 2003, the rainfall was 919 mm, which accounted for 68.7 % of the annual precipitation. At the same time 80% of the water was leached (Liu, 2003). In contrast, in the second half of the year, leaching losses are only minor.

Table 9:
Sulfur uptake by some crop rotations in southern China.

Rotation system	S uptake (kg ha^{-1})
Rapeseed-peanut-rice	45.9
Rapeseed-rice	36.7
Wheat-rice	31.0
Rice-rice	25.4
Peanut-rice	21.9
Mean	32.1

Table 10:
S leaching losses during the growing season of some crops

Crop	Treatment**	S added (kg S ha^{-1})	S leaching loss (kg S ha^{-1})	% of S added	Location
Wheat (Nov-Mar)*	Control	0	13.0	21.0	Jiangsu (2002)
	SSP	70	27.7		
Rice (Jun-Oct)*	Control	0	22.7	29.4	Jiangsu (2002)
	SSP	48	36.8		
Rapeseed-Peanut rotation (Nov-Jun)*	Control	0	25.9	14.9	Jiangxi (2003)
	ES	75	37.0		
Peanut (Apr-Jun)*	Control	0	12.3	20.6	Jiangxi (2003)
	GYP	71.5	27.0		
Average			32.1	21.8	

*Growing season
**SSP, single superphosphate; ES, elemental S, GYP, gypsum

In southern China, during the growing season of rice, wheat, oilseed rape and peanuts, the leaching losses of S ranged from 13 - 37 kg ha^{-1}. If no S was applied, the average leaching loss was 18.5 kg ha^{-1}. When S was added, the average loss was 32.1 kg ha^{-1}, accounting for 21.8% of the applied S (Table 10). Apparently the type of fertilizer influences leaching losses, too. In the tea garden fertilizer trials in Zhejiang in 2002, potassium sulfate yielded the highest S leaching losses with 35.2 kg ha^{-1} yr^{-1}, which was 3 times higher than in the control plots with 10.6 kg ha^{-1} yr, and about 2 times higher than in the gypsum treatment with 15.7 kg ha^{-1} yr^{-1}. Elemental S applications resulted in the lowest sulfate leaching losses with 12.4 kg ha^{-1} yr^{-1} as it needs to be oxidized by microorganisms.

Sulfur losses by runoff and soil erosion

According to the determination made by the Red Soil Station at Yingtan, Jiangxi Province, the average annual runoff volume for the Orthic Acrisols (Ao) cultivated land < 5º was 115 mm (Zhang and Zhang, 1995). The average S content in the runoff was 0.30 mg l^{-1}, and S lost by runoff was 3.5 kg ha^{-1} yr^{-1}. For the cultivated land < 5º, the mean quantity of the eroded soil was 33 t ha^{-1} yr^{-1}. The eroded soil had an average total S content of 111mg kg^{-1}, and the eroded soil throughout the year contained 3.7 kg ha^{-1} S. Consequently, on the red soil sloping land < 5º the annual quantity of S lost by runoff and by the soil erosion was 7.2 kg ha^{-1} S.

Sulfur balance on agricultural soils in southern China

In southern China, the S input in the S balance comes from S fertilizers (25.8 kg ha^{-1}), rainfall (13.4 kg ha^{-1}), and irrigation water (9.2 kg ha^{-1}), with a total input of 50.8 kg S; the main S output parameters are S-removal by harvest products (32.1 kg ha^{-1}), leaching (19.9 kg ha^{-1}), and runoff (7.2 kg ha^{-1}), with a total removal of 59.2 kg ha^{-1} (Table 11). If one does not take into accounts the sulfur input from dry deposition, the input and the output of sulfur are nearly balanced. Nevertheless, the contribution of dry S deposition is presumably in the range of the wet deposition, however, these estimates as well as those for the gaseous S losses need verification by corresponding measurements.

Table 11:
S balance in soils of Southern China (kg ha^{-1} yr^{-1}).

Input		Output	
Mineral S fertilizer	25.8	Crop removal	32.1
Organic manure	2.4	Leaching	19.9
Wet deposition	13.4	Runoff	7.2
Irrigation	9.2		
Total	50.8		59.2

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An Agricultural Sulfur Information System for China

Youhua Ma[1], Hongxiang Hu[1], Qiang Wang[1], Xiaoli Liu[1], Yanping Zhao[1], Hongxia Liang[1] and Zhaoming Zhu[1]

Abstract

An Agricultural Sulfur Information System was developed for China by using Visual Basic, API function of MapGis software and Access database software in Chinese Windows 98. According to the data and the demand of production, five application modules were developed, including modules of soil sulfur states, sulfur nutrition in crop, sulfur fertilizer effect, balance of soil sulfur, update and setting. The developed system is an effective information management tool for managers of fertilizer producers and sale departments and agricultural scientific research departments.

Key words: geographic information system, soil, sulfur fertilizer

Introduction

The incidence of soil S deficiency is increasing rapidly throughout China and the agronomic benefits of plant nutrient S are now widely known. Numerous field experiments conducted in China have clearly shown that crop yields increased by adding S fertilizers and the sulfur fertilization improved crop quality and increased their market value. According to statistics of S field experiments from many Chinese provinces, Sulfur fertilization averagely increased crop yields by over 10% for most major agricultural crops such as rice, rapeseed, wheat, soybean, peanuts (TST, 2001; Zhang J, 2001; Huang et al., 2002).

An Agricultural Sulfur Information System for China was built in order to supply information management tool about Chinese soil sulfur status, plant sulfur nutrition and effective application of S fertilizers for the departments of agricultural technological extension, fertilizer production and distribution, agricultural research and education so that they can distribute S fertilizers suitable to local soil, crop kinds and climate conditions. Meanwhile the farmers could learn related agricultural S knowledge such as diagnoses of S deficiency and could decide whether, how and when to apply S fertilizers.

[1] Department of Soil Science and Agrochemistry, Anhui Agricultural University, Hefei, 230036, China

Materials and methods

Data sources, design outline, hardware and software

The basic data from different regions, soil types and crops were obtained from the results of the Chinese agricultural sulfur research for past twenty years. Based on GIS as the core technology, the spatial data and related attribute were combined and analyzed and were linked and treated with GIS. MapGis, popular GIS software in China, was selected as development platform and Visual Basic as development language

Hardware: CPU (central processing unit) basic frequency 733M, memory 128M, HD 20G, scanner (Uniscan A600). Software: Windows 2000 Chinese Operating System, Visual Basic 6.0, Photoshop 6.0, Chinese Office 2000.

System construction, economy and society benefit analysis

In the current Geographic Information System, spatial objects can be described from three aspects, namely positional information, non-positional information (attribute information) and time information. Spatial data in computer are characterized with encoding technique in mode of point, line and area. And data sets are built among objects. Positional information is recorded with positioning data (also called geometry data), which reflects geographic distribution of the phenomena of nature. Non-positional information is recorded in attribute data, which describe the nature phenomena, the characteristics of object quality and quantity. A piece of farmland, for example, the concrete position can be known by its geometry data, longitude and latitude, while the content of sulfur in corresponding farmland is called attribute data.

There are several popular and wonderful GIS software in China such as MapGIS from China Geology University, GeoStar from Wuhan University etc. These GIS software supply a great deal of API functions and controls. It is easy and convenient to transfer these functions and controls with advanced programming language such as Visual Basic to develop the system (Gong Jianya, 2002). This system was developed with MapGIS as developing platform and implemented by transferring the API functions and controls with Visual Basic.

As mentioned above, the incidence of soil S deficiency is increasing rapidly throughout China and serious S deficiency is emerging in Chinese agriculture, which would result in the decrease of crop

yields and productivity. It is estimated by TSI, that the S demand deficit is projected to increase to 2.7 million tons by 2010 with the continuous increase of agricultural production and S-free high-analysis fertilizer use. The loss of grains would reach 30 million tons for the S deficiency, which should not be neglected (total Chinese grain: 430.7 million tons in 2003).

Agricultural Sulfur Information System for China could make people understand the importance of sulfur in Chinese agriculture and find out the Chinese soil sulfur status and the response of crops to S fertilizer so that the research results of S fertilizer could be extended quickly throughout China.

System design

Founding on GIS, the system design would solve the problem how to build on the base of demand and feasibility analysis. The objective of general function design is to solve how to carry out the system, the main task of which is to divide function modules in subsystems and to determine the links among modules and their descriptions On the selection of coordinate system, national coordinate of the system in 1980 was chosen as the system horizontal coordinate and the height datum of national geodetic coordinate system in 1980 was selected as the system height datum.

Basing on the system analysis, four function modules were determined in the system according to the purpose and demand of the system building.

(1) Data Management module: Management on input of soil sulfur data and related spatial data, and operation for modifying, updating, appending, deleting and view build.

(2) Specialty Management Module: Management on diagnosing of the corps S deficient, symptom identification of crop S deficiency, analyses of reason for S deficiency and the build of specialty knowledge base

(3) Application Function Module: According to the aim of the system, select similar information system as reference to build the corresponding function module based on.

(4) Assistant Function: Establishment of the help system with detailed content and convenient usage

Classification and transformation of data

The data sources from many channels lead to inconsistency in using nomenclature and criterion of partial data. Nomenclature and measure criterion are unified and standardized on the condition of same meaning and content. For example, the "acreage" and "ppm" were transformed into "hm^2" and "mg kg^{-1}" in measure unit; and "slope" in definition of topography was changed into "the middle and bottom part of hills" according to current standard.

Since Access is a relative database, its development environment and language are characterized with relational database. But some data are not relational and must be classified, sorted and transformed first. If they were input without treatment, there would be many problems. Firstly, characters are double byte occupying much space of dish and memory, that results in unnecessary resource waste and it is difficult to carry out maintenance and to update. Secondly, fields are described by Chinese characters with much repetitiveness, which breaches the principle of the smallest redundancy, the relationship among fields are also not clear with ineffective utilization by system.. Therefore these fields in system should be transformed, e.g. long fields are split or given corresponding coding.

Database construction

The independent soil sulfur database was constructed in this system for the querying, updating and modifying. In the database, with sampling place as key word the fields such as soil code, soil type, soil parent material, the sampling regions (province, city county), available S content, pH, organic matter, sampling depth, remark were set up. The sample place was given four fields in order to query with different rank administrative districts

In China due to incomplete foundational digital materials compared with developed countries, the most spatial data were obtained by scanning of maps with scanner and digitizing with input edit module of MAPGIS . In the system it, the maps of province boundary of 1:1 million were selected. The map base management subsystem supplies the flexible and intuitionistic way to input data and some approaches to query data with effective management for various maps.

Attribute data, the important parts of spatial data, are edited, modified and stored with the sub-system of attribute management of MAPGIS. In this way, it ensures data integration, compatibility and unification to reduce development difficulty and convenient to use.

Database management

Management of database includes definition of data, query of data, renewal of data, construction of data and usage of view as well as construction and application of index. Definition of data means definition of the built database structure and determination relative mode. Query of data is the content search of built database. Because soil S contents are main objectives to be queried, the kinds of data are built separately. The update includes inserting, modifying and deleting. These functions are included in data input module

Results and Discussion

Based on database, application system manages and utilizes the data resource in system. According to existent data and production requirements, this system develops five application modules including soil S status, crop S nutrition, fertilizer effects, soil S balance, update and setting.

Soil S status subsystem is mainly used to query soil S status from different regions and parent materials. This system provides two approaches to inquire the soil S status. One is by the maps of administrative area, and another is by the maps of soil parent materials. Based on MAPGIS platform, the operating platform of the sub-system was built by calling control and API (Application Programming Interface) function supplied by MAPGIS with Visual basic. Soil S contents and other related information could be searched by selecting language in SQL (Structured Query Language) through the location of mouse in administrative map.

Because of complexity of soils, diagnose of plants for S deficiency was more reliable than soil test. Plants diagnoses of S deficiency have made great progress in recent years. Plant sample position is important to S deficiency diagnose for plants weak S recycle and mature leaves accumulate more S than younger organs .It is thought that the total S content in full unfold younger leaves or leaves developed well on 1/3 upper could reflect plant S nutrition status. In system volumes of pictures for crop S deficiency were chosen to build special picture database. With abundant pictures and text, the system provided the symptoms of S deficiency of crops and analyzed the reason of S deficiency for farmers.

Farmers are more concerned about effect of S application. The S fertilizer effect subsystem provides effects of S application on crop yields, quality and market value in different regions of China, and gives S fertilizer recommendation (e.g. S fertilizer type, application rates and methods) special for different regions and crops in China. In the soil S balance subsystem the data for soil S balance such as atmosphere sulfur, soil sulfur, irrigation water sulfur

Figure1:
Primary interface of the system.

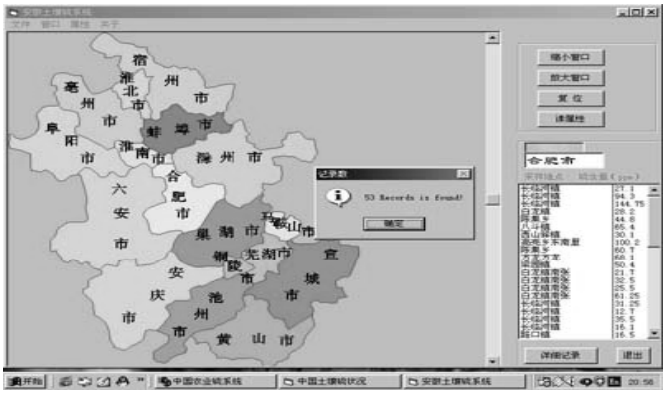

Figure 2:
Example of interface for querying soil S in Anhui province, China.

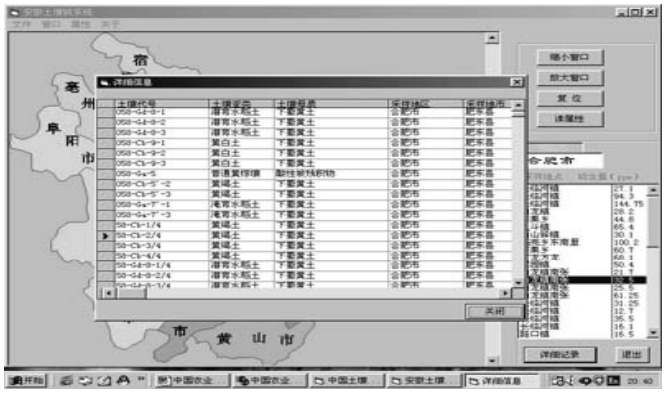

Figure3:
Example of interface for the detailed soil S status.

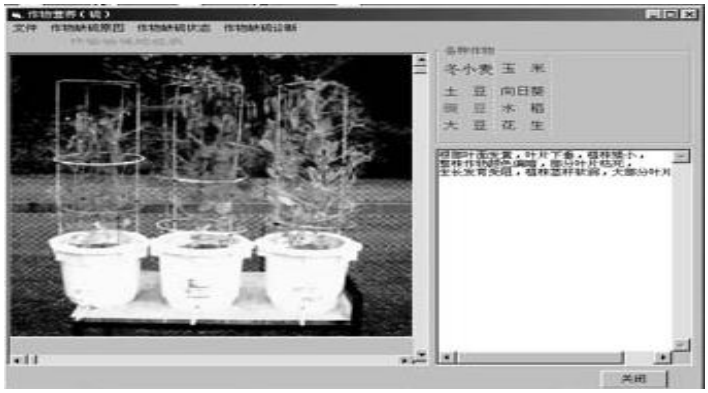

Figure 4:
Example of crop S nutrition interface.

and ground water sulfur etc were analyzed to obtain local soil S balance in Chinese farmlands. The database in the system could be updated to ensure the practicability of this system, but only administrator could update database, and different users are given different power limits

An example of the primary interface is presented in Figure 1. Examples of querying for soil S status are presented in Figures 2 and 3, and of crop S nutrition interface in Figure 4. As the outcome of combining information technology and soil-fertilizer and plant-nutrition technology, this system is explored for its developing outline and implemented methods. By this system, soil S deficiency status, effects of S fertilizer application and soil S balance of input and output in Chinese different regions could be directly queried, and for S deficiency regions the fertilization recommendation of NPK should be transferred to that of NPKS to supply balance nutrients to improve soil fertility, to increase yields and to meet the increasing requirement of grain by China.

With increasingly maturation and popularization of the internet technology, WebGis (World-Wide-Web Geography Information System) is paid attention and welcome by more and more people, for it not only solves the problem of expensive price for GIS software, but also reduces the cost of collecting geography spatial data and improves the sharing degree and extension of the geography information. As the developing direction of GIS it is necessary to integrate this system with internet in the future.

Acknowledgments

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Global sulfur requirement and sulfur fertilizers

D.L. Messick[1], M.X. Fan[1] and C. de Brey[1]

Abstract

The world sulfur fertilizer deficit is projected at close to 11 million tons per year by 2012, with Asia and the Americas as the most sulfur deficient regions in the world. Pollution controls, intensified cropping, and the absence of sulfur from high-assay fertilizers are combining to produce a growing worldwide deficiency in soil sulfur, needed for high crop yields and crop quality, that only the use of plant nutrient sulfur products will solve. Sulfur deficiency is increasingly becoming one of the major limiting factors to further sustainable increases in agricultural production and fertilizer use efficiency, and also is stimulating growth in farmers' demand for sulfur-containing fertilizers, which is becoming a greater potential market for the fertilizer industry to develop innovative technologies and products for this market potential. Most sulfur-containing fertilizer materials can be divided into three groups: 1) Sulfate-containing; 2) Sulfur-containing, and 3) Liquid. Sulfate-containing fertilizers provide most of the fertilizer sulfur applied to soils. The most significant and popular sources are ammonium sulfate, single superphosphate (SSP), potassium sulfate, potassium-magnesium sulfate and gypsum. These materials have the advantages of supplying sulfur primarily as a component of multi-nutrient fertilizers in a sulfate form that is immediately available for plant uptake. Elemental sulfur-containing fertilizers are the most concentrated sulfur carriers. Elemental sulfur has to be oxidized into the sulfate form before plant uptake, which limits its availability immediately after application to soil. Micronized sulfur products have improved the effectiveness of elemental sulfur by providing elemental sulfur in a physical form so that it can be used for direct application and bulk blending with little dust and be more readily converted to the sulfate form in soil. Fertilizer manufacturers are introducing new products to meet the increasing demand of the sulfur fertilizer market, including specially formulated sulfate-containing compound fertilizers or elemental sulfur enriched compound fertilizers based on specific crop and soil needs. These sulfur modified or enriched compound fertilizers using either sulfate or elemental sulfur or a combination of the two have several advantages, including improved chemical and physical properties; and providing multi-nutrients with balanced ratios for plant nutrition. The increasing demand for sulfur fertilizers and their use in agriculture will provide significant benefits to both fertilizer manufacturers and farmers through the next decade.

Key words: sulfur, sulfur deficiency, sulfur fertilizers, sulfate-containing compound fertilizers, elemental sulfur enriched fertilizers, micronized sulfur products

Introduction

Sulfur (S) is one of the major essential plant nutrients, and it contributes to an increase in crop yields by providing direct nutritional value and improving the use efficiency of other essential plant nutrients, particularly nitrogen (N) and phosphorus (P). As agricultural productivity has increased, the demand for all nutrients has increased. While N fertilization, in particular, and to lesser degrees, P and potassium (K) fertilization needs have been addressed, S has emerged as the fourth major nutrient for the fertilizer industry. This trend will only continue and will be exacerbated with the reduction of sulfur dioxide emissions, which have served as a significant source of S for crop production for a number of years. Furthermore, the increased trend to use high-analysis fertilizers devoid of sulfur, combined with declining levels of soil organic matter, a significant potential source of S, have reduced soil S content to levels where S is increasingly becoming a limiting factor to higher yields and production.

Ammonium sulfate and single superphosphate (SSP) dominate the current available worldwide supply in so far as volume of fertilizers used containing S, representing 83% of the approximately 10 million tons of S applied in fertilizers annually. While these traditional sources will be in use for a number of years to come, production is limited and future availability may diminish due to competing production processes. These materials, including potassium sulfate as well, belong to a broader group of what are termed "S fertilizers with sulfate carriers" as opposed to "elemental S-based S fertilizers." The elemental S-based S fertilizers are newer on the scene and refined production technologies for a series of these types of products have gained attention in recent years. These products are gaining market share, and a growing array of S fertilizers are available to accommodate different soil, crop and application conditions and situations.

[1] The Sulphur Institute, 1140 Connecticut Avenue, N.W., Suite 612, Washington, DC 20036, U.S.A.

The rising demand in sulfur fertilizer requirements

In 2001, almost 10 million tons of S was applied to soils worldwide through fertilizers. The current potential S fertilizer market is estimated to accommodate an additional 9.4 million tons annually. With increased food production raising S requirements, and assuming slower expansion rates for S application in accordance with recent history, the unfulfilled requirement for S fertilizers is projected to grow to 11.0 million tons by 2012.

A regional breakdown of world S deficits is shown in Figure 1. Asia is the region manifesting the greatest S shortfalls. Intensified agricultural production, pressured by the backdrop of food self-sufficiency goals and limited land resources in the globe's two most populous nations, China and India, has created the S nutrient imbalance. Asia's annual S fertilizer deficit, currently estimated at over 5 million tons, will increase to 6 million tons by 2012, with over 70% represented by China and India. China currently applies about 3 million tons S to agricultural soils every year, mostly from SSP (12% S) and ammonium sulfate (24% S), at an average rate of 15 kg S/ha sown area. However, the total annual crop requirement for plant nutrient S is about 4.5 million tons, resulting in a total 1.5 million tons S deficit, which will increase to 2.4 million tons by 2012 indicating the need for corrective measures. In India, the total S containing fertilizer production in 2001 was close to 5 million tons, providing 700,000 tons S, and the total crop requirement was about 2.2 million tons, which resulted in a 1.5 million tons deficit. This deficit is projected to increase to 1.9 million tons in 2012, which will provide a large market for the fertilizer industry.

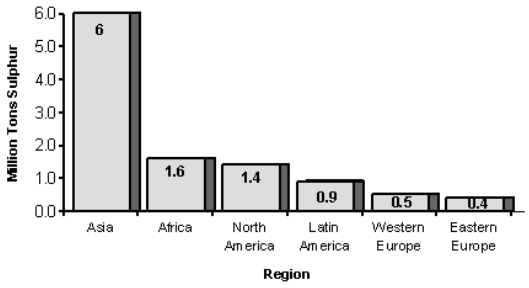

Figure 1:
Regional plant nutrient sulfur deficit in 2012.

To develop these two biggest Asian S markets, The Sulphur Institute (TSI) has continuously worked on promotion of S fertilizer use, increasing public awareness and knowledge about the role of S in agriculture at various levels, including governmental, agricultural research and educational institutes, the fertilizer industry and local farmers by different research, extension and education programs. These programs have made great progress. For example, since 1993, TSI, collaborating with 15 institutions throughout China as a cooperative network has achieved significant advances in evaluation of S fertilizer requirements and promotion of S fertilizer use in Chinese agriculture, identified more than 30% of arable soils in China, equivalent to about 40 million hectares, are S deficient. Sulfur fertilizer significantly increased crop yields in 468 field trials, 87% of the total trials completed, with average yield increases from 7% to 30%. With the increased awareness of the importance of S in agriculture, S fertilizer production and use in China is growing. In 1999, the Chinese government recognized S as a plant nutrient, encouraging production of S-containing NPK compound fertilizers. In 2002, the total S-based NPK compound fertilizer output reached 4.6 million tons, providing 500,000 tons S. It is estimated that S containing compound fertilizer production capacity will increase to over 7.0 million tons, supplying about 700,000 tons S by 2005.

In India, following the recognition of the benefits of S fertilizer for Indian agriculture from the TSI-Fertilizer Association of India (FAI)-International Fertilizer Industry Association (IFA) cooperative project, the Indian government amended the Fertiliser Control Order (FCO) by including the S content of fertilizers as a part of product specifications in the FCO. According to the new amendments of FCO, all manufacturers must specify the minimum guaranteed S content for listed fertilizers and print the S content on the fertilizer bag. This change in the FCO has helped bring S into the mainstream of balanced nutrient application. It is expected that S fertilizer use in India will increase significantly over the coming decade and make a greater contribution to increasing agricultural production through balanced fertilization, including S.

The Western European S market is one of the most advanced in the world. The significant drop in sulfur dioxide (SO_2) emissions since the 1970s, coupled with intensive agronomic practices including the use of high-analysis, S-free fertilizers spurred the region to action to correct the deteriorating S nutritional status. Sulfur deficiency was qualified as a major nutritional problem in arable crops. Comprehensive agricultural research and extension systems facilitate farmers' response to the deficit. It is projected that the market will have a deficit level of 500,000 tons in 2012 within Western Europe, as the increased need for S, becomes more prominent particularly in the North. Additional commercial opportunities are expected to arise in Eastern Europe, as several countries project SO_2 reductions in part resulting from their entry into the European Union.

The current Eastern European S deficit of over 300,000 tons is expected to rise to 400,000 tons by the end of the decade.

In North America, the reduction in atmospheric deposition of SO_2 combined with crop intensification continues to determine S deficiencies. The U.S. Environmental Protection Agency recently estimated that SO_2 emissions decreased 33% between 1983 and 2002, and by 31% between 1993 and 2002, indicating an acceleration of emission reduction. Continued reductions in SO_2 emissions and increased yields are expected to expand areas of S deficiency. The North American deficit for S fertilizers is expected to increase from the current 1.2 million tons to 1.4 million tons by 2012. Some research institutions are evaluating the need to increase current S fertilizer recommendations in line with existing trends. Currently about 1.6 million tons S was applied annually in North America through fertilizers, mostly as ammonium sulfate. The level of S consumption is expected to increase, as numerous fertilizer concerns are developing marketing efforts to increase new sulfate and elemental S fertilizer production.

Latin America is developing as a market for S fertilizer. Agricultural production increased significantly over the last decade, which in conjunction with the rising use of high-analysis fertilizers leads to increasing instances of S deficits, particularly in Argentina. The largest fertilizer consumer, Brazil, is an important and growing user of ammonium sulfate and SSP. The current increased market opportunity in Latin America is estimated at 700,000 tons, and is projected to rise to at least 900,000 tons by the end of the decade.

Sulfur fertilizer sources

There are two types of S fertilizers: those that are in the sulfate form and those that need to go through a chemical reaction to get into the sulfate form for plant uptake. The bulk of S fertilization comes from multi-nutrient fertilizers that are already in the sulfate form. Ammonium sulfate, SSP, and potassium sulfate (K_2SO_4) are the leading products by volume. Although these products were originally applied for their N, P, and K content, respectively, they are increasingly recognized for their S content in its own right. Sulfur is not called the fourth nutrient in vain. All major multi-nutrient S fertilizers provide S in the form of the sulfate anion, readily available for uptake by plants. Adding to the array and sophistication of available S products, elemental S in various formulations and liquid fertilizers are capturing increasing shares of S fertilization, mainly in the developed world, at present.

The trend to increase the N, P, and K analyses of fertilizers over the last four decades gradually squeezed out most of the S in the major N, P and K fertilizers, urea, diammonium phosphate (DAP) and potassium chloride (MOP), respectively. What was once removed because it was considered incidental, is now required.

Multi-nutrient sulfur fertilizers

Ammonium sulfate is mostly produced as a co-product of other industries. An estimated 70% of global output originates from the production of caprolactam, an intermediate for the manufacture of synthetic fibers. A small amount is recovered from coke oven gas, with most of the remainder produced synthetically from sulfuric acid and ammonia. In 2000, approximately 18 million tons of ammonium sulfate fertilizers were produced, equivalent to over 4 million tons of S. Over 3 million tons of S equivalent are used directly, with the remainder used for blending with other fertilizers. The main advantages of ammonium sulfate are low hygroscopicity and chemical stability. It is a good source of both N and S. The acid-forming reaction of ammonium sulfate can be advantageous in high pH soils and for acid-requiring crops. When ammonium sulfate is used for direct application as a N source, much more S is applied incidentally than is typically required. In addition to this N/S imbalance, excessive soil acidity can develop when frequent high rates are applied to poorly buffered soils.

Improvements in the ammonium sulfate formulation processes allow for increasing shares of larger-sized granular material, which is easy to handle and desirable for bulk blending. This has greatly increased application options and spreading performance. Ammonium sulfate is also popular in Europe in the manufacture of compound fertilizers, such as ammonium nitrate plus ammonium sulfate. One grade of 26-0-0-14S is very popular in the European market. Other specialty grades with differentiated N/S ratios also exist. The 26-0-0-14S grade is made by granulating ammonium sulfate in the presence of ammonium nitrate solution or neutralizing sulfuric acid with ammonia in an ammonium nitrate solution and then granulating.

Mixtures of ammonium nitrate and ammonium sulfate are affected by United Nations transportation classification limits (Annon, 2001). In the current regulations, if the ammonium nitrate content is less than 45% the product is deemed non-hazardous for land transport. If the ammonium nitrate content is greater than 45% but less than 70%, and the total combustible/organic material content is less than 0.4%, then the product is UN Class 5.1 (an oxidizer) under UN2067, SP 307. Materials with ammonium nitrate contents above 70% and containing ammonium sulfate are prohibited as fertilizers under these

regulations. Other local regulations may also apply. All are subject to change and current regulations should be reviewed regularly by those intending to formulate products containing ammonium nitrate.

In production of these materials, limiting ammonium nitrate content can frequently result in a grade that has S content too high for the crop requirement. This adds to increasing use of these materials in bulk blends. Approximately 2.7 million tons of ammonium sulfate is produced annually in Western Europe from all manufacturing processes. Historically, this material was shipped to developing countries for use as a fertilizer, but today, increasing amounts have been used within Western Europe for the development of sulfur-containing fertilizers. Recently, some new urea based sulfate-containing fertilizers, (40-0-0-9S) and granulated urea sulfur (38-0-0-13S) were launched in the European market. These sulfate containing multi-nutrient compound fertilizers have several advantages, including lower hygroscopicity than either constituent individually, and have a satisfactory N/S ratio for direct application purposes.

Ammonium sulfate can also be used in clear liquids to make solutions of fertilizer containing N and S. Sulfur concentrations in solutions based on ammonium sulfate solution can vary from 1 to 9%. In liquid formulations made with ammonium sulfate and containing P, the typical S concentrations range from 1 to 3%, although S concentrations can be reached from 5 to 7% with lower P.

In other developments relating to by-product ammonium sulfate production, a new nickel production process is expected to co-produce ammonium sulfate: high-pressure acid leach of nickel lateritic ores came on stream in Oceania. Moreover, in North America and Europe, ammonia-based flue-gas desulfurization technology will produce ammonium sulfate at a coal power plant and an oil sands project. The increasing availability of the inexpensive by-product sulfuric acid may encourage increased production of ammonium sulfate - particularly if credit can be obtained for its S values.

Single superphosphate was once the most important phosphate source in the world and still is a major fertilizer in China, India, Brazil, Australia and New Zealand due to its P and S contents. Single superphosphate contains 12 to 22% phosphate and 10 to 14% S and is an excellent source of P, S and calcium. The occurrence of S deficiencies has been delayed in many areas of the world because of the involuntary addition of S when large amounts of SSP were used to supply P. Its calcium content, ranging from 18 to 21%, can be important in soils low in this nutrient.

Total S content in SSP used in 2000 was 4.0 million tons, mostly produced in Brazil, China, India, Australia and New Zealand. Production of SSP is relatively stable with a tendency to decline; the majority of P capacity expansion plans include tradable compound fertilizers and ammoniated phosphates; this contributes further to S deficiencies and the need to replace the foregoing S source.

Potassium sulfate is the main S-containing potash fertilizer. It contains 42 to 44% K (50 to 53% K_2O) and 17 to 18% S. For purposes of this discussion potassium-magnesium sulfate is also included. The current global market for these materials is approximately 1.6 million tons of products, equivalent to close to 300,000 tons of S per year. About half of global production is mined directly from potash and sulfate salts or brines requiring no additional S. Potassium sulfate can also be produced based on the reaction between potassium chloride and sulfuric acid, known as the Mannheim Process. Potassium sulfate is normally used for situations and crops susceptible to high chloride and salt concentrations; it is facing increased competition from potassium nitrate as a chloride-free potash fertilizer, thus signaling another potential source of S deficit. Potassium-magnesium-sulfate is a double salt and contains 22% K (27% K_2O), 11% Mg and 22% S. It has the advantage of supplying multi-nutrients, K, Mg and S and is frequently included in mixed fertilizers on soils deficient in these three nutrients. They are particularly useful when low levels of chloride are desired, as is often the case for crops such as tobacco, potatoes, peaches, some legumes and turf grass.

Kieserite ($MgSO_4.H_2O$), usually listed as a sulfate based Mg fertilizer, is produced from a natural salt deposit and is a highly concentrated two-nutrient fertilizer containing 15 to 17.5% Mg and 20 to 23% S. Kieserite has a neutral reaction regarding soil acidity, and thus it is suitable for all soil types. Owing to its high solubility, both the Mg and the S are immediately available to the plant. Kieserite is a suitable fertilizer for either direct or blended application, and can also be used in clear liquids and foliar sprays. Commercial kieserite products are available in both fine and granular forms in the European market.

Gypsum (calcium sulfate) is not as widely used as a fertilizer compared to ammonium sulfate. Most calcium sulfate is commercially available in forms that are not as easy to handle, blend, and spread. A more important reason for its limited use as a fertilizer, however, is its relatively low analysis. One notable exception is the use of gypsum in peanut (groundnut) production. The calcium is required for proper plant pegging.

Within Europe, there is another group of fertilizers that can contain S. These are compound fertilizers that are produced by the nitrophosphate process and/or the mixed acid route. Nitrophosphate fertilizers are, as the name implies, fertilizers produced

by a process involving treatment of phosphate rock with nitric acid.

After separation of the major part of the calcium nitrate, phosphoric acid is neutralized with ammonia to produce a fertilizer. The remaining calcium nitrate, not typically recognized for its nutritive value, and known for its effect on phosphorus availability, can be converted into calcium sulfate nitrate by sulfate addition.

While not a necessarily common method, the solution obtained by reaction of nitric acid with phosphate rock can also be treated by the addition of a soluble sulfate to precipitate part or nearly all of the calcium as calcium sulfate. In commercial processes, ammonium sulfate, potassium sulfate, and sulfuric acid have been used. The calcium sulfate may be separated by filtration and removed to form a higher grade product, or allowed to remain in the product. Compound fertilizers produced by the nitrophosphate process can have S concentrations varying from 2 to 21% according to a recent survey conducted by TSI.

Ammonium sulfate, SSP, and potassium sulfate materials remain important S sources; however, their stable to declining production base, against the backdrop of growing S deficiencies, and the increasing sophistication and understanding of fertilizer actions have attracted new S sources that are increasing market share. Sulfur fertilizer producers are introducing new products to meet diversified and specific application requirements. These can be categorized broadly into elemental S-based fertilizers and liquid S formulations.

Elemental sulfur fertilizers

The use of elemental S as a fertilizer is increasing mostly in the developed world and is projected to continue. Limited, if any, expansion of sulfate-containing carriers has resulted in industry giving attention to elemental S as a means to correct S deficiencies. Two features of elemental S highlight its use as a controlled-release fertilizer for permanent pastures and crops. First, it is the most concentrated S form, which lowers transport and application costs. Secondly, it offers reserve availability. Elemental S is converted to sulfate over time. Thus, availability is a function of this process, which depends on the elemental S particle size, soil microorganism activities, and environmental factors. Elemental S fertilizers are now manufactured in Oceania, North America, Western Europe and West Asia.

The effectiveness of elemental S as a fertilizer is governed by its oxidation rate, which is a biological process carried out principally by bacteria of the genus *Thiobacillus*. The bacteria feed on elemental S and oxidize it to the sulfate form, making S available to plant roots. Physical factors, including soil temperature and moisture, play an important role in determining rates of S oxidation. A third critical physical factor influencing oxidation is particle size of the applied elemental S. Finer particle size increases the oxidation rate, as the greater specific S surface area provides for greater access and action by microbes. The application of coarse elemental S historically produced low yield response in S-deficient annual crops, attributable to low oxidation rates associated with large particle size. The elemental S fertilizer industry has come a long way since those early days.

Elemental S can be readily incorporated into N/P fertilizer materials to provide 5 to 20% sulfur with various technologies. However, the use of elemental S in combination with ammonium nitrate should be avoided, and is prohibited in some jurisdictions, for safety reasons. Monoammonium and diammonium phosphates (MAP or DAP) containing from about 5 to 20% S can be made by applying a hydraulic spray of elemental S at 1.4 kg/cm^2 during drum or pan granulation. Recently, a new sulfate and elemental sulfur-enriched MAP fertilizer was developed in North America, containing 15% sulfur, ammonium-nitrogen and phosphate. This granular fertilizer containing 50% elemental S and 50% sulfate-S provides readily available S for early plant uptake and residual S for later in the growing season. It is suitable for bulk blending with other granular fertilizers or direct application.

New Zealand and Australia, along with the United States and Canada, were at the forefront in elemental S fertilizer research and technology, with S deficiencies recognized and addressed since the 1950s. Most research was oriented to areas of deficiency, suitable diagnostic tests, plant S requirements, S cycle modeling, oxidation modeling of elemental S, and development of effective S fertilizers. This research led to the development of suitable elemental S fertilizers including the methodologies to incorporate elemental S with fertilizers, either during processing or into the finished product. Sulfur enriched SSP is one of the examples, which is popular in Australia and New Zealand. Single superphosphate is enriched with elemental S to make mixtures containing 18 to 35% sulfur. The added S is superior in its residual effect to the sulfate in the SSP. This S-enriched SSP has received attention in the area with high leaching losses of plant nutrients because of its potential for reducing sulfate leaching loss and also providing available sulfate to meet crop needs during the whole growing season. More recent S fertilizer research in New Zealand was directed towards the development of technology to produce fine-particle elemental S suitable for incorporation into high-analysis P fertilizers or as a degradable granulated product appropriate for dry blending. An emulsifying process was developed to overcome the

spontaneous ignition problem when grinding elemental S.

Sulfur bentonite products are manufactured by a number of processes, with molten S blended with swelling bentonite clays and solidified into useable forms, usually granules or pastilles. This material has gained popularity in North America and to a limited degree in Western Europe. Generally, research results indicate that particle sizes of 0.15 mm to 0.20 mm or smaller are required if elemental S is to be fully effective during the growing season in which it is applied. The modern concept behind S bentonite fertilizers is that after application the bentonite or other binding agent absorbs moisture from the soil, causing it to expand and subsequently dispersing the material into minuscule elemental S particles that oxidize rapidly. A product with a range of particle sizes is preferable in many circumstances, allowing for short-term and long-term release. A water-degradable product containing 90% S granulated with bentonite clay is most widely produced. Produced in pastille and granular forms, these products can be used in bulk blends, direct soil applications, and suspensions as a plant nutrient S source. Recent innovations in production technology and anti-dusting agents resulted in the marketing of more effective products, such as a combination of S and sulfate product that offers both immediate available sulfate and slow release S together to maximize S supply for plant nutrition.

Alternative formulations of elemental S, particularly tried in Oceania, included mixtures with phosphate rock, SSP, either molten or in dry form. Adhesion of elemental S to finished products, such as triple superphosphate (TSP), DAP, and urea, offered new opportunities. This approach is an alternative to the methodology to form elemental S into granules or prills using bentonite or other binders. A new process was developed, which solved some problems regarding S fertilizer application in flooded and non-flooded crops and pastures, including improved S dispersion from the granule and better spatial distribution characteristics. A product, with micronized S bonded with special binders onto granules of high-analysis TSP is also available. The process establishes an elemental S coating on the surface of the TSP's granules. The S is non-leachable, but in a form that is readily oxidized by soil microorganisms. The special coating process involves the creation of an adhesive film on the surface of the granules by spraying minute quantities of water into a tumbling bed. The S-based dry coating material is applied after the adhesive film is established. This product offers a valid combination for situations requiring high-analysis fertilizers and the need to apply S. An expanded product line is available using other granular fertilizers, including DAP, MAP, and urea.

In conclusion, the demand for micronized elemental S and elemental S-modified compound fertilizers is increasing worldwide, especially in Oceania, North America and Western Europe. In North America, elemental S consumption for fertilizer use was estimated at close to 300,000 tons in 2000, and is projected to climb to 500,000 tons by the end of the decade, assuming a modest annual growth rate of 6%. Western European efforts to reduce atmospheric S also have created a huge market for the S based fertilizer industry in the coming decade, with elemental S expected to take a portion of this market.

Liquid sulfur fertilizers

Low water solubility hampers the use of mainstream sulfate fertilizers such as ammonium sulfate and potassium sulfate, in liquid or suspension fertilizer formulations, which have gained importance. Ammonium thiosulfate solution (ATS) is a popular source of S for use in liquid fertilizers because of its solubility and compatibility with various ions. Fertilizer-grade ATS in its commercial form is in a 60% aqueous solution with a (12-0-0-26S) analysis. It is compatible in any proportion with neutral to slightly acidic phosphate-containing solutions or suspensions, as well as with aqueous ammonia (NH_3) and N solutions. It is not compatible with anhydrous NH_3 or strong acids; thus, a wide variety of N-S, N-P-S, and N-P-K-S formulations are possible utilizing this material. Ammonium thiosulfate can be applied directly by drip, sprinkler or flood irrigation. It does not corrode metal piping nor clog spray nozzles. Thiosulfate S is unique in that it exists in two oxidation states, making it more suited to the S uptake patterns of most plants; it decomposes in the soil to form approximately equal amounts of sulfate and elemental S. The sulfate is available immediately whereas the elemental S is gradually converted to sulfate by bacterial oxidation. Ammonium thiosulfate may be synthesized by reacting SO_2 and NH_3 in aqueous solution forming at first ammonium sulfite, which reacts further with elemental S to form ATS solution. Alternatively, NH_3 may be absorbed in an ATS solution, reacted with SO_2, then further reacted with hydrogen sulfide to form ATS solution and S.

Ammonium thiosulfate has gained prominence in North America and is growing in use and importance in Europe, because of its versatility and high S concentration in fluid formulations. It is estimated that the total production capacity in North America reached about 1.4 million tons in 2000 and 700,000 tons of ATS (180,000 tons S) were consumed. Future North American demand for ATS is expected to continue to grow due to overall increasing recognition of the sulfur benefits and higher recommendation rates.

Table 1:
Product nutrient analysis.

Sulfur Fertilizers	Content (%)			
	S	N	P_2O_5	K_2O
Ammonium nitrate with ammonium sulfate or ammonium nitrate sulfate	7 to 16	up to 30	0	0
Ammonium nitrate with gypsum	3 to 6	24 to 27	0	0
Ammonium phosphate sulfate	6 to 17	variable	variable	0
Ammonium polysulfide	40 to 45	20 to 21	0	0
Ammonium sulfate	24	21	0	0
Ammonium sulfate liquid	9	8	0	0
Ammonium thiosulfate solid	43	19.5	0	0
Ammonium thiosulfate solution	26	12	0	0
Calcium nitrate with sulfur	1 to 5	15	0	0
Calcium sulfate (dihydrate gypsum)	17 to 18	0	0	0
Calcium sulfate (hemihydrate gypsum)	19 to 22	0	0	0
Calcium sulfate (anhydrite gypsum)	22 to 24	0	0	0
Fortified SSP	28 to 50	0	5 to 16	0
Iron pyrites	54	0	0	0
Magnesium sulfate (Epsom salt)	13	0	0	0
Magnesium sulfate (Kieserite)	10 to 23	0	0	0
Micronized sulfur*	50 to 99	0	0	0
Mixed-grade NKs with sulfur	5.2 to 10	variable	0	variable
Mixed-grade NPs with sulfur	2 to 21	variable	variable	0
Mixed-grade NPKs with sulfur	2 to 17	variable	variable	variable
Mixed-grade PKs with sulfur	2 to 15	0	variable	variable
Nitrogen-sulfur solutions	2 to 6	7 to 35	0	0
Potassium magnesium sulfate	22	0	0	22
Potassium sulfate	17 to 18	0	0	48 to 52
Potassium thiosulfate	17	0	0	25
Single superphosphate - SSP	11 to 14	0	16 to 20	0
Sulfur (elemental)	50 to100	0	0	0
Sulfur bentonite	90	0	0	0
Sulfur-coated DAP	12	12 to15	40	0
Sulfur-coated MAP	12	8 to 10	44	0
Sulfur-coated TSP	10 to 20	0	38 to 43	0
Sulfur-coated urea	10 to14	38 to 40	0	0
Sulfur with micronutrients	2 to 80	0	0	0
Urea with sulfur	5 to 6	40	0	0
Urea sulfuric acid	9 to 18	10 to 28	0	0
Zinc sulfate	11	0	0	0

*Includes wettable/dusting powders (dry powder) and flowable sulfur (liquid suspension)

The largest producer of ATS has developed other liquid S fertilizers: ammonium polysulfide solution (20-0-0-40S), potassium thiosulfate (0-0-25-17S, particularly suited as a starter fertilizer) and calcium thiosulfate solution, for crops and situations requiring these other nutrients besides S. Thiosulfates ($S_2O_3^{2-}$) are non-corrosive and non-hazardous to handle; they also are well adapted to the methods used to apply fertilizer solutions. They are clear, liquid fertilizers that are suitable for direct applications or blending, offering versatility to farmers and fertilizer retailers. Manufacturers produce thiosulfates in North America and Western Europe. New liquid formulations include (26-0-0-3.1S), for early season use and suitable for all crops, particularly cereals, oilseed rape, and grass. For foliar applications, (35-0-0-1.7S) and (20-0-0-1.7S) are marketed, as are other fertilizers with S, based on ATS tailored to individual requirements.

Potassium sulfate tends to react with ammoniacal N, phosphates, and metal ion impurities to form insoluble deposits. The largest producer of potassium sulfate in North America developed a grade twice as soluble as ordinary potassium sulfate, produced as a dry, fine crystalline material with a (0-0-49-17S-1Mg) analysis. This breakthrough increased the use of potassium sulfate in liquid formulations and fertigation. The product also has a low salt in-

dex, reducing the impact on salt-sensitive soils and crops. It is more stable in solution at low temperature than potassium nitrate, thus reducing problems of salting out during storage, transport, and application.

Conclusions

The S fertilizer industry has developed materials adapted and suited to particular crop and soil management situations. (Table 1) Traditional sources, ammonium sulfate and SSP will continue to lead in consumption for S fertilizers in the near-term. However, elemental S-based materials will become more readily available for dry fertilizer applications and thiosulfates will continue to gain in popularity for fluid fertilizer applications. Sulfur, unlike N, P, and K fertilizers, offers a much wider range of products, which provide versatility for a variety of applications. However, farmers, fertilizer dealers, extension agronomists, and others in the agricultural community need to better understand how these products work for optimal performance. The fertilizer industry needs to invest more on education and promotion programs to accelerate commercialization of S products as both a fertilizer and soil amendment, which will provide significant benefits to both fertilizer manufacturers and farmers.

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Sulfur in organic farming

Hans Marten Paulsen[1]

Abstract

Beyond the natural role of sulfur as plant nutrient, in organic farming it is an important fungicide and acaricide. S as plant nutrient has to be kept at a sufficient level because it can help in saving nitrogen and in reducing nitrogen leaching. S influences the nitrogen fixation of legumes, which is the essential microbiological process for plant production in organic farms. S is determining quality aspects of feedstuffs and other products. An adequate S nutrition of plants is therefore essential. But in organic farming practice negative S balances are found. To decide about fertilization needs, organic farmers need to know about S flows in soils, S supply to plants, necessary S contents in plants and also about S availability in soils, in organic materials and in different fertilizers. Various S-containing fertilizers are approved in organic farming and could be used to correct S imbalances. Due to its low S content and low S availability manure application is of low importance for the S nutrition of plants.

Keywords: Acaricide, elemental sulfur, fertilizers, fungicide, organic farming, sulfur fertilization, sulfur fertilizers

Introduction

In organic farming the input of chemo-synthetic fertilizers is forbidden. Sulfur (S) in organic farms can be supplied together with S containing approved fertilizers or raw S from natural sources. Even if S deficiencies in plant nutrition are reported in conventional agriculture, S fertilization in organic farms is not of practical importance up to now.
In organic farming the use of pesticides is strictly limited to natural sources and has to be certified by the control bodies in advance (IFOAM 2002; EU, 1991). S used as fungicide and acaricide is of special importance in organic vine- and pomefruit-production. In the following article the importance of S, S balances and S use in organic agriculture are reviewed and described. The legal base used for the discussion and description is the Council Regulation (EEC) No 2092/91 of 24 June 1991 on organic production of agricultural products and indications referring thereto on agricultural products and foodstuffs (EU, 1991).

Sulfur as fungicide and acaricide

Limiting legislation on pest-, disease- and weed-control in organic farming is given as guideline of worldwide validity by the IFOAM Basic Standards of Organic Production and in European law by the Council Regulation (EEC) No 2092/91 of 24 June 1991 (EU, 1991). Additional restrictions are given by different organic grower associations in the whole world, which are listed in Willer and Youseffi (2004). According to the EEC 2092/91 pests, diseases and weeds shall be controlled by a combination of the following measures: Choice of appropriate species and varieties, appropriate rotation program, mechanical cultivation procedures, protection of natural enemies of pests through provisions favorable to them (e.g. hedges, nesting sites, release of predators) and flame weeding. Only in cases of immediate threat to the crop may recourse be had to direct measures with products referred to in Annex II of the regulation. In organic viniculture, organic fruit and vegetable growing elemental S (S^0) is a main and essential agent of plant protection to keep the internal and external quality (Palm and Klopp, 2004; Kienzle, 2004; Hofmann, 2004; Table 1).

Table 1:
Target organisms for elemental S (S^0) application and common doses used in organic vine- and pomefruit production according to Palm and Klopp, 2004; Kienzle, 2004; Hofmann, 2004.

S^0 as fungicide:
Powdery mildew in vine (*Uncinula necator*, *Oidium tuckeri*)
Powdery mildew in tomatoes (*Oidium lycopersicum*)
Apple scab, pear scab (*Venturia spp*.)
Cherry leaf spot (*Blumeriella jaapii*)
Leaf rust on plum (*Tranzschelia pruni spinosae*)
S^0 as acaricide:
Pear bud, grape bud (*Eriophyes piri*, E. viti)
Rust mite in vine (*Phyllocoptes vitis*)
S^0-dosage per year:
Pome fruits: 21-27 kg S^0 per meter crown height divided in up to 30 applications
Vine: up to 9 applications between 3.6 an 4.8 kg ha^{-1}

[1] Institute of Organic Farming, Federal Agricultural Research Station (FAL),Trenthorst 32, 23847 Westerau, Germany

Winkler and Stein (2004) summarized risk assessments and findings for S^0 in the environment when used as plant protection agent as follows. S^0 has low toxicity for mammals, birds and fish and high no-observed-effect-concentration (NOEC) values for plants. Soil application of 10 and 100 kg ha^{-1} S^0 lowered N- and C-mineralization. The legislative limit of a level of 75 % of the N- and C-mineralization in S^0-treated soil in comparison to untreated soil after 100 days was reached after 14 and 66 days respectively. S^0 is relatively immobile in soils and is leached as sulfate (SO_4) after incorporation and oxidation in the soil sulfur cycle. S^0 is hydrophobic and nearly not watersoluble. When reaching surface waters it is incorporated in the soil after sedimentation. Additional SO_4-loads to waters from oxidation under aerobic conditions are irrelevant under consideration of natural water contents. S^0 is toxic for green algae (e.g. *Scenedesmus subspicatus*) and water fleas (e.g. *Daphina magna*). Therefore safe distances to waters are necessary when S^0 is applied. S^0 is toxic for different non-target terrestial arthropodes (e.g. *Trichogramma cacoecie*) but further studies on the toxicity of S^0 for arthropodes are necessary. Due to this restricted knowledge on the effects of S^0-application on non-tagret-terrestial arthropods, Winkler and Stein deduced, that a final risk assessment for S^0 in the environment according to the rules of the German plant protection law (PflSchG, 1998) is not possible at the moment. Several S^0-products have a new admission for the use as plant protection agent. Even if in organic farming legislation no limits in dosage is given, German organic farmers have to keep to the application restrictions of the German plant protection act. But a natural limit on S^0 application used as acaricide e. g. in organic apple production is set by biological balances because high S^0 doses are killing beneficial mites (e.g. *Amblyseius spp.*) as well. Those mites are natural predators of spider mites that are non controllable in organic farming (Palm and Klopp, 2003) and are urgently needed to keep a natural balance.

But still S^0 as fungicide is of high importance in organic pest management and is an essential tool in organic vine and fruit production. The legal restrictions are under discussion but the lacks in knowledge on environmental effects have to be filled to ensure a reasonable future use of S^0 in organic agricultural systems (Kühne and Friedrich, 2003). Research on alternatives to S^0 as fungicide is focusing on direct measures like different plant strengtheners based on SiO_2, different plant extracts, milk products, $NaHCO_3$, lactic acid bacteria and other microorganisms and on resistant plants. As indirect control measures supporting of soil antagonist populations and removal of plant residues are reported (Berkelmann-Löhnertz and Kauer, 2003; Hofmann, 2003). S^0 used as acaricide in organic farming can not be substituted up to now (Pfeiffer, 2003).

Sulfur as plant nutrient

S is an essential plant nutrient influencing internal and external quality, plant growth, health and nutrient efficiency of agricultural crops. In plants S is involved in the composition of amino acids, in the determination of the protein content, in aspects of baking quality, in the formation of secondary plant components and pharmaceutical components, in the nitrogen metabolism of plants and in the resistance of plants against pests and diseases.

According to the Council Regulation (EEC) No 2092/91 the fertility and the biological activity of the soil must be maintained or increased, in the first instance, by the cultivation of legumes, green manures or deep-rooting plants in an appropriate multiannual rotation program, incorporation of livestock manure from organic livestock production and by incorporation of other organic material, composted or not, from holdings producing according to the rules of this regulation. Other organic or mineral fertilizers, mentioned in Annex II, may, exceptionally, be applied, as a complement to the extent that adequate nutrition of the crop being rotated or soil conditioning are not possible by the methods mentioned before. In organic farming S can be applied as a component of approved fertilizers (Table 2) to compensate expected or acute S deficiencies. S from sulfate (SO_4) sources is readily plant available whereas S^0 has to be oxidized in soil before plant uptake. The oxidation speed of S^0 is limited by high particle sizes (Fox et al., 1964, Gupta et al., 1998, Paulsen, 1999) and small populations of thiobacteria in soil (Schnug and Eckhardt, 1981).

Table 2:
Approved S containing fertilizers in organic farming according to the Council Regulation (EEC) No 2092/91.

Fertilizer	S content
Potassium sulfate	18 % SO_4-S
Kieserite*	22 % SO_4-S
Epsom salt	13 % SO_4-S
Gypsum (from natural sources)	14 % SO_4-S
Calcium carbonate with S (gypsum from natural sources)	2-4 % SO_4-S
Elemental S (from natural sources)*	80 % S^0-S

* Use has to be authorized by the inspection body

Table 3:
Dry matter- (DM), N- and S-contents of cattle slurry (n=14) and cattle farmyard manure (n=43) from organic farms in England (Shepherd et al., 2002).

	Slurry				Farmyard manure		
	Mean	Range	SD		Mean	Range	SD
DM (%)	7.9	1.0-12.0	3.57	DM (%)	21.0	13.0-38.0	5.83
Total N (kg m^{-3})	2.5	0.3-4.1	1.19	Total N (kg t^{-1})	5.2	2.9-7.8	1.16
S (kg m^{-3})	0,29	0.03-0.53	0.139	S (kg t^{-1})	0.8	0.3-1.8	0.30

Values expressed on a fresh volume or weight basis

Organic materials used in fertilization have low S contents and low S availability (Eriksen et al., 1995). Ranges of S and N contents of manure and slurry from organic farms in England were surveyed by Shepherd et al., 2002 (Table 3). The N/S ratios of slurry (1/0.12) and farmyard manure (1/0.15) are wide.

Furthermore the mineralization of organic S from organic materials added to soils is mainly dependent of the C/S ratio of the materials (Figure 1). From manures with C/S ratios between 430 and 735 between 47 % and 127 % from the organic S were mineralized to SO_4-S respectively. Mean values ranged between 5 % (horse manure) and 31 % (chicken manure). Digested materials had a relatively constant S mineralization of up to 97 %, decreasing with increasing C/S ratio (Tabatabai and Shae, 1991). According to the values given in Table 3 and figure 1 from 16 kg S applied together with 20 t farmyard manure per hectare only 2.6 kg S would be plant available. Farmyard manure and slurry therefore are only poor S sources in organic plant nutrition.

Due to the lower yield level in organic farms compared to conventional farming, S uptake and S demand of the crops are lower as well. Therefore S fertilization is not common in organic farms up to know. But S balances determined in a survey in Denmark (Table 4) are showing that normal organic crop rotations already have negative S balances (Erikson et al., 2002).

So it must be expected that in high S demanding crops or in years with favorable growth conditions and with high yield levels an insufficient S nutrition, at least in parts of the vegetation time, will likely to be occur in organic plant production as well. Because soil structure and water movement are determining the S supply to a large extent (Bloem et al., 1998) it is necessary to have a close look on site specific conditions influencing the S supply to plants.

Because organic farms rely on mineralized soil-nitrogen, temporary N-deficiency in early spring is widespread and can be mixed up with S deficiency symptoms (Schnug and Haneklaus, 1997). Therefore in organic farms for the identification of S deficiency expert knowledge is needed to avoid misinterpretations.

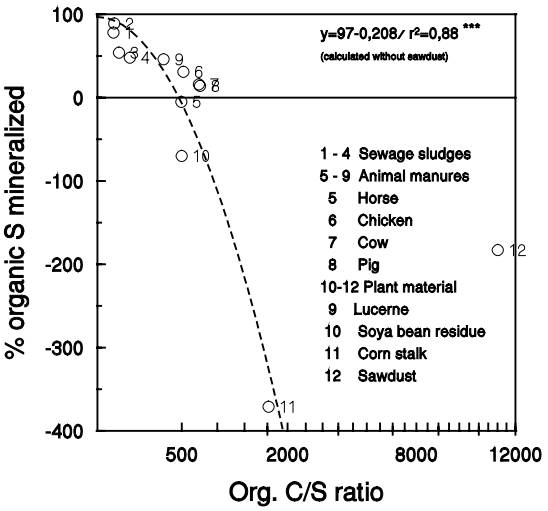

Figure 1:
Mineralization of organic S from waste materials with different C/S ratios added to soils. Mean values of five soils as difference between treated and untreated soil (after Tabatabai and Chae, 1991).

Additionally due to the lower yield levels in organic production critical nutrient thresholds for S and other plant nutrients extracted from field surveys and fertilization trials (Schnug et al., 1997, Haneklaus and Schnug, 1998; Bergmann 1993) have to be revised and must be adopted to yield expectations of organic production. Only an exact knowledge on S demands of crops grown in systems with lower yield expectations can result in an adequate S fertilization strategy in organic farms.

S and N nutrition of plants are metabolically linked (Hawkesford et al., 1994; Amâncio et al., 1998). In grassland and crops the application of S has been shown to increase the efficiency of N use by plants. Adequate S supply is increasing the N-recovery and reduces N losses from the system (Brown et al., 1999; Schnug and Haneklaus, 1994). So also in organic farming the control of the S nutri-

Table 4:
Sulfur balance (kg ha^{-1}) in an organic crop rotation as average of year, location and crop[a] (Eriksen et al., 2002).

	Input[b]			*Output*		*Balance*[c]
	Deposition	Manure	Irrigation	Plants	Leaching	
Year						
1997-1998	10	4	9	3	34	-13ab
1998-1999	10	3	6	3	34	-18ab
1999-2000	10	3	0	2	19	-7a
Location						
Jyndevand	10	4	15	2	32	-6a
Foulum	7	2	0	3	34	-28b
Flakkebjerg	13	5	0	2	20	-4a
Crop						
Barley	10	7	5	3	31	-12a
Grass-Clover	10	0	4	0	22	-8a
Winter Wheat	10	8	6	4	30	-11a
Parley/pea	10	0	5	4	33	-22b

[a]Main effects did not interact
[b]Assuming no variation between replicates
[c]Values with the same letter are not significantly different within the group ($P<0.005$)

tion of plants could help in saving nitrogen and in reducing nitrogen leaching. Also in nodule formation of legumes S has an important role (Howieson et. al., 2000). It is part of a metal-sulfur-cluster, acting as catalyst during nitrogen fixation (Schneider and Müller, 1999). So S deficiency can induce N-deficiency of legumes (Mason and Howieson, 1988). S as key component in nitrogen fixation, which is the essential microbiological process for plant production in organic farms, therefore should be carefully kept in mind in organic production.

The S balance of organic plant production also has consequences for organic animal production. Organic farming aims at the use of local feedstuffs in livestock production. Therefore oilcakes are valuable energy and protein sources and are used as substitute for imported soy (Zollitsch et al., 2000). S in excess can lead to increased glucosinolate concentrations in different oilseeds (Zhao et al, 1994) and may limit their use as component in feedstuffs (Jeroch et al., 1997).

On the other hand S-containing amino acids - mainly methionine - are limiting factors in home grown organic feedstuffs for monogastric animals, especially in rations for poultry (Zollitsch et al., 2000). The use of synthetic amino acids to correct imbalances of feed rations is not allowed in organic production. An adequate S nutrition of plants helps maintaining the methionine and cysteine content of plants (Eppendorfer et al., 1992).

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Sulfur nutrition and its significance for crop resistance – a case study from Scotland

Ioana Salac[1], Silvia H. Haneklaus[1], Elke Bloem[1], Elaine J. Booth[2], Karene G. Sutherland[2], Kerr C. Walker[2] and Ewald Schnug[1]

Abstract

Severe sulfur deficiency causes a decrease in yield and has a negative impact on crop quality. Besides this, a higher susceptibility of crops to certain diseases was observed. Sulfur fertilization proved to lower disease incidence and severity of fungal infections in different crops. The sulfur metabolism provides several potential mechanisms by which plants are able to tackle biotic stress. The identification of these processes and adaptive control of sulfur induced resistance (SIR) against fungal diseases offers the opportunity to develop natural plant protection measures by means of targeted fertilization strategies. In the present paper, the results from a field experiment in Scotland are summarized, which reflect the influence of the sulfur nutritional status on sulfur-containing metabolites and infection with fungal diseases.

Key words: cysteine, glutathione, glucosinolate, Pyrenopeziza brassicae, sulfur induced resistance (SIR)

Introduction

Since the beginning of the 1980s severe sulfur (S) deficiency can be observed regularly under field conditions in the northern parts of Europe because of continuously decreasing S inputs to agroecosystems (Schnug and Pissarek, 1982; Schnug and Haneklaus, 1994). High S demanding cruciferous crops reacted first to a reduced S supply (Schnug and Pissarek, 1982) and about 10 - 15 years later, low demanding crops such as cereals and sugar beat also showed S deficiency (Schnug et al., 1993; Schnug et al., 2000). In Scotland, the infection of oilseed rape plants by fungal pathogens such as *Pyrenopeziza brassicae* (anamorph: *Cylindrosporium concentricum*) (*P. brassicae*) and *Leptosphaeria maculans* (anamorph: *Phoma lingam*) increased during the 1980s (Brokenshire et al., 1984). This phenomenon was attributed to the drastically reduced atmospheric S depositions in this region (Dore et al., 2003) as S was found to play a key role in the defense system of plants (Schnug and Ceynowa, 1990; Schnug et al., 1995a). Soil-applied S increased the resistance against various fungal diseases in different crops under greenhouse (Wang et al., 2003) and field conditions (Schnug et al., 1995a; Bourbos et al., 2000; Klikocka et al., 2004; Bloem et al., 2004; Salac et al., 2004). Based on these findings the concept of SIR (Sulfur Induced Resistance) was developed (Schnug, 1997).

The concept of Sulfur Induced Resistance (SIR)

The S metabolism of plants offers different possibilities to tackle with biotic stress. It includes an increased synthesis of natural compounds (e.g. H_2S, cysteine, methionine, glutathione), the degradation of glycosides (e.g. glucosinolates) and the synthesis of new compounds (e.g. phytoalexins; Figure 1) (Haneklaus et al., 2004). Supposedly, these S-containing defense compounds are released in a chain reaction triggered by the pathogen and controlled by the S status of the plant (Haneklaus et al., 2004, Figure 1).

Cysteine is the precursor of all relevant S-containing metabolites putatively involved in SIR (Figure 1) and therefore it might be assumed that cysteine is one of the cornerstones of plant resistance against pathogens. Previous studies have shown that the cysteine concentration in plant tissues is strongly related to the S nutritional status of plants (De Kok, 1990; Schnug, 1997) and that cysteine is enriched in resistant plant tissues (Vidhyasekaran, 2000). Cysteine can be rapidly degraded to H_2S or metabolized to other compounds that are putatively involved in pathogenesis (Figure 1).

H_2S is fungitoxic and plants have the ability to release H_2S and other gaseous S compounds into the atmosphere by different enzymatic reactions (Schroeder, 1993; Burandt et al., 2001; Bloem et al., 2004). Glutathione (γ-glutamyl-cysteinyl-glycine) (GSH) was found to accumulate rapidly in response to fungal attack (Vanacker et al., 2000; Bloem et al., 2004; Salac et al., 2004) and this was proven to be related to pathogenesis (Gullner and Kömives, 2001). Glucosinolates (GSLs) undergo hydrolysis, catalyzed by the enzyme myrosinase, to produce an array of products of which isothiocyanates are a major component (Luethy and Matile, 1984). These compounds, and other products of GSL hydrolysis, have been shown to be toxic or inhibitory to many

[1] Institute of Plant Nutrition and Soil Science, Federal Agricultural Research Centre (FAL), Bundesallee 50, D-38116 Braunschweig, Germany
[2] Scottish Agricultural College, Ferguson Building, Craibstone Estate, Bucksburn, Aberdeen, AB21 9YA, Scotland

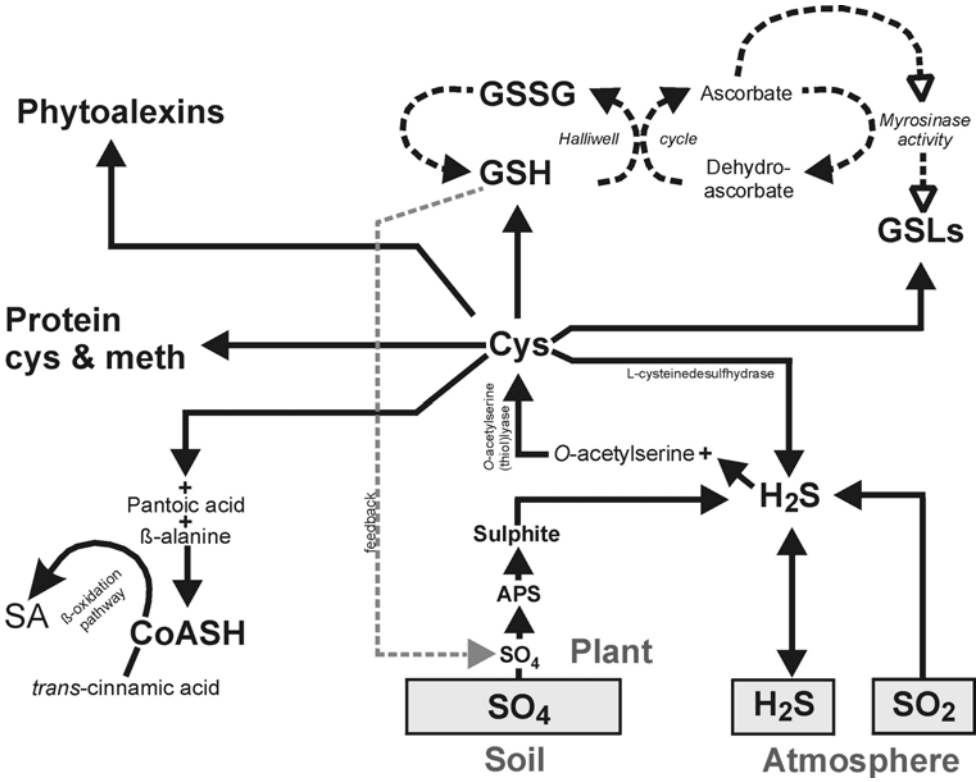


Figure 1:
Sulfur metabolites and pathways putatively involved in chain reactions of SIR in *Brassica* species (Haneklaus et al., 2004).

species of fungi and bacteria (Greenhalgh and Mitchell, 1976; Mithen et al., 1987; Doughty et al., 1991). Phytoalexin synthesis is induced after infection, involving *de novo* synthesis in the infected plant tissue (Hammerschmidt and Nicholson, 2000). The involvement of phytoalexins in SIR is obscure and can only be speculated from the dependency of their precursors on S. High levels of pathogenesis-related proteins were found to be related with enhanced disease resistance in plants (Bohlmann, 1999; van Loon, 1999). However, their possible role in SIR still requires empirical proof. The significance of the formation of elemental S in plants for defense has been discovered only recently (Williams et al., 2002), but the exact mode of action is still unclear.

Most investigations on the putative role of S-containing compounds in SIR were carried out *in vitro* and in pot experiments. Factors governing initialization and strength of SIR need to be tested, however, under field conditions in order to identify and regulate resistance mechanisms by means of targeted S applications. In a field experiment in Scotland, the influence of soil-applied S fertilization on disease incidence and severity of fungal diseases was tested and set in relation to extent and variation of S-containing metabolites in order to perceive triggers and magnitude of SIR.

Material and methods

Design of the field experiment

A quadri-factorial field experiment was carried out in 2001/2002 in Aberdeen, Scotland (W 2° 13`, N 57° 12`; 60 m a.s.l) on a loamy sand (Humic Podzol according to the FAO classification system). The plot size was 40 m^2. Plots were arranged in a split-plot design in four blocks. Two oilseed rape cultivars with different susceptibilities against *P. brassicae* were grown: *Bristol* (B; susceptible) and *Lipton* (L; resistant) (HGCA Recommended List WOSR 2003). For defining the growth stages (GS) of oilseed rape the BBCH scale was used (Strauss et al., 1994).

S was applied as K_2SO_4 to the soil at rates of 0 (S_0) and 100 kg S ha^{-1} (S_{100}). The K supply was balanced by fertilizing adequate amounts of KCl. The S

dose was split in two equal parts in autumn (GS 04) and in spring (GS 14 - 15). N was supplied as NH_4NO_3 at rates of 100 and 200 kg N ha^{-1}. 100 kg N ha^{-1} was applied to all plots at the start of the vegetation period (GS 14 - 15) and an additional 100 kg N ha^{-1} was fertilized at the beginning of stem elongation (GS 30) to those plots receiving a higher N dose.

Specific fungicides were used against *P. brassicae* infections. Either no fungicides were applied or the plots received 0.4 L ha^{-1} flusilazole (250 g L^{-1}) plus carbendazim (125 g L^{-1}) in autumn (GS 12) and in spring (GS 30), respectively.

Disease assessment

The development of *P. brassicae* was followed up during the whole growth period. Since visible symptoms of *P. brassicae* do not usually occur before February/March, during autumn-winter samples were taken every 1 to 2 weeks by randomly choosing 10 plants from non-treated fungicide plots. After incubating them in a damp chamber over night, the parameters disease incidence (%-age of plants infected) and disease severity (%-age of leaf area infected) were visually assessed. When macroscopic symptoms of infections by *P. brassicae* became visible in the field, the level of fungal infection was assessed visually and directly in all plots at monthly intervals. Besides assessing infections caused by *P. brassicae*, plants were also rated for other major fungal diseases (e.g. *Leptosphaeria maculans*, *Peronospora parasitica*, *Alternaria brassicae*, *Sclerotinia sclerotiorum*, *Botrytis cinerea*).

Sampling procedure

Younger, fully developed leaves of winter oilseed rape were randomly taken from each plot at the beginning of stem elongation (GS 50 - 53). Whole-leaf samples were split and either shock frozen in liquid nitrogen and finally freeze-dried, or dried in a ventilated oven at 60° C until constancy of weight. Additionally, leaf disc samples (16 mm) from leaf areas with visible symptoms of *P. brassicae* infections (+ infection) and without visible symptoms (- infection) were taken from the upper third of the crop. Leaf disc samples were shock frozen in liquid nitrogen before being freeze-dried.

Plant analysis

Oven-dried leaf samples were fine-ground to a particle size < 0.12 mm using a *Retsch* ultra-centrifugal mill and the total S content was determined by X-ray fluorescence spectroscopy according to Schnug and Haneklaus (1999). Freeze-dried leaf material and leaf disc samples were fine-ground in a coffee mill or a mortar, respectively prior to the analysis of organic S compounds. The free cysteine, GSH and GSL content were determined by HPLC analysis according to Hell and Bergmann (1990) and Rosa (1992), respectively.

Statistical calculations

For statistical analysis the SPSS software package version 10 was employed (SPSS, 1999). The GLM multivariate procedure was applied to assess the influence of the treatments on individual parameters. Cultivar, S, N and fungicide were tested as fixed factors. N and fungicide treatment delivered no statistical differences with respect to the investigated parameters and therefore their effect is not shown in the present paper. In order to test the influence of infections by *P. brassicae* on the cysteine and GSH content a one factorial ANOVA was carried out. The Student-Newman-Keuls test was used to determine which means were significantly different from each other at the 5 % significance level ($LSD_{5\%}$).

Results and discussion

Infections by *P. brassicae* were the most important fungal disease in winter oilseed rape in 2001/2002. Infections by *L. maculans* and *P. parasitica* were also found, but only at low levels. Disease progression throughout the vegetation period is illustrated for *P. brassicae* in Figure 2. Usually, *P. brassicae* infects winter oilseed rape plants soon after emergence of the seedlings (Gilles et al., 2000). During experimentation, first infections were found in mid-late October and maximum values for disease incidence and severity were determined in late March/April. At this time, the disease incidence was 91 % if no fungicides were applied (Figure 2). Values of > 25 % plants infected by *P. brassicae* at stem extension indicate a severe infection (Steed and Fitt, 2000). The corresponding value for disease severity was 13 % (Figure 2). In plots where fungicides were applied the disease incidence and severity of *P. brassicae* were lower compared to non-treated fungicide plots, but differences were not consistently significant. *P. brassicae* is a hemi-biotrophic fungus, which means that it becomes necrotrophic in the late developmental stage (Ashby, 1997), a characteristic that might be significant with view to processes involved in SIR (see below). At the time of leaf sampling (GS 50 - 53), additional infections by *L. maculans* (3 % plants infected; 0.01 % leaf area infected) and *P. parasitica* (13 % plants infected; 0.2 % leaf area infected) were found (data not shown).

Disease incidence and severity of *P. brassicae* were independent of the cultivar (Figure 2), though differences had been expected because of their divergent rating (HGCA Recommended List WOSR 2003). There is circumstantial evidence that resis-

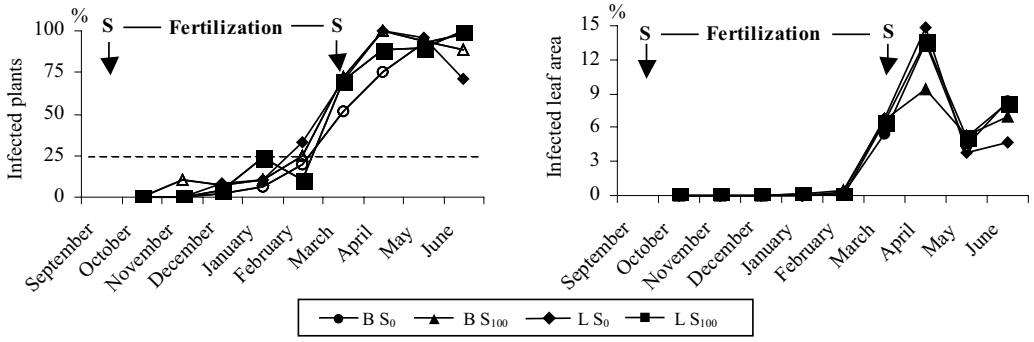


Figure 2:
Disease progression of *Pyrenopeziza brassicae* expressed by the percentage of infected plants (left) and the percentage of the infected leaf area (right) in winter oilseed rape in plots without fungicide applications in relation to cultivar and S rate.

tance against *P. brassicae* in new cultivars is overcome after a few years by changes in the metabolism of pathogen (Karolewski et al., 2004).

Since the S nutritional status of the plant was reported to have a strong impact on its natural resistance against pathogens (Schnug et al., 1995a), S was applied in autumn and spring in order to sufficiently supply the crop and to promote resistance mechanisms. However, in the present study the S applications did not influence disease progression of *P. brassicae* traceably (Figure 2), which indicates that S supply, S uptake, S resistance mechanisms and virulence of the pathogen did not fully coincide. Nevertheless, the data reflect changes in the plant S metabolism caused by S fertilization in combination with fungal infections, which contribute to uncover mechanisms underlying SIR. In this experiment special attention was paid to the metabolites cysteine, GSH and GSLs because of their direct dependence on the S supply (De Kok et al., 1981; Schnug, 1988; Schnug et al., 1995b; Schnug 1997) and their apparent link to SIR (Haneklaus et al., 2004).

The efficacy of S fertilization can be best verified by determining the total S content (Figure 3). S fertilization significantly increased the total S content from 4.2 mg S g^{-1} to 7.9 mg S g^{-1} in *Bristol* and from 4.2 mg S g^{-1} to 7.3 mg S g^{-1} in *Lipton* (Figure 3). In the control plots, the total S content in the leaf tissue was in the range of latent S deficiency (3.5 - 6.5 mg S g^{-1}), i.e. that though no macroscopic symptoms were visible, the S status was not sufficient for a high yielding crop (Schnug and Haneklaus, 1998). The S supply had no influence on disease incidence and severity of *P. brassicae* (Figure 2). This might indicate a temporal discrepancy between S fertilization and S uptake. Another explanation could be that the S doses were not sufficiently high to initiate SIR. Here, a regular S fertilization throughout the growing season might yield the desired effect.

Free cysteine and GSH are S-containing compounds of the primary plant metabolism. These metabolites were found to be involved in plant resistance against fungal pathogens (Vidhyasekaran, 2000; Gullner and Kömives, 2001). The effect of S fertilization and cultivar on the cysteine and GSH content in leaf discs infected and non-infected by *P. brassicae* is shown in Table 1. S fertilization increased the cysteine and GSH content in leaf discs, whereby differences were not consistently significant (Table 1). In greenhouse and field experiments, De Kok et al. (1981), Schnug et al. (1995b) and Bloem et al. (2004) found a significant relationship between S status and the cysteine and GSH content. Relevant in this context is that effects in Aberdeen might have been masked due to the smaller range of variation of the plant S status.

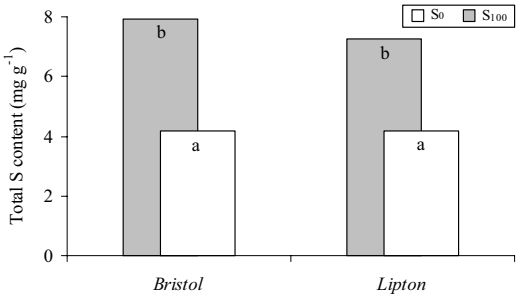


Figure 3:
Influence of S fertilization on the total S content in younger, fully developed leaves (d.w.) of two winter oilseed rape varieties at the start of stem elongation.

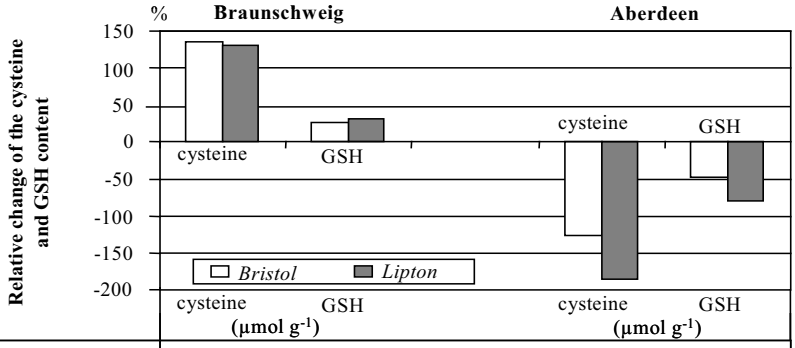
The increase in the content of cysteine and GSH was higher in infected leaf discs compared to non-infected leaf discs (Table 1). Differences in the cysteine and GSH content between the two cultivars were not significant (Table 1).

Table 1:
Influence of S fertilization on the cysteine and glutathione content in leaf discs (d.w.) of two winter oilseed rape varieties at the start of stem elongation.

Treatment		Cysteine (µmol g^{-1})			Glutathione (µmol g^{-1})		
		min	max	mean	min	max	mean
				+ Infection			
Bristol	S_0	0.10	0.44	0.27	4.2	10.3	7.2
	S_{100}	0.30	0.64	0.47	7.6	13.7	10.7
Lipton	S_0	0.01	0.35	0.18	2.6	8.7	5.6
	S_{100}	0.22	0.56	0.39	7.1	13.1	10.1
$LSD_{5\%}$				0.24			4.26
				- Infection			
Bristol	S_0	0.73	0.83	0.78	11.3	13.3	12.3
	S_{100}	0.84	0.94	0.89	13.1	15.1	14.1
Lipton	S_0	0.70	0.80	0.75	12.5	14.5	13.5
	S_{100}	0.83	0.93	0.88	13.8	15.8	14.8
$LSD_{5\%}$				0.74			1.41

In Figure 4, the influence of infections by *P. brassicae* on the cysteine and GSH content at two experimental sites, in Aberdeen (Scotland) and Braunschweig (Germany) is shown. When plant material was visually infected by *P. brassicae*, a significant 2.5-fold and 1.6-fold decrease of the cysteine and GSH content, respectively was found in Aberdeen (Figure 4; Table 1). In contrast, in experiments with the same cultivars in Braunschweig in 2002, infections by *P. brassicae* resulted in an increase of the cysteine and GSH content at the site of infection (Figure 4; Bloem et al., 2004). Additionally, the activity of the enzyme L-cysteine desulfhydrase increased (Bloem et al., 2004). Other researchers also showed that fungal infections generally yield an increase in the GSH content (Vanacker et al., 2000; Gullner and Kömives, 2001; Williams et al., 2002). Two scenarios are possible which could explain these different findings. Firstly, on sites with a higher S supply, reflected in higher total S concentrations (4.8 mg S g^{-1} in Braunschweig *vs*. 4.2 mg S g^{-1} in Aberdeen), a correspondingly higher cysteine (0.7 µmol g^{-1} in Braunschweig vs. 0.5 µmol g^{-1} in Aberdeen) and GSH content (12.1 µmol g^{-1} in Braunschweig vs. 9.7 µmol g^{-1} in Aberdeen) can be found in the leaf tissue. Besides this, an increased synthesis of GSH on the Braunschweig site was obviously related to a certain disease severity (Salac et al., 2004). In comparison in Aberdeen, where a continuous and consistently high infection severity for *P. brassicae* existed, particularly from the start of the vegetation period onwards, and the S status being sub-optimum, more S is bound in cysteine and GSH in non-infected tissues. In the infected plant tissues these metabolites were eventually consumed during metabolic protection processes thus yielding significantly lower values.

Secondly, the possibility exists that the plant tissue was severely and lastingly damaged by the pathogen resulting in a shift of anabolic in the favour of catabolic processes. Previous investigations revealed no differences between dry weights of leaves in inoculated and non-inoculated pea leaves by *Mycosphaerella pinodes* (Garry et al., 1996). Necrotic leaf areas are composed of dead cells and assuming a complete degradation and/or translocation of cysteine and GSH in/from necrotic plant tissue, this would imply that if ≤ 50/60 % (*Bristol/Lipton*) and ≤ 17/33 % (Bristol/*Lipton*) of the leaf disc area is impaired by *P. brassicae* at the time of sampling (see Figure 2), a significant decrease in the cysteine and GSH content might be expected in visually infected leaf discs whereby causal reasons remain speculative (see above). In other words, only if > 60 % of the leaf disc area in case of cysteine and > 32 % in case of GSH is severely impaired by *P. brassicae*, reflected in corresponding necroses, the decreases may be attributed to metabolic changes in



		Braunschweig (2002)		Aberdeen (2002)	
		cysteine (μmol g^{-1})	GSH (μmol g^{-1})	cysteine (μmol g^{-1})	GSH (μmol g^{-1})
Bristol	- Infection	0.59 a	10.8 a	0.84 a	13.2 a
	+ Infection	1.39 b	13.7 b	0.36 b	8.95 b
Lipton	- Infection	0.57 a	11.6 a	0.82 a	14.2 a
	+ Infection	1.31 b	15.3 b	0.28 b	7.86 b

Figure 4:
Influence of infections by *P. brassicae* on the cysteine and GSH content in leaf discs (d.w.) of two winter oilseed rape varieties at the start of stem elongation in Braunschweig (2002) and Aberdeen (2002) (source for Braunschweig: Bloem et al., 2004).

the decaying leaf tissue. These simple calculations reveal that the latter scenario may apply for leaf tissues severely impaired by the pathogen.

Glucosinolates are S-containing secondary compounds, which are protective against fungal pathogens (Mithen et al., 1987; Schnug and Ceynowa, 1990; Doughty et al., 1991; Zukalová and Vašák, 2002). Alkenyl GSLs are supposed to take part in the general resistance of plants against fungal pathogens, whereas the synthesis of indole and aromatic GSLs may be involved in the induced resistance (Zukalová and Vašák, 2002). So far, however, no relationship between GSL content or GSL profile in vegetative tissues and crop resistance has been verified (Chen and Andreasson, 2001). Three predominant alkenyl GSLs were detected in leaves of winter oilseed rape in the present study: glucobrassicanapin (4-pentenyl glucosinolate), gluconapin (3-butenyl glucosinolate) and progoitrin (2-hydroxy-3-butenyl glucosinolate) (Table 2). Glucobrassicin (3-indole methyl glucosinolate) and gluconasturtiin (2-phenyl ethyl glucosinolate) were the main indole and aromatic GSLs, respectively found in the vegetative tissue (Table 2). S applications increased the individual and total GSL content in younger leaves of winter oilseed rape at the start of stem elongation in both varieties, but differences proved to be statistically not significant (Table 2). Schnug (1997) found a significant close correlation between S status (from severe to excess S supply) and GSL content (from 3 μmol g^{-1} to 52 μmol g^{-1}) in younger, fully developed leaves of *B. oleracea*. The total GSL content ranged from 2.8 μmol g^{-1} to 5.4 μmol g^{-1} in *Lipton* and *Bristol* (Table 2), which is fairly low compared to values of up to 7.8 μmol g^{-1} found for the variety *Cobra* by Booth et al. (1991).

Glucobrassicanapin, which was found to have biocidal properties (Peterka and Schlosser, 1989), was the predominant alkenyl GSL in the leaf tissue of winter oilseed rape (Table 2). As its concentration was not influenced by the S supply, its significance in preformed resistance appears negligible. As a response to infection an increased indole and aromatic GSL content was determined in the plant tissue (Doughty et al., 1991; Giamoustaris and Mithen, 1995). Besides the degradation of GSLs, a selective accumulation of indole and aromatic GSLs could be mediated physiologically and might contribute to the resistance of plants (Haneklaus et al., 2004).

The cultivars *Bristol* and *Lipton* differed significantly in the progoitrin content (Table 2), but this GSL has no antifungal properties (Mithen et al., 1987; Peterka and Schlosser, 1989). The mean progoitrin content in the leaf tissue was 0.7 μmol g^{-1} for *Lipton* and 0.4 μmol g^{-1} for *Bristol* (Table 2).

Conclusions

Alternative plant protection measures are gaining increasing interest for conventional and organic farming systems. Up till now nutrient induced resistance mechanisms are well known (Datnoff et al., 2003), but still of minor importance in agricultural production. Sulfur induced resistance (SIR) was first observed for oilseed rape by Schnug et al. (1995a) and will be of high relevance in S-deficient production areas. This, however, requires targeted S fertilization strategies, which prompt SIR on production

Table 2:
Influence of S fertilization on the individual and total glucosinolate (GSL) content in younger, fully developed leaves (d.w.) of two winter oilseed rape varieties at the start of stem elongation.

Treatment		Glucobrassicanapin (µmol g^{-1})			Gluconapin (µmol g^{-1})			Progoitrin (µmol g^{-1})		
		min	max	mean	min	max	mean	min	max	mean
Bristol	**S_0**	1.9	3.1	2.5	0.38	0.63	0.51	0.12	0.46	0.29
	S_{100}	2.1	3.3	2.7	0.40	0.65	0.53	0.25	0.59	0.42
Lipton	**S_0**	1.5	2.7	2.1	0.32	0.56	0.44	0.50	0.83	0.67
	S_{100}	1.9	3.2	2.6	0.31	0.57	0.44	0.58	0.98	0.76
$LSD_{5\%}$				0.83			0.17			0.23

Treatment		Glucobrassicin (µmol g^{-1})			Gluconasturtiin (µmol g^{-1})			Total GSL (µmol g^{-1})		
		min	max	mean	min	max	mean	min	max	mean
Bristol	**S_0**	0.11	0.19	0.15	0.19	0.33	0.26	2.8	4.8	3.8
	S_{100}	0.15	0.24	0.19	0.26	0.40	0.33	3.3	5.3	4.3
Lipton	**S_0**	0.10	0.18	0.14	0.22	0.36	0.29	2.9	4.8	3.9
	S_{100}	0.11	0.20	0.15	0.25	0.39	0.32	3.3	5.4	4.4
$LSD_{5\%}$				0.06			0.09			1.34

fields. In this context, the presented research work revealed that:
- S fertilization increased the cysteine, GSH and GSL content;
- disease incidence and severity during the vegetative period obviously play a major role in SIR as changes in the GSH and cysteine content showed corresponding variations;
- for initializing SIR, the S supply needs to follow the actual metabolic demand, which means that: (a) doses higher than the physiological demand might be required; (b) split doses need to be applied in order to match the S demand for S induced processes against fungal infections.

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Metabolic background of H_2S release from plants

Ahlert Schmidt[1]

Abstract

Sulfate nutrition has been shown to be beneficial for plant health. Emission of H_2S has been analyzed as one possible target for a defense mechanism. In this review the possible reactions leaving to sulfide are summarized and the recent developments for sulfide generation from either the sulfite reductase or different cysteine-specific desulfhydrases are analyzed. Mechanisms for the formation of COS (carbonyl sulfide) and its degradation to CO_2 and sulfide in plants are discussed. It is shown that sulfide is toxic for plants itself inhibiting mitochondrial respiration. The present paper summarizes the possible reactions concerning sulfide formation and emission.

Key words: H_2S emission, cysteine, O-acetyl-L-serine (thiol)lyase, cysteine catabolism, cysteine desulfhydrase, COS metabolism, sulfide toxicity

Introduction

Plant health is influenced by sulfur starvation; sulfur nutrition therefore is mandatory for good plant growth. It has been shown, that the reduction of SO_2 emission has led to sulfur shortage especially in Cruciferae leading to higher susceptibility towards infection, which has been shown especially for rape (Schnug et al., 1993, 1995). This observation has been coined SIR (sulfur-induced resistance). Whereas the mechanisms involved in the SIR are not understood so far, is seems clear that more than just one specific metabolic process seems to be involved in this SIR syndrome. It has been shown, that plants can emit sulfide to the environment (Schmidt et al., 1980; Sekiya et al., 1982a; Rennenberg, 1984, 1989). Therefore it was speculated that release of H_2S by plants could affect bacterial and fungal growth and contribute to SIR in rape fertilized with a surplus of sulfate. Possible reactions leading to H_2S have been analyzed and will be discussed here in some detail.

Sulfide formation catalyzed by the sulfite reductase

Sulfide is generated in the process of sulfate assimilation and the whole reaction sequence from sulfate to cysteine is localized in the chloroplast. The needed energy in form of ATP and ferredoxin is generated by the electron transport chain. As sulfate activation is discussed already in the general introduction section, I will give some information to the sulfite reductase. This enzyme catalyzes the reduction of sulfite to sulfide using reduced ferredoxin as electron donor (Figure 1). The sulfite is bound to siroheme and is reduced without free intermediates to siroheme-bound sulfide (Murphy et al., 1974), which than is liberated to free sulfide. H_2S will be assimilated by the cysteine synthase (*O*-acetyl-L-serine thiol(lyase) [OASTL]) or it could be emitted from the plant to the environment. Some aspects to be mentioned: 1) Some plants have only one gene for the sulfite reductase and this might be a limiting step for assimilatory sulfate reduction. 2) Sulfide formed is effectively bound to cytochromes thus affecting respiration and possibly other iron-containing complexes as well, which will be discussed later. 3) These K_M-data for sulfide binding are in the same range as cyanide (µM), which suggests that the plant should control the free H_2S-pool in order to avoid toxic side effects of H_2S. Therefore the incorporation of sulfide to L-cysteine using the cysteine synthase should be the most efficient way to keep its concentration low to avoid inhibitory effects. However, the reported K_M-data for the cysteine synthase have been too high so far for this explanation (Schmidt and Jäger, 1992). Only recently our understanding of low sulfide concentrations and efficient cysteine formation seems to be resolved due to finding exceptionally low K_M-data for the cysteine synthase in the micromolar range (Wirtz et al., 2004)

If sulfide assimilation will not consume all sulfide generated by the sulfite reductase it could be emitted to the environment. Some aspects of H_2S toxicity will be discussed in the last section. It might be speculated here that the sulfide generated by the sulfite reductase due to the reduction of sulfate is primarily used for cysteine formation and that sulfide needed for biosynthesis of iron-sulfur centers and coenzymes is handled as sulfide generated from cysteine by metabolic steps discussed later. The regulation of the sulfite reductase and thus regulation of sulfide formation by this enzyme is not understood so far, but possibly the generation of sulfite by the APS reductase might be the limiting step for H_2S-generation by this pathway (Schmidt and Jäger, 1992).

Sulfide formed in this way would be localized

[1] Institut für Botanik, Universität Hannover, Herrenhäuser Str. 2, 30419 Hanover, Germany

within the chloroplast, since the sulfite reductase is a chloroplastic enzyme (Schmidt, 1969, Schmutz and Brunold, 1985; Brunold and Suter, 1989). Furthermore it was found, that sulfide was light dependent (Sekiya et al., 1982b), which would be expected if the energy needed for assimilatory sulfate reduction is regenerated by the electron transport chain.

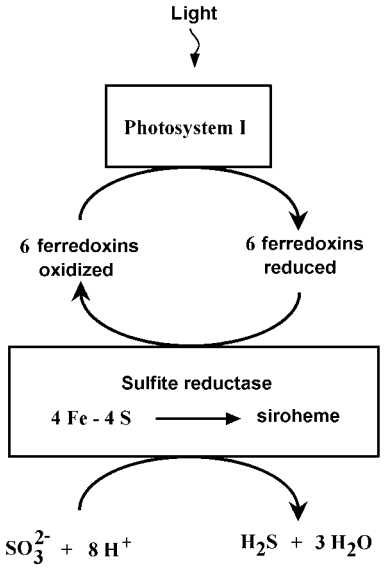


Figure 1:
A general scheme for sulfide formation by the sulfite reductase.

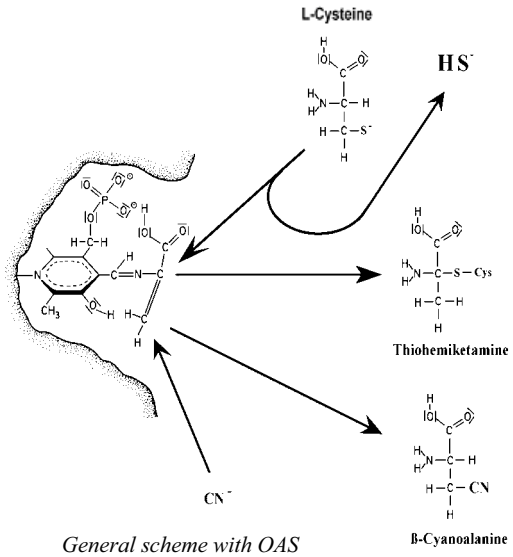
Sulfide formation catalyzed by cysteine synthase

The cysteine synthase catalyzes the formation of L-cysteine from *O*-acetyl-L-serine and H_2S as shown in Figure 2. Plants contain isoenzymes of the cysteine synthases in the cytosol (OASTL a), the chloroplast (OASTL b) and the mitochondrion (OASTL c). The isoenzymes have different catalytic properties; especially the K_M-data for the substrates sulfide and *O*-acetyl-serine vary and the pH-optima are different as well (Table 1). For L-cysteine synthesis an aminoacrylate bound to the pyridoxal phosphate is generated at the active site of the cysteine synthase. This amino acrylate contains an activated C-C double bond, which can accept free sulfide (HS^-) to generate L-cysteine (Figure 2). It has been found by isotopic exchange reactions (Schmidt, 1977) that L-cysteine can be used as a donor for the aminoacrylate intermediate as well (a partial back reaction; Figure 2) which leads to H_2S formation using cysteine as a substrate. Therefore the cysteine synthase has an inherent capability to H_2S-formation from L-cysteine. The K_M for the back-reaction using L-cysteine as a substrate is about 10-fold lower as its original substrate for cysteine formation with *O*-acetyl-L-serine (see however the new resultz by Wirzt et al., 2004). Since the cysteine synthase in plants is present in high amounts (at least using the assay with *O*-acetyl-L-serine and H_2S; Schmidt and Jäger, 1992) this side reaction of L-cysteine degradation is clearly a possibility for H_2S-formation in plants.

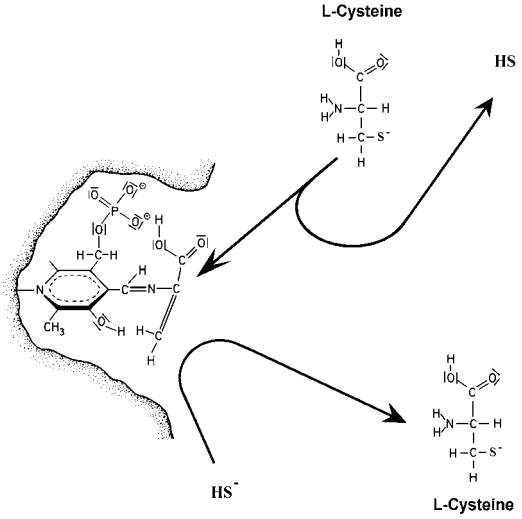
However, there are differences of the cysteine synthases to cysteine desulfhydrases to be discussed later. The intermediary enzyme-bound amino-acrylate is not hydrolyzed by water to decompose to ammonia and pyruvate (Burandt, 2002) but it is stable and can only be released by addition of either sulfide, cyanate or other thiol groups (Figure 2). Especially L-cysteine itself can be used instead of H_2S forming a thiazolidine derivative (Figure 2) and the dithiotreithol (DTE) can be used as well leading to the corresponding DTE-cysteine compound with the release of H_2S as shown for bacteria (Mino and Ishikawa, 2003) and seems to be valid for plants as well; glutathione is not active in this reaction, possibly favoring GSH as a mass thiol in plants (unpublished data).

Sulfide formation catalyzed by L-cysteine desulfhydrase

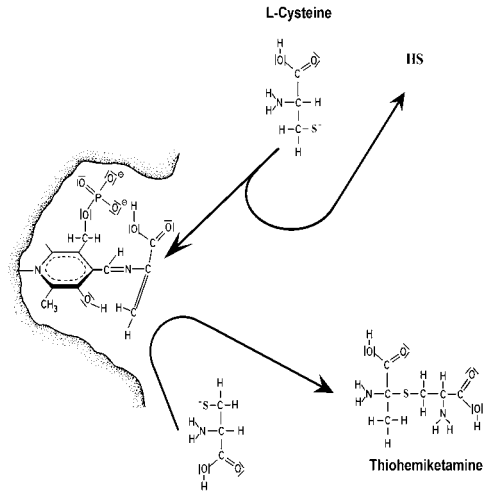
Cysteine degradation by an L-cysteine desulfhydrase catalyzes the formation of sulfide, ammonia and pyruvate in a stochiometric relation of 1:1:1 as shown in Figure 3. This reaction is well characterized from the bacterium *Treponema* where it has been crystallized (Chu et al., 1999; Bertoldi et al., 2003). It shows, that L-cysteine reacts with a pyridoxal phosphate, forming a bound aminoacrylate similar to the situation discussed above for the back reaction of the cysteine synthase. However, this aminoacrylate intermediate is not stable but hydrolyzed directly with water to pyruvate, ammonia and sulfide in a stochiometric way (Figure 3). Whereas such enzymes are found in bacteria and are normally used for cysteine degradation under energy shortage (cysteine catabolism). Such activities have been found in higher plants as well, however, the differentiation between back reactions of a cysteine synthase and correct L-cysteine desulfhydrase activity has been difficult. Therefore the corresponding genes have to be isolated and the pure protein has to be at hand for critical examination of the reactions involved and the corresponding products formed. Genetic evidence from *Brassica rapa* genotypes however, clearly shows, that cysteine synthase and L-cysteine desulfhydrase activity are different in analyzed lines showing that these reactions can be dissected by careful analysis (Burandt et al., 2002). The



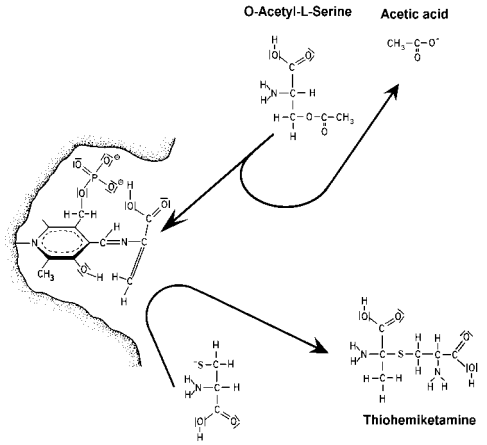
General scheme with OAS



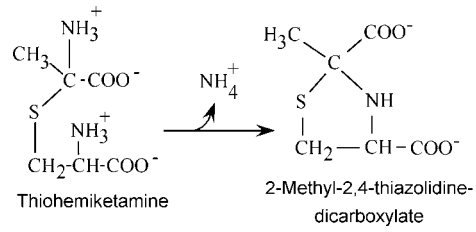
Isotopic exchange reaction



L-Cysteine desulfhydrase reaction



Direct thiohemiketamine formation



2-Methyl-2,4-thiazolidine-dicarboxylate formation

Figure 2:
Reactions around the cysteine synthase protein.

characterization of a plant L-cysteine desulfhydrase by recombinant proteins is still missing, however an enzyme from *Synechocystis* has been characterized recently (Kesser, 2004).

Sulfide formation catalyzed by L-cysteine desulfhydrase (NifS) forming alanine

Another reaction of a possible L-cysteine desulfhydrase has been found during

Isoform	K_M für OAS-TL-Reaktion: *O*-Acetyl-L-Serin [mM]	K_M für OAS-TL-Reaktion: *S*-Methyl-L-Cystein [mM]	K_M für DES-Reaktion: L-Cystein [µM]	K_M für CAS-Reaktion: L-Cystein [µM]
OAS-TL A	2,06 ± 0,30		25,05 ± 2,48	98,63 ± 15,63
OAS-TL B	0,69 ± 0,17	38,4 ± 2,98	28,17 ± 3,23	113,80 ± 9,34
OAS-TL C	3,94 ± 0,50		47,59 ± 10,34	52,63 ± 9,29

OAS-TL = L-Cysteine synthase reaction; DES-Reaktion = L-Cysteine desulfhydrase-reaction; CAS-Reaktion = ß-Cyanoalanine-reaction

Table 1:
K_M-data for cysteine synthases from *Arabidopsis* (Burandt, 2002).

characterization of the nitrogenase biosynthesis in bacteria where reduced sulfur is needed for iron-sulfur centers. The donor for the labile sulfide in these centers has been shown to be L-cysteine and this activity is termed as "NifS-type reaction". This enzyme was needed for the iron-sulfur cluster formation in nitrogenase formation. It catalyzes the formation of elemental sulfur and alanine, according to Figure 4. This NifS protein is a L-cysteine desulfhydrase with a pyridoxal phosphate as cofactor. Again an aminoacrylate should be formed with the release of sulfide. Obviously the aminoacrylate is not hydrolyzed to ammonia and pyruvate but instead the double bond seems to be reduced thus forming alanine. Therefore an electron donor is needed. These electrons obviously could come from sulfide being oxidized to elemental sulfur. However, recent evidence shows the formation of free sulfide in the presence of DTT (Mühlenhoff et al., 2004). This could indicate that dithiothreitol functions as an electron donor for alanine formation. It is suggest that the formation of elemental sulfur is a side reaction when no other electron donor is available; so this reaction should be analyzed in more detail. However, the formation of alanine is a good indication for NifS related activity. A gene for NifS-type L-cysteine desulfhydrases of *Arabidopsis* has been found with a signature for chloroplasts. The recombinant protein was shown to specifically form alanine as discussed above (Leon et al., 2002). Therefore this enzyme could, after reduction of elemental sulfur to sulfide, attribute to the formation of free sulfide.

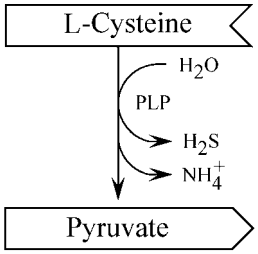


Figure 3:
The L-cysteine lyase reaction: L-cysteine reacts with water and the products formed are free sulfide, pyruvate and ammonia.

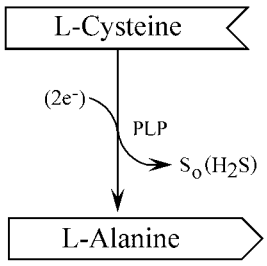


Figure 4:
Cysteine catabolism by a stereospecific L-cysteine desulfhydrase (NifS). Such genes have been found for mitochondria and chloroplasts of *Arabidopsis* and are correlated to the NifS type proteins. The catalyze the formation of L-alanine and elemental sulfur.

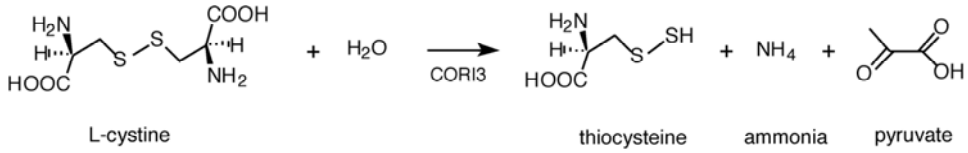


Figure 5:
The cystine lyase reaction forming L-cysteine-persulfide.

Sulfide formation catalyzed by D-cysteine desulfhydrase

Besides the L-cysteine desulfhydrase plant do contain a cysteine desulfhydrase which specifically uses D-cysteine as substrate which is abbreviated here as D-cysteine desulfhydrase (lyase) as shown in Figure 7 (Schmidt, 1982, 1987; Schmidt and Erdle, 1983). This enzyme activity is present in each plant analyzed so far with prominent activities within plant species used for agriculture such as *Zea mays*, *Triticum aestivum*, *Avena sativum, Secale cereale, Oryza sativa, Solanum tuberosum*, *Beta vulgaris*, *Brassica napus*, *Arabidopsis* and in suspension cultures of *Nicotiana tabacum* (Schmidt, 1982; Schmidt and Erdle, 1983; Rennenberg, 1983; Rennenberg et al., 1987) The activity of the D-cysteine enzyme is different in *Brassica napus* strains showing clearly that it genetically coded (Burandt et al., 2001). This enzyme catalyzes the formatin of H_2S, ammonia and pyruvate as shown for *Escherichia coli* (Nagasawa et al, 1985, 1988). The gene for the D-cysteine desulfhydrolase has been identified in *E. coli* as yedO (Soutourina et al., 2001). The corresponding gene for a plant D-cysteine desulfhydrase has been identified in our laboratory recently (At1g48420; Riemenschneider et al., 2004). We have so far no function assigned for D-cysteine nor do we know how it is synthesized. One might speculate that it could be formed with a transaminase from ß-mercaptopyruvic acid (see later discussion) or by a racemase transforming L-cysteine to D-cysteine. Even a synthesis via *O*-acetyl-D-serine and sulfide according to the cysteine synthase cannot be ruled out so far, but we hope to clarify these possibilities having the recombinant enzyme available.

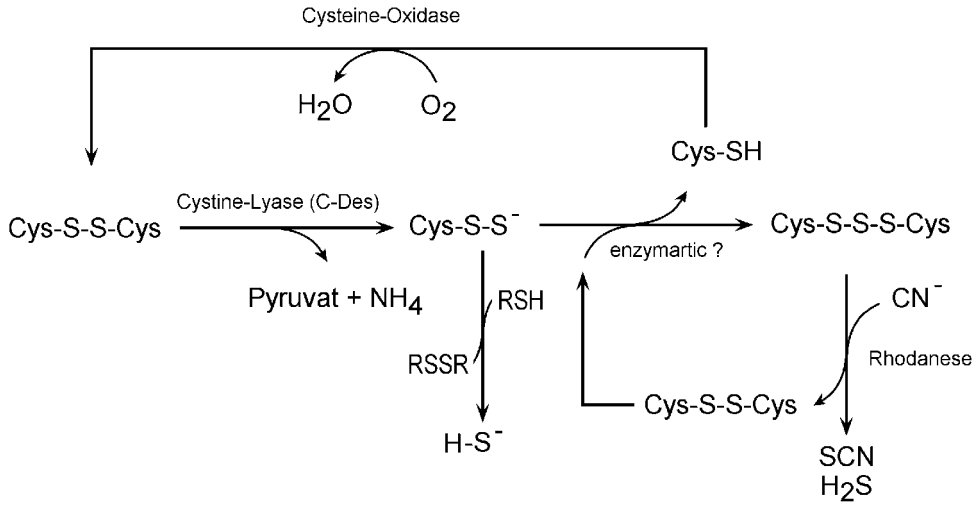


Figure 6:
Cystine metabolism involving cystine lyase and a sulfurtransferase.

Sulfide formation catalyzed by ß-mercaptopyruvate metabolism

An important metabolic intermediate for sulfur metabolism is ß-mercaptopyruvic acid (ß-MEP); it is an excellent donor for sulfurtransferases (Papenbrock and Schmidt, 2000). Although the formation of ß-mercaptopyruvic acid has not been shown in plants so far, we can speculate its formation either by a cysteine transaminase, a cysteine aminooxidase or a cysteine dehydrogenase according to Figure 8. Once formed, ß-MEP can be

used as sulfur donor for ß-MEP sulfurtransferases. In the genome of *Arabidopsis* a gene family for sulfurtransferases has been characterized with 18 members (Bauer and Papenbrock, 2002) These enzymes contain a cysteine in its active site wich accepts the sulfide from the donor forming a persulfide. This persulfide than can be used for different biosynthetic pathways including free sulfide formation according to Figure 9 (Papenbrock and Schmidt, 2000) The function of only one sulfurtransferase has been identified so far for the molybdate cofactor biosynthesis (Matthies et al., 2004). So we can speculate, that other functions of sulfurtransferases (rhodaneses) for sulfur metabolism should be discovered in the future.

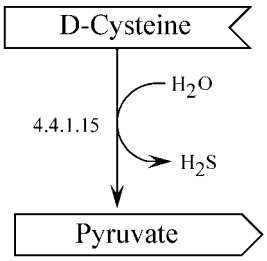
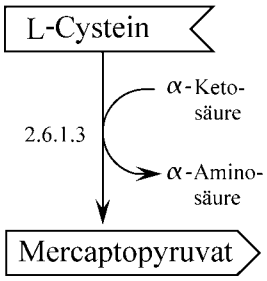


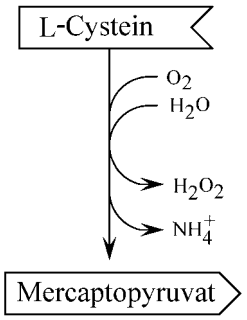
Figure 7:
The D-cysteine desulfhydrase (lyase) reaction.

Sulfide formation from COS catalyzed by carbonic anhydrase

COS is a substance, which is emitted from plants in low concentrations. COS will react with a carbonic anhydrase yielding H_2CO_3 and sulfide (Protoshill-Krebs and Kesselmeier, 1992). This reaction favors sulfide formation, the backward reaction has not been demonstrated so far. One might speculate about this pathway in the following context: H_2S has been shown to react with ribulose bisphosphate carboxylase forming thioglyceric acid 3-phosphate besides the normal PGA (Brändle and Martin, 1971; Lorimer, 1989). Thus thioglyceric acid is a normal intermediate within the chloroplast if H_2S is formed. So it might be speculated, that the corresponding thiopyruvic acid is formed as well. It has been shown further, that the Rubisco enzyme forms directly pyruvate without a PGS intermediate in the range of 1 % of the CO_2 fixation rate (Andrews and Kane, 1991). By decarboxylation of PEP acetyl-CoA is formed and if the thiopyruvic acid is used, than COS should be formed. This activity has been shown to be localized within the chloroplast as well (Kubis et al., 2004). COS formed in that way would leave the chloroplast; than it will be picked up by the CO_2 hydratase in the cytoplasm yielding H_2CO_3 and sulfide as discussed in the beginning of this chapter. This might be a salvage pathway to capture H_2S losses caused by COS formation.



Mercaptopyruvate formation by a transaminase



Mercaptopyruvate formation by an L-amino acid oxidase

Figure 8:
Possibilities of cysteine catabolism by mercaptopyruvic acid.

Some remarks on sulfide toxicity

Sulfide is toxic to microorganisms and it was shown that H_2S retards growth of *E. coli* if concentrations exceed µM concentrations (Sohn 2000). H_2S has a binding affinity to chelated iron in the same range as cyanide or oxygen. It will bind to hemes of the mitochondria, thus blocking respiration and ATP-formation. The observed growth inhibition in *E. coli* is obviously due to H_2S binding to the cytochrome a3 for oxygen uptake partly inhibiting ATP-formation. Due to shortage of ATP there is less sulfate reduction and H_2S is removed by assimilation and the growth inhibition is thus releaved leading to a growth cycling in *E. coli*. The K_M data for H_2S inhibition are in the in the µM range and this similar to cyanide inhibition. Therefore inhibition of oxygen uptake can be expected for plant mitochondria. We have analyzed sulfide inhibition of oxygen uptake with isolated mitochondria from potatoes and pea leaves. These

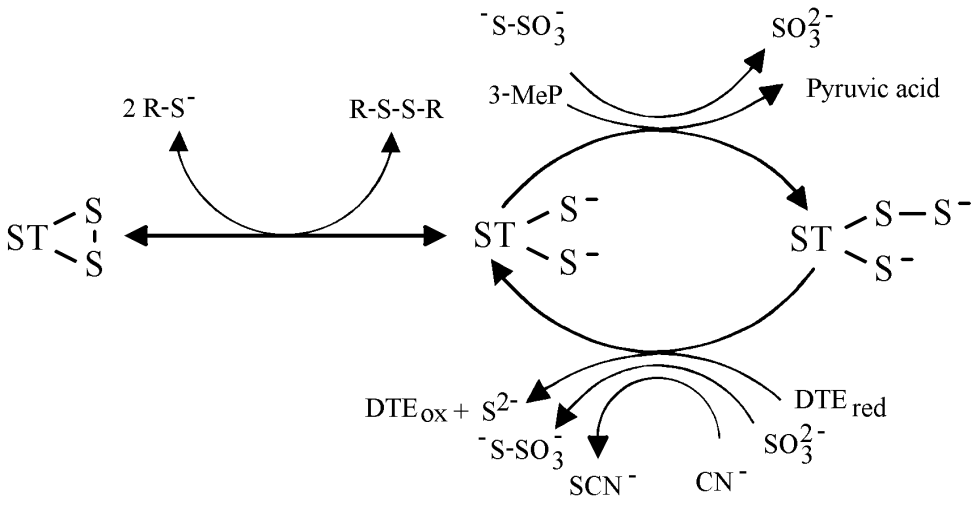


Figure 9:
Possibilities of cysteine catabolism by sulfurtransferases.

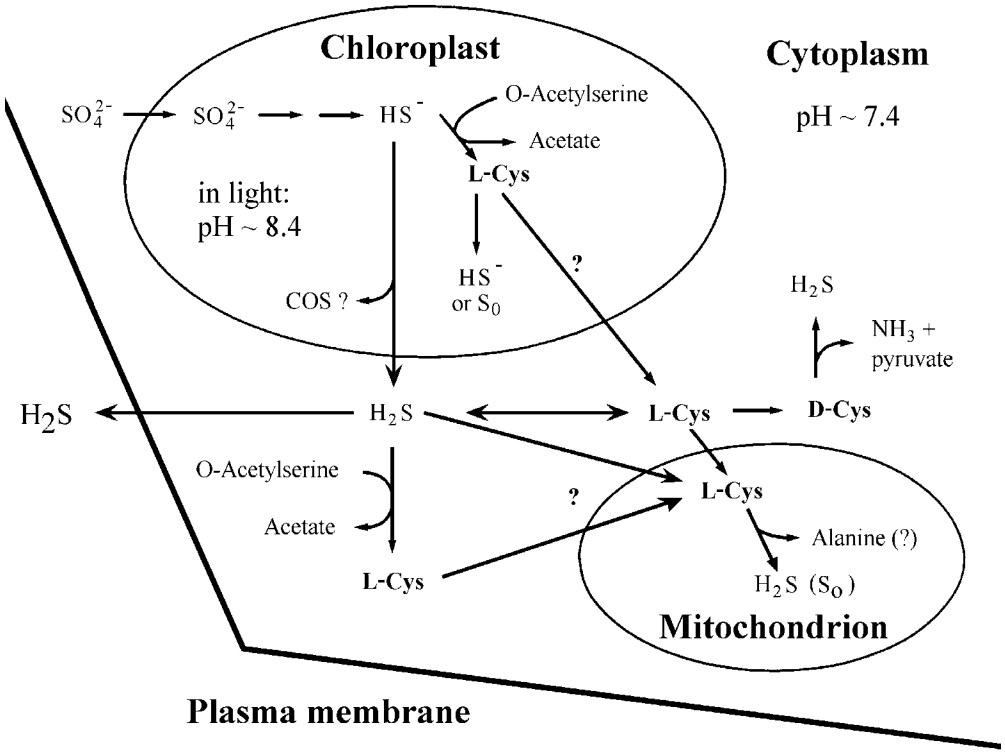


Figure 10:
A generalized scheme for cysteine-dependent formation of sulfide.

data are summarized in Table 2 (Huchzermeyer and Schmidt, unpublished results). As can be seen the K_i data for oxygen uptake inhibition are in the range of 10 µM, showing that H_2S is inhibiting oxygen uptake by mitochondria and thus ATP-formation in plants as well. It should be expected that H_2S will bind to other heme-type iron as well including the siroheme of the sulfite reductase and the nitrate

reductase, since these can be inhibited by cyanide as well. This would suggest that H_2S takes an indirect control over nitrate reduction as well, if the siroheme of the nitrite reductase is blocked by H_2S.

Table 2:
Toxic effects of sulfide on respiration.

Potato mitochondria:	**K_i for H_2S**
without addition	40 µM
1 mM GSH	11 µM
100 µM SHAM	14 µM
Pea leaf mitochondria: (+30 µM DCMU)	**K_i for H_2S**
without addition	35 µM
1 mM GSH	9 µM
100 µM SHAM	19 µM

An overview of reactions involved

The possible reactions leading to free sulfide are summarize in Figure 10, showing the close correlation of cysteine metabolism and sulfide formation in plants. Although we can not give a precise mechanism of enhanced plant tolerance to microbial damage by efficient sulfur nutrition, the data accumulated so far clearly support the concept of sulfate induced resistance (SIR) in plants (Bloem et al., 2004). Therefore the possible reactions of cysteine metabolism and H_2S formation have to be analyzed in more detail for a better understanding of SIR induced plant health.

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The role of sulfur in sustainable agriculture

Ewald Schnug[1] and Silvia Haneklaus[1]

Abstract

The term 'sustainability' has been used so many times on facets of agriculture that it is meanwhile difficult to understand its true origin. “Sustainable development” has been defined in 1987 by *The Brundtland Commission* as: “development that meets the needs of the present without compromising the ability of future generations to meet their own needs”. For agriculture this implies primarily the sustainable use of natural resources such as water, soil and atmosphere. This contribution highlights the role of a single plant nutrient in achieving sustainability in agriculture. A sufficient sulfur supply secures level and quality of yields, improves plant health through stimulation of natural resistance processes and alleviates the ecologically hazardous side effects of nitrogen fertilization on surface and groundwater bodies as well as on the quality of the atmosphere. Beside this the sulfur supply of agricultural crops affects also neighboring compartments of agro-ecosystems by providing indirectly food for insects.

Key words: atmosphere, fertilization, food safety, food security, nitrogen losses, ozone, sulfur, sustainability

Introduction

Few words have been so often used and few words have been so often abused as the word 'sustainability'. In many cases claiming for 'sustainability' is simply claiming for 'profitability'. In a world where making profit is the key indicator for being successful, the true meaning of sustainable development is often forgotten. 'Sustainable development' has been defined in 1987 by *The Brundtland Commission* as: “development that meets the needs of the present without compromising the ability of future generations to meet their own needs”. Fact is that our society is far away from being on the track towards sustainable development like Wendel Berry stated in 2002: “We currently live in the economy and culture of the “one-night stand“. Industrialism has provided us innumerable commodities, amusements, and distractions, but these offer us little satisfaction. Instead we suffer ever-increasing alienation from our families, our communities, and the natural world.

In a way agriculture may be a special segment within human societies as its sustainability is intrinsic under any circumstances, simply because no food, no man! Fertilizers provide food for plants but still fertilizers are often named together with pesticides as 'agrochemicals' which is to a great extent misleading: fertilizers provide essential minerals without no plants can grow. In contrast, pesticides are as essential for plants as aspirin to man. Several authors have identified pesticides as key issues counteracting sustainability, for instance Friedrich Engels, who wrote already in 1876: "Schmeicheln wir uns nicht so sehr mit unseren menschlichen Siegen über die Natur. Für jeden solchen Sieg rächt sie sich an uns“, followed years later by the famous Rachel Carson who wrote in her famous book “Silent Spring“ (1954): “The chemical war can not be won, and a life is caught in its violent crossfire.“

But also fertilization has its black spots in view for sustainability like for instance the loss of nitrogen and phosphorous from agro-ecosystems, the pollution of atmosphere and water-bodies with nitrogen compounds, the waste of non-renewable P-resources through inefficient fertilization strategies, the charging of soils with heavy metals and radioactivity through fertilization of waste materials and P-fertilizers) and the charging of soils with hazardous organic compounds, pharmaceuticals and infectious materials.

This contribution highlights the role of a single plant nutrient in achieving sustainability in agriculture: sulfur. A sufficient sulfur supply secures level and quality of yields, improves plant health through stimulation of natural resistance processes and alleviates the ecologically hazardous side effects of nitrogen fertilization on surface and ground water bodies as well as on the quality of the atmosphere. Beside this the sulfur supply of agricultural crops affects also neighboring compartments of agro-ecosystems indirectly by providing food for insects.

Sulfur fertilization and agricultural economy

One often used criterion for justifying fertilization as a component of sustainable development in agriculture is the allegation that fertilization alleviates world hunger. Kimbrell (2002) reveals this as a

[1] Institute of Plant Nutrition and Soil Science, Federal Agricultural Research Center (FAL), Bundesallee 50, 38116 Braunschweig, Germany

great mistake, because "world hunger is not created by lack of food but by poverty and landlessness, which deny people access to food. Industrial agriculture actually increases hunger by raising the cost of farming, by forcing tens of millions of farmers off the land, and by growing primarily high-profit export and luxury crops".

The general contribution of fertilization to sustainability is addressed directly to the success of the farm enterprises and simply aims at improving the profit of production.

In this context sulfur plays an extraordinary role in the history of fertilization: free and in surplus, amounts delivered by atmospheric pollution until the beginning of the 1980s sulfur deficiency is today the most common nutrient disorder in Northern European crop plants. The reason are the stringent clean air acts introduced at the end of the last century, which caused atmospheric sulfur depositions to drop from over 100 kg ha^{-1} S down to 10 kg·ha^{-1} S within only 20 years. The positive effect of sulfur fertilization to a sulfur-starving crop can easily be demonstrated in field experiments. Difficulties arise when trying to upscale results from field experiments to assess the impact of sulfur deficiency on crop production in an entire country. Table 1 shows an assessment of potential yield losses and their monetary value for two federal counties of Germany, where extended soil survey and hydrogeological information allows the classification of the cropping area according to the potential risk for S deficiency. Applying the same calculation model to the 7.600 km^2 of grassland in this area (assuming a loss of 10% under moderate and 20% under severe S deficiency and an average N content of 2% in the dry matter the potential N losses for this type of farming amounts to an additional 19.8 million kg N. Those two counties comprise roughly 17% of Germanys cereal, and 27% and 12% of the entire oilseed rape and grassland area. Extrapolating the results from table 1 according to these figures, German agriculture faces a potential monetary loss (potential means a scenario without any sulfur fertilization) of about 1.200.000.000 € per annum alone from yield losses in oilseed rape and cereal cropping.

Contribution of sulfur to sustainability in agriculture

Crops not only provide food and profit for man, but also have also ecological functionalities. In the context of this paper ecological functionality is defined as the beneficial contribution of crops to ecosystems. As far as S is concerned, three examples shall be presented here: the contribution of crops to the degradation of surface ozone, non-point nitrogen losses from agriculture and the function of oilseed rape as a forage crop for honey bees.

Surface ozone concentrations

Over the last decade surface ozone concentrations in rural areas increased on average by 1.8 µg m^{-3} yr^{-1} (Schnug, 1997). At the same time S concentrations declined at a constant rate of 0.45 mg yr^{-1} (Schnug, 1997). Assuming that: a) H_2S emissions from plants decline together with the sulfur supply (Collins, 1997; Rennenberg, 1984) linearly on a rate of 0.57 nmol m^{-2} h^{-1} (calculated from data given by Schroeder (1993)); b) crops have an average leaf area index of 1; c) crops assimilate and reduce sulfur on average of 100 days a year and 10 h a day; and d) H_2S degrades O_3 in a 1:1 ratio; then up to 75% of the observed increase in surface ozone could be attributed to the decrease in the total amount of S turnover in the 'green part' of the ecosystem. The figures given here are only an estimate and may change depending on the factors considered, but they still outline the important function of sulfur assimilation and reduction in the ecosystem. Despite the importance of this for air quality, the higher sulfur inputs in the past century enabled plants to adapt to increasing environmental stress caused by increasing surface ozone concentrations and, vice versa, the decline of the sulfur supply within only one decade (Schnug, 1997; Schnug and Haneklaus, 1994) may have serious consequences for the stability of recent ecosystems. For example, sulfur deficiency is thought to be one of the reasons why 50% of all forests are damaged, although sulfur emissions have been cut down drastically over the past 10 years (Umweltbundesamt, 1993). The effect is thought to be due to the combination of reduced resistance (due to sulfur deficiency) and, at the same time, increased environmental stress (Will et al., 1997; Zhang and Rennenberg 1997).

Nitrogen losses to the environment

Via the metabolism of amino acids, the utilization of nitrogen and sulfur depend on each other, which means that for the efficient use of high nitrogen levels in agriculture, a sufficient sulfur supply is required. Therefore, increased ecological problems from agricultural crop production are expected because the utilization of fertilizer nitrogen is diminished in sulfur deficient crops (Schnug et al., 1993). This may result in increased nitrogen losses to the environment, particularly by nitrate leaching into the hydrosphere, or gaseous losses to the atmosphere. On average, each kg of sulfur unavailable to satisfy the plant's demand causes 15 kg of nitrogen with the potential to be lost to the environment. From the basic data presented in table 1 it was calculated that the potential annual loss of nitrogen due to insuffi-

Table 1:
Assessing the impact of sulfur deficiency on cop production in Brandenburg and Mecklenburg-Western-Pomerania (Germany).

		Brandenburg	Mecklenburg-Western Pomerania	Σ	Yield loss (10^3 t) [1]	Monetary loss (10^6 €)[2]	Potential N loss (10^6 kg)[3]
Cereals							
Total area	(km^2)	5650	5890	11540			
Potential yield	(t ha^{-1})	7					
Modelled yield	(10^3 t on 30% of area)						
no S deficiency		1316	1568	2884	0		
moderate S deficiency		1184	1411	2595	-289	-67	-11.5
severe S deficiency		1052	1254	2307	-577	-34	-5.8
Oilseed rape							
Total area	(km^2)	1110	2330	3440			
Potential yield	(t ha^{-1})	3	4				
Modelled yield	(10^3 t on 30% of area)						
no S deficiency		111	312	423	0	0	
moderate S deficiency		89	250	339	-84	-20	-3
severe S deficiency		67	187	254	-169	-40	-6

[1]calculated yield losses for cereals/oilseed rape: moderate S deficiency 10/20 and severe S deficiency 20/40 % of potential yield; [2]prices (€ t^{-1}): 116 for cereals and 235 for oilseed rape; [3]calculated for yield losses with 2% N in seeds

cient sulfur supply amounts to at least 300 million kg of nitrogen, which is equal 10% of the total nitrogen consumption of German agriculture.

Forage crops for honeybees

Although oilseed rape is self-pollinating (Saure 2002), the cross-pollination rate, predominately by honeybees, was estimated to be about 20% (Dan et al., 1980). According to Olsson (1960) the cross-pollination rate may vary in relation to genotype and climatic conditions between 5 % and 95 %. By comparison, on fields where composite hybrid oilseed rape varieties are grown or male-sterile lines for breeding of restored hybrid cultivars, these plants depend on pollination by vectors (Steffan-Dewenter, 2003). First observations in field-grown composite hybrids show increased problems with pollination of hybrids in low sulfur environments. This problem can be attributed to the processes discussed next. Oilseed rape provides an important source of nectar and pollen for honeybees, which are attracted by the bright yellow color of the crop in bloom (Pierre et al., 1999). Oilseed rape is one of the most important European melliferous crops for beekeepers as it is an important foraging plant in early summer. The main pollinators in oilseed rape are insects of the family Apidea (e.g. honey bees, wild bees and bumble bees) (Corbet, 1992; Williams, 1996) and the significance of honeybees as pollen vectors for seed set and yield has been described in the literature (Steffan-Dewenter, 2003).

Honeybees are attracted by scent, color and form of the honey-bearing plants, but it is the scent, which has the fastest and strongest impact (Menzel et al., 1993). Honey bees might assess the amount and concentration of nectar in each flower by employing different senses: directly by visual access to the nectar (Throp et al., 1975; Willmer et al., 1994), or by olfactory sensation (Heinrich 1979, Galen and Kevan, 1983); indirectly by an indicator of the reward for foraging such as color (Gori, 1983; Weis ,1991), flower size (Galen and Neport, 1987; Eckhart, 1991), or the particular floral structures (Bell et al., 1984; Gonzalez et al., 1995).

Volatiles released during flowering of plants facilitate flower recognition by the honeybee and thus increase their foraging efficiency. The chemical analysis of volatiles from various plant species revealed a multiplex composition of floral scents with more than 700 different compounds that were found in 60 families of plants (Knudsen et al., 1993). The mechanisms by which honeybees process this complex chemical information and adapt their behavior accordingly are as yet unknown (Wadhams, 1994). A total of 34 different compounds were found in volatiles of oilseed rape (Tollsten and Bergström, 1988, Robertsonet al., 1993; McEwan and Smith, 1998). The main volatiles from oilseed rape flowers were 3-hydroxy-2-butanone > 2,3-butanedione > dimethyl disulfide >> formaldehyde > 3-methyl-2-butanone > dimethyl trisulfide (Robertson et al., 1993). Omura et al. (1999) determined nitriles and isothiocyanates in large quantities in the floral volatiles of *Brassica rapa*. Honeybees use volatiles for discrimination whereby a conditioning threshold was determined for individual components (Pham-Delégue et al., 1993). Previous studies have shown that the S supply increases the glucosinolate in vegetative plant tissue, seeds and petals of oilseed rape (Schnug, 1988, 1993). Additionally, 2-phenylethyl isothiocyanate yielded limited conditioned responses in honeybees, but was an active component after being learned in a complex mixture of volatiles (Laloi et al., 2000). Thus a relationship

between the S-containing compound, intensity of the scent and finally the attractiveness to honey bees seems possible.

Crops visited by bees show earlier petal fall, probably because they set flowers earlier, resulting in a more uniform pod ripening and ease of harvest. Nectar, however, is the bee's source of carbohydrate and their hovering is the one of the most energy expensive forms of flight. The reflective pattern of flowers provides visitors with clues as to the age of the flowers and presence of food rewards (Kevan and Baker, 1983). During senescence of rapeseed flowers, which begins immediately after pollination, the yellow petal color vanishes and the petals shrink quickly before falling to the ground. A pollinated and fading rapeseed flower is therefore similar to an unpollinated S deficient one and thus less attractive to honey bees. Barth (1982) reported that bees prefer yellow flowers to white ones and consequently in S deficient fields, much lower bee activity has been observed than in S sufficient crops, which are bright yellow.

Smaller, whiter flowers may be less attractive to bees only after previous experience and not because of a specific signaling. Even if sufficiently with S supplied rapeseed flowers would be 'instinctively' more attractive to honey bees, the animals are known to adapt their behavior rapidly, in this case in favor of white(r) and smaller flowers if the reward will be satisfying. De Jong (1998) emphasized that bees are extremely fast in associating relevant cues with a reward. S-deficiency in rapeseed, therefore, will probably only have the negative bee-related effects when the bees can not distinguish pollinated from non-pollinated flowers as reliable as they can in rapeseed that is sufficiently supplied with S.

Who could have imagined at the beginning of the 1980s that the reduction of SO_2 emissions from burning fossil fuels (Sendner, 1985) would have an impact on honey production twenty years later?

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Metabolism and catabolism of glucosinolates

Dirk Selmar[1]

Abstract

Glucosinolates are sulfur containing natural products with numerous metabolic specialties. In this chapter, a brief overview on various aspects on its metabolism and catabolism is presented. The biosynthesis of glucosinolates is similar to that of cyanogenic glucosides. It involves the conversion of amino acids via aldoximes to corresponding thiohydroximates and the attachment of glucose. Glucosinolates are accumulated in the central vacuole and are stored without any decay. When the cells are desintegrated, the glucosinolates are hydrolyzed by myrosinases. The resulting decomposition products comprise a complex mixture of thiocyanates, isothiocyanates and nitriles, referred to as mustard oils. Due to their toxicity, these compounds exhibit an ecological significance as protective agents against herbivores and microorganisms.

Keywords: glucosinolates, mustard oil, myrosinase, secondary sulfur compounds

Introduction

Glucosinolates are sulfur containing natural products which have achieved their scientific popularity because their biology and metabolism represent interesting systems to study various aspects of biochemistry and general biology of secondary plant products (Figure 1). The knowledge of liberation of toxic reaction products from nontoxic precursors - known as *the mustard oil bomb* - has contributed significantly to an understanding of major principles in compartmentation as well as so important aspects of ecological biochemistry. Several special reviews focus on different aspects of glucosinolate research, such as taxonomy (Rodman, et al., 1996), chemistry and ecology (Louda and Mole, 1991), biosynthesis (Halkier, 1999), genetics (Mithen, 2001), degradation (Bones and Rossiter, 1996), methodology (Poulton and MØller, 1993), and anti-carcinogenic potential (Jongen, 1996; Verhoeven et al., 1997). Some more general reviews covering the entire field of glucosinolate research, are presented by Bennet et al. (1998), Selmar (1999) and Wallsgrove et al. (1999). This present paper, which is based on my previous review (Selmar, 1999) is designed to provide a brief overview of the entire biology and biochemistry of these natural products.

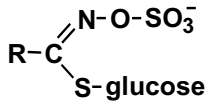

Figure1:
General structure of glucosinolates

Glucosinolates resemble cyanogenic compounds in many aspects, however, this group of compounds contains sulfur atoms in the molecule. They are characterized by the liberation of thiocyanates (mustard oils) or related nitrile compounds after being decomposed (for review see Bones and Rossiter, 1996). Decomposition takes place when tissues of glucosinolate-containing plants are damaged and cells are destroyed. Similarly to cyanogenesis, this *post mortem* process is initiated by the loss of cell integrity, leading to contact of glucosinolates with their hydrolytic enzymes. In contrast to widespread cyanogenic glucosides, the occurrence of glucosinolates is restricted. Most of these compounds are found in the Capparales, however sporadic occurrences also have been recorded for members of other families, e.g. Caricaceae, Euphorbiaceae, Sterculiaceae (Rodman et al., 1996). Glucosinolates and their degradation products are important factors in plant defence against herbivores, as well as against pathogens (for review see Louda and Mole, 1991). In addition, they have significant allelopathic potential and are thought to be effective in defense against ephemeral, unapparent plants or plant parts (Feeny, 1976).

The presence of glucosinolates in the agriculturally important crop plant, rape (*Brassica napus*), is of great economic importance, because glucosinolates reduce the feeding quality of rapeseed meal drastically. However, with regard to our health, glucosinolates also reveal positive effects based on their anticarcinogenic potential. As a consequence of the wide range of interest, glucosinolates are presently being studied in many different fields of biology, biochemistry, agriculture, and medicine.

[1] Technical University Braunschweig, Institute for Plant Biology, Section for Applied Plant Biology, Mendelssohnstrasse 4, D-38106 Braunschweig, Germany

Chemical structures

Glucosinolates consist of a ß-thioglucose moiety, a sulfonated oxime moiety, and a variable side chain. The parent compound "glucosinolate" according to the semisystematic nomenclature introduced by Ettlinger and Dateo (1961), is presented in Formula 1, where R = H. The various glucosinolates are derived by naming the side chain R as a prefix. Some examples are given in Figure 2.

Up to now, about 100 different structures of glucosinolates are known. Presumably, all are derived biosynthetically from amino acids (Kutachek et al., 1962; Underhill and Chisholm 1964). In analogy to cyanogenic glucoside biosynthesis - the carboxyl group is lost and the α-carbon is transformed into the central carbon of the glucosinolates (see chapter biosynthesis). The side chain R, therefore is identical to the substituent of the α-carbon of the amino acid. Only seven glucosinolates correspond directly to protein amino acids. In addition to the five amino acids that also are utilized for cyanogen biosynthesis (valine, leucine, isoleucine, phenylalanine and tyrosine), alanine and tryptophan also serve as precursors for glucosinolates. The large variety of additional glucosinolates is either a consequence of modification of the side chains, apparently taking place at the glucosinolate level, or has its origin in non-protein amino acids that are produced from protein amino acids by chain-lengthening processes. As an example 2-phenylethylglucosinolate is synthesized from homophenylalanine, which, in turn, is derived from phenylalanine by chain elongation (Underhill et al., 1962). Glucosinolates synthesized from methionine by side chain elongation, may have up to 11 methylene-groups introduced (Kjær and Schuster, 1972a, b). In addition, oxidation of the methionine sulfur to a sulfinyl or a sulfonyl group (Dalgaard et al., 1977), or the loss of the methylthio group accompanied by the introduction of a terminal double bond can lead to further modifications. These modifications at the amino acid level alone result in four series of methionine-derived glucosinolates (Figure 2). Additionally, glucosinolate side chains may be altered by hydroxylation, desaturation, or methoxylation. Further diversifications are achieved by esterification or acylation of the hydroxyl groups of the side chain. This can be demonstrated by the pattern of glucosinolates present in *Arabidopsis thaliana*: 23 of the identified glucosinolates correspond to various benzoyl esters of the hydroxyl groups of the side chain (Hogge et al., 1988). In the most comprehensive list of structures, Ettlinger and Kjær (1968) presented 74 different glucosinolates.

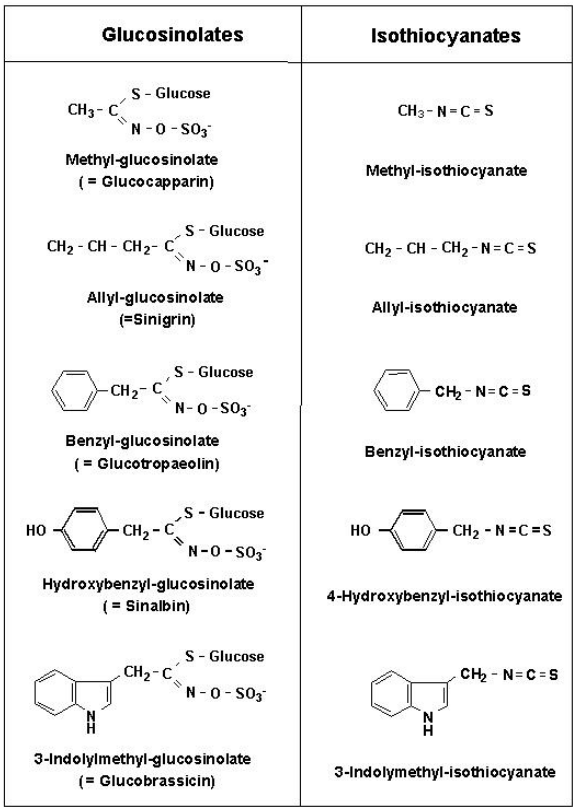

Figure 2:
Glucosinolates and related isothiocyanates. Structures of some common glucosinolates corresponding isothiocyanates.

In contrast to cyanogenic glucosides, variations of the sugar moiety are not common in glucosinolates. All known glucosinolates contain glucose bound as a thioglucose derivative. The only variations known to occur within the sugar moiety are esterifications with several organic acids, e.g., sinapinic acid (Linscheid et al., 1980; Sørensen, 1990), and in very few cases, additional glycosylation is observed. In *Hesperis matronalis*, various apiosyl derivates of hydroxybenzyl- and dihydroxybenzylglucosinolates have been detected. Interestingly, these compounds with a substituted thioglucose moiety are not hydrolyzed by myrosinases, suggesting a possible significance of these compounds in being protected against hydrolysis (SØrensen, 1990). Thus, in analogy to the diglucosidic cyanogens, these compounds might represent metabolites that can occur within the apoplastic space without being hydrolyzed, e.g. in the course of translocation processes.

Biosynthesis

The biosynthesis of glucosinolates includes three independent stages. First, the chain elongation of amino acids, secondly, conversion of the precursor amino acid into glucosinolates, and, finally, further modifications of the resulting glucosinolates. Detailed information on glucosinolate biosynthesis is given in the excellent review of Halkier (1999).

Side chain elongation of precursor amino acids

Elongation of amino acid side chains prior to glucosinolate biosynthesis has been studied in several plants. The mechanisms involved are believed to be similar to the formation of leucine from valine and acetate (Figure 3). First, through transamination, the amino acid is converted to the corresponding α-keto acid, followed by an incorporation of an acetyl residue from acetyl-CoA. After isomerization, the compounds are oxidized. In the course of this NAD mediated oxidation, the intermediate is decarboxylated. The α-keto acid produced is transaminated to yield an amino acid that, in comparison to the original compound, is elongated by a methylene group. The biochemical evidences for this scheme are based on the analysis of ^{14}C-labelled glucosinolates isolated from plants to which either ^{14}C-labelled protein amino acids, or [2-^{14}C] acetate, had been administered (Matsuo and Yamazaki, 1964; Chisholm and Wetter, 1964) A corresponding mechanism for the chain elongation for methione as precursors for the methionine derived glucosinolates in *Arabidopsis thaliana*, was recently elucidated by Textor et al., 2004).

Biosynthesis of basic glucosinolates

In contrast to the biosynthesis of cyanogenic glucosides, the intermediates involved in the conversion of the amino acids to glucosinolates are not yet unequivocally identified. However, *in vivo* studies with seedlings from various plants indicated that *N*-hydroxyamino acids, nitro compounds, oximes, thiohydroximates, and desulfoglucosinolates are putative precursors of glucosinolates (for review see

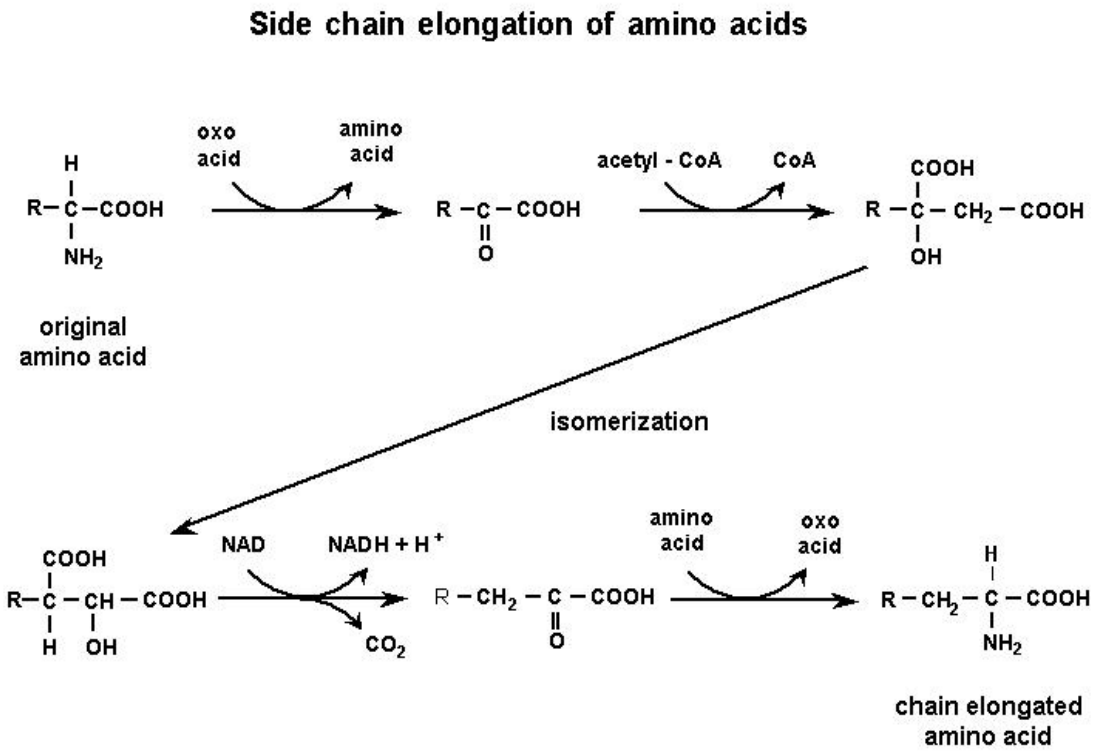

Figure 3:
Side chain elongation of amino acids. In analogy to the conversion of valine to leucine, the methene group is introduced to various other amino acids, which subsequently serve as precursors of glucosinolates.

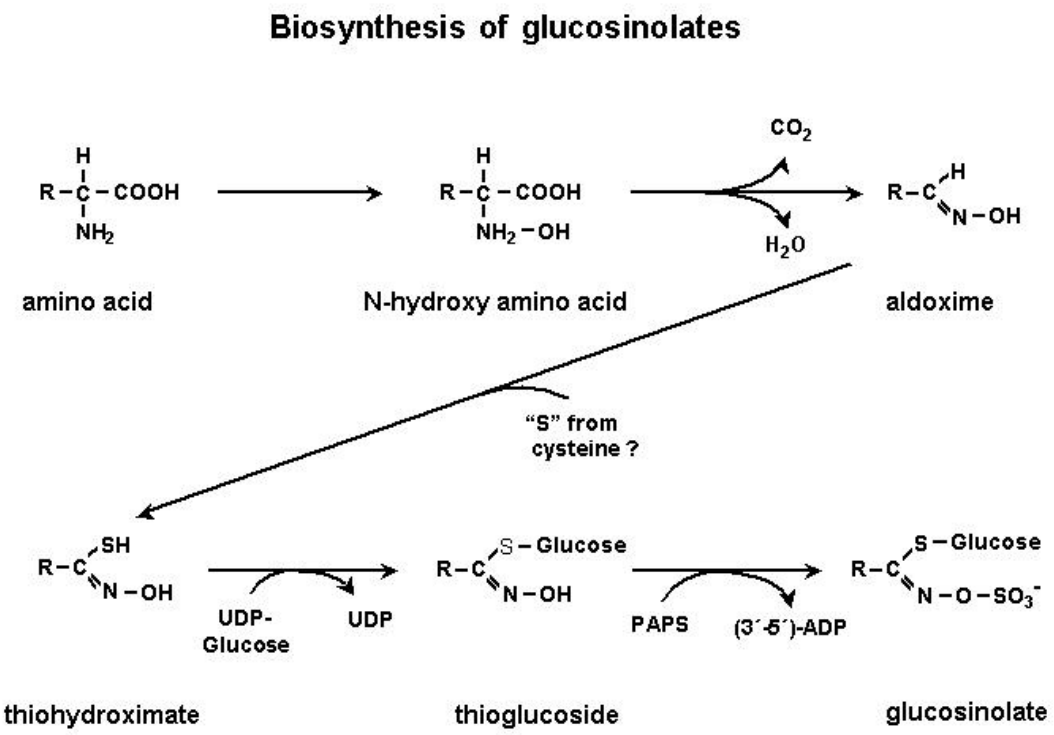

Figure 4:
Biosynthesis of glucosinolates. Postulated scheme for the glucosinolate biosynthesis. Detailed information on is given by Halkier (1999).

Underhill et al., 1973; Larsen, 1981; Halkier, 1999).

Based on various experimental data, it is evident that aldoximes are the final products of the first set of reactions leading to glucosinolates (Bennett et al., 1993; Du et al., 1995). Nevertheless, the subsequent steps in the biosynthetic pathway have not been elucidated: neither the intermediates between aldoximes and thiohydroximates have been identified nor is any biochemical evidence available for potential enzymes involved in this transformation (Halkier, 1999). Moreover, the sulfur donor for the thiol sulfur is not known, although thioglucose can be excluded (Wetter and Chisholm, 1968). *In vivo* studies reveal that several inorganic and organic sulfur compounds are incorporated into thiohydroximates. Since cysteine was incorporated most efficiently in these experiments, this amino acid is thought to be the sulfur donor (Wetter and Chisholm, 1968). Following the introduction of sulfur, the thiohydroximates produced are glucosylated by a soluble UDPG dependent transferase. In the final step of glucosinolate biosynthesis, the resulting thioglucoside is sulfurylated by PAPS. The putative biosynthetic pathway of glucosinolates is outlined in Figure 4.

Conversion of amino acids to oximes

Independent studies of various glucosinolate-containing plants indicate that, depending on the species, different enzyme systems are involved in conversion of the amino acids into aldoximes.

Analysis of microsomes isolated from young leaves of *Brassica napus* established that chain elongated amino acids are converted into the related aldoximes (Dawson et al., 1993; Bennett et al., 1993). As this reaction is not inhibited either by carbon monoxide, or by other cytochrome inhibitors, nor by antisera toward NADPH-cytochrome P450-reductase, involvement of a cytochrome P450 could be excluded. However, inhibitors of flavin dependent enzymes (e.g., copper salts, diphenyl iodonium sulfate) were effective in inhibiting aldoxime synthesis (Bennett et al., 1993; Bennett et al., 1995a). Based on these results, it is concluded that, at least in the biosynthesis of chain elongated glucosinolates in *Brassica napus*, flavin-containing mono-oxygenases are involved. Further characterization by the means of various substrates indicated that chain elongated methionine homologues inhibit competetively oxidation of homophenylalanine. In contrast, the oxidation of chain elongated methion-

ine homologues was not influenced by the corresponding aromatic and aliphatic amino acids. Thus, in *Brassica napus*, at least two flavin containing mono-oxygenases are involved in the biosynthesis of glucosinolates: one is responsible for the oxidation of elongated aromatic and aliphatic amino acids, and the other is specific for oxidation of chain elongated methionine derivatives.

In contrast, the corresponding enzyme systems isolated from young seedlings of *Sinapis alba* and *Tropaeolum majus* turned out to be cytochrome P450 monooxygenases (Du et al., 1995; Du and Halkier, 1996). These enzymes have now been purified and cloned. Also in *Arabidopsis thaliana* the phenylacetaldoxime, which represents a precursor of the benzylglucosinolate, is produced by the action of a cytochrome P450 (Wittstock and Halkier, 2000). A detailed presentation of these data and corresponding conclusions on the evolutionary relations are given by Bak et al. (1998). Based on great homology to cytochrome $P450_{tyr}$, involved in the biosynthesis of cyanogenic glucosides, it can be assumed that the reaction mechanisms of these two enzymes are very similar. As the aldoxime synthesis involved in cyanogenic glucoside biosynthesis is perfomed via *N*,*N*-dihydroxyamino acids, aldoxime synthesis leading to glucosinolates, which is catalyzed by similar cytochrome monooxygenases from *S. alba* and *T. majus*, should also include *N*,*N*-dihydroxyamino acids as intermediates (Halkier, 1999).

In seedlings of Chinese cabbage (*Brassica campestris*), conversion of tryptophan into indole acetaldoxime, representing the first step in the biosynthesis of indole glucosinolates is catalyzed by a membrane bound peroxidase (Ludwig-Müller and Hilgenberg, 1988). Because the corresponding enzymatic activity was also detected in several species that do not contain glucosinolates, it was concluded that the enzyme involved in the biosynthesis of indole acetic acid in Chinese cabbage, is also involved in indole acetaldoxime production (Ludwig-Müller et al., 1990). Various comparative studies demonstrated a good correlation between the content of indolyl glucosinolates and peroxidase activity on one hand, and the concentration of chain elongated glucosinolates and the activity of flavin-containing mono-oxygenase on the other. These correlations suggest that aldoxime production in biosynthesis of the two different groups of glucosinolates present in *Brassica* is catalyzed by distinct enzyme systems (Ludwig-Müller et al., 1990; Bennett et al., 1995b). It appears that enzymes catalyzing conversion of amino acids into aldoximes within the glucosinolate pathway have evolved at least three times in a non-homologous manner. This opens many doors for speculation and discussion about the evolutionary origin of glucosinolate biosynthesis and the manner by which it was optimized (Bak et al., 1998).

Glucosylation and sulfurylation of thiohydroximates

The final steps in glucosinolate biosynthesis are represented by the glucosylation ot the sulfhydryl group of the thiohydroximates and subsequent attachment of sulfate to the aldoxime function. Glucosylation is performed by a soluble UDP-glucose: thiohydroximate glucosyltransferase. Corresponding enzymes from *Brassica juncea* (Jain et al., 1990a), *Brassica napus* (Reed et al., 1993), and *Arabidopsis thaliana* (Guo and Poulton, 1994) have been purified and characterized. While these enzymes seem to be specific for thiohydroximates, they do not reveal a marked substrate specificity with regard to differences in the side chain.

Little is known about sulfation of desulfoglucosinolates. The sulfate is introduced by PAPS (3′-phosphoadenosine-5′-phosphosulfate). Only two corresponding sulfotransferases have been detected and purified: first from cress seedlings, *Lepidium sativum* (Glendening and Poulton, 1988), and, secondly, from *Brassica juncea* cell cultures (Jain et al. 1990b). Both enzymes investigated have very similar properties. They catalyzed the sulfation of several different desulfoglucosinolates. Despite their low substrate specificity for desulfoglucosinolates, they do not catalyze the transfer of sulfate to other potential substrates, e.g. flavonoids, and phenylacetaldoximes.

Side chain modification of basic glucosinolates

In addition to the side chain modification of the precursor amino acid, also the side chain of the synthesized glucosinolates can be modified. These modifications consist of hydroxylations and transformations of methylthio groups into methylsulfinyl groups, into methylsulfonyl groups, and, by elimination, into terminal double bonds. The enzymes involved in these modifications have not been identified, however, based on comprehensive genetic studies it can be deduced that chain modifications of aliphatic glucosinolates depend on three loci (Parkin et al., 1994; Mithen et al., 1995; Giamoustaris and Mithen, 1996). In spite of the great variation in aliphatic side chain structures, the genetic results indicate that the diversity is the result of genetic variations of these three major loci.

Biochemical studies indicate that the enzyme that is responsible for the hydroxylation of 3-butenylglucosinolate to yield 2-hydroxy-3-butenylglucosinolate in *Brassica napus* corresponds to a cytochrome P450 mono-oxygenase (Rossiter et al., 1990).

The introduction of a *Brassica*-dioxygenase gene, whose protein seems to be responsible for side chain

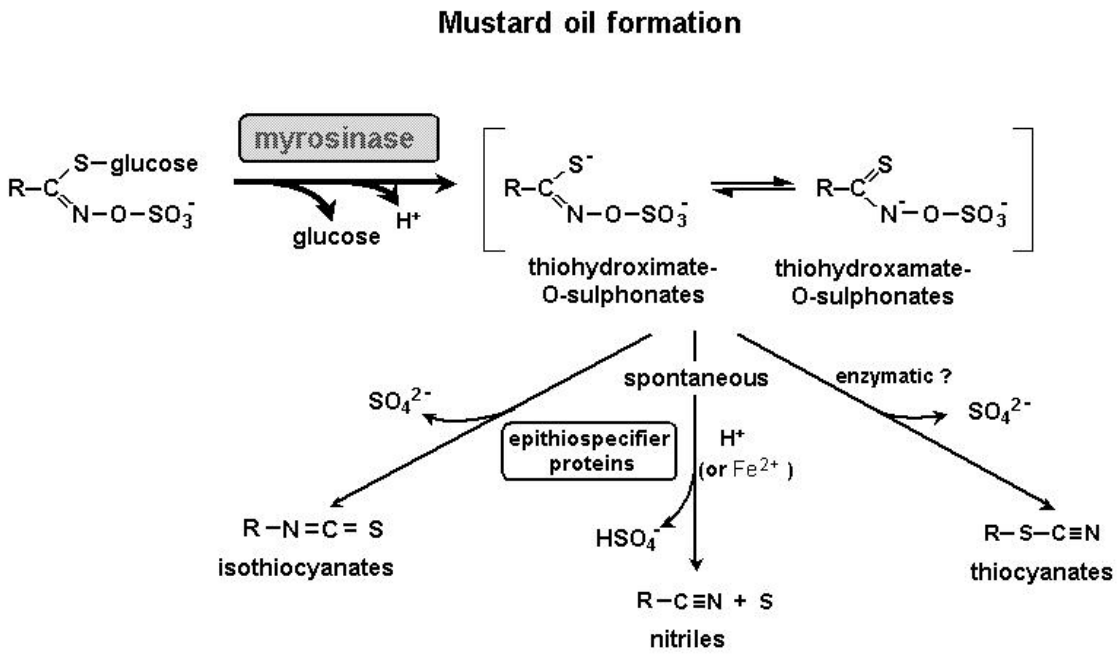

Figure 5:
Mustard oil formation. After hydrolysis of glucosinolates, the unstable intermediates rearrange. In general, the main reaction products are isothiocyanates, but also nitriles and thiocyanates are produced.

modification of glucosinolates, into *Arabidopsis thaliana* resulted in significant changes of the glucosinolate profile in the transformed plants (Li and Quiros, 2003).

Mustard oil formation

All plants containing glucosinolates also contain enzymes that are capable of decomposing these compounds. These ß-glucosidases are generally called myrosinases. The enzymatically catalyzed loss of glucose yield in thiohydroxamate-*O*-sulfonates which isomerize to thiohydroxamate-*O*-sulfonates. These compounds rearrange by Loessen-type reaction with a concerted loss of sulfate to yield isothiocyanates. However, not only isothiocyanates, but also the corresponding nitriles are formed in greater or lesser amounts along with the concomitant liberation of elemental sulfur (Figure 5). Nitrile formation is favored by low pH values and is also promoted by ferrous ions (for review see Larsen, 1981). Under *post mortem* conditions after tissue disruption, isothiocyanates normally are the predominant products, accompanied by smaller amounts of nitriles. In contrast, the aglycones of some glucosinolates (e.g., allyl, benzyl, and 4-(methylthio)-butyl glucosinolates) undergo enzymatic degradation to thiocyanates. The mechanism for thiocyanate formation is still unknown. The enzyme presumably responsible for the corresponding rearrangement to yield thiocyanates has neither been isolated nor properly characterized. The presence of ß-hydroxylated side chains results in spontaneous cyclization of isothiocyanates to produce oxazolidine-2-thiones. A terminal double bond in the side chain may result in the formation of epithionitriles, although for this reaction an epithiospecifier protein is necessary (Figure 5). The complex mixture of isothiocyanates, thiocyanates, nitriles and possibly some other reaction products is termed as mustard oil.

When tissues of glucosinolate-containing plants are injured and cells are disrupted, myrosinases and glucosinolates come into contact and mustard oil formation is initiated. This process has been described graphically as a *mustard oil bomb* (Matile, 1980). Consequently, under *in vivo* conditions, hydrolytic enzymes and glucosinolates are efficiently partitioned. Glucosinolates are localized in vacuoles (Grob and Matile, 1979; Helmlinger et al., 1983). In contrast, the localization of the myrosinase remained unclear. It has long been known that myrosinases are localized in special cells, so-called myrosin cells (Guignard, 1980). Myrosin cells are scattered throughout most tissues of glucosinolate-containing plants. As myrosin cells contain special granular structures, called myrosin grains, and the presence of myrosinase activity was detected in vacuolar fractions (Matile, 1980), it was concluded that myrosinase is localized inside the myrosin grains. Presently, the localization of myrosinase in myrosin

cells has been confirmed by immunocytochemical studies. Myrosinase is localized in the cytosol, although it is associated with the membrane surface of myrosin grains (Thangstad et al., 1990; Thangstad et al., 1991). Apart from the presence of myrosinase in the cytosol, enzyme activity also can be detected in cell walls, corresponding to an apoplastic localization (Matile, 1980).

Certainly, degradation of glucosinolates is initiated by the mixing of enzymes and substrates; however, mustard oil formation is accelerated by concomitant activation of the myrosinase by ascorbic acid, which is localized in the vacuoles of intact cells (Grob and Matile, 1980). The stimulation by ascorbic acid appears to be due to conformational changes of the enzyme, probably as a consequence of the reduction of disulfide bridging in the protein (Bones and Rossiter, 1996).

The estimation of myrosinase activity in the presence of ascorbic acid causes various difficulties. Up to now, a wide array of methods for the determination of myrosinase activity has been described. These vary from the simple photometric estimation to highly sophisticated assays using radioactively labeled substrates. However, ascorbic acid - the effective activator of myrosinases - interferes with most of these enzyme tests. Unfortunately, in the past such interferences were disregarded in many scientific examinations of myrosinases. Whereas such failings have less effects when the activation of myrosinases is not very distinctive, they are quite relevant in all cases where myrosinases are completely inactive in the absence of ascorbic acid (Kleinwächter and Selmar, 2004). The authors presented an interference-free HPLC-based quantification method of the enzymatically produced glucose, by which the activation by ascorbic acid could be estimated exactly (Kleinwächter and Selmar, 2004).

Interestingly, various other proteins have been identified in relation to myrosinases, namely myrosinase binding proteins, myrosinase binding protein-related proteins and myrosinase-associated proteins (Falk et al., 1995; Taipalensuu et al., 1996). The localization and putative function of these proteins has not yet been clarified, but it has been speculated that they are important for the activation process of myrosinase as cell integrity is destroyed (Geshi and Brandt, 1998).

Myrosinases are the only known *S*-glucosidases; they exhibit a pronounced substrate specificity towards glucosinolates. The hydrolysis of other *S*- or *O*-glucosides is only poorly catalyzed by these enzymes (Lein, 1972; Durham and Poulton, 1990). Ascorbic acid activates most myrosinases at concentrations at about 1 mmol/l, whereas higher concentrations inhibit myrosinase activity (Ohtsuru and Hata, 1973). In the meantime, cDNAs of several myrosinases have been cloned and sequenced, e.g., from *Sinapis alba* (Xue et al, 1992) *Brassica napus* (Thangstad et al., 1993), and *Arabidopsis thaliana* (Chadchawan et al., 1993). Myrosinases are encoded by multigene families: 14 genes have been estimated to be present in *Brassica napus* (Thangstad et al., 1993). Recently, a myrosinase from *Sinapis alba* was crystallized (Burmeister et al., 1997). This enzyme folds into a structure very similar to that of cyanogenic ß-glucosidases from white clover (Barrett et al., 1995), which supports the assumption that myrosinases have been evolved from ancestral *O*-glucosidases (Burmeister et al., 1997).

Ecological significance of glucosinolates

In a manner similar to cyanogenic glucosides, glucosinolates can be considered as preformed defense chemicals that are activated in case of emergency. Many experimental data demonstrate the protective role of glucosinolates and their degradation products, respectively (For review see Louda and Mole, 1991; Oleszek, 1995). The pungent smell and taste of glucosinalates reduce the palatability of plants that conatin them to generalist herbivores, e.g., birds, slugs and insects (Chew, 1988; Glen et al., 1990). Because isothiocyanates can easily penetrate biomembranes, they can interact with epidermal and mucosal skin, leading to painful irritations. In addition, isothiocyanates can lead to various complaints (e.g., bronchitis, pneumonia, gastroenteritis, kidney disorders). Consequently, high concentrations of glucosinolates and isothiocyanates are toxic to animals; although in general, adapted specialists such as the white cabbage butterfly (*Pieris brassicae*) can handle these toxins (Siemens Mitchell-Olds, 1996). For the imagines of these specialized butterflies, glucosinolates are even attractants that stimulate oviposition. Interestingly, the oviposition stimulus has its origin in the glucosinolates rather than in the isothiocyanates. This was clearly demonstrated by application of allyl glucosinolate and allyl isothiocyanate, respectively, to non-host plants of the butterfly (Stadler, 1978).

In addition to their protective function against herbivores, glucosinolates and their degradation products also are important factors for the interactions of plants with microorganisms. In most cases reported, the presence of glucosinolates enhances the resistance of the plant against numerous pests (Giamoustaris and Mithen, 1996; Mayton et al., 1996). In *Brassica napus*, the content of glucosinolates increased significantly after being infected with various pathogens (Doughty et al., 1991). However, the resistance is not caused by the glucosinolates themself, but by their degradation products, i.e., the isothiocyanates (Mayton et al., 1996; Manici et al., 1997; Smolinska et al., 2003). In addi-

tion to numerous data on the protective function of glucosinolates against pathogens, there are also quite opposite findings: high glucosinolate contents in Chinese cabbage enhanced its susceptibility to *Plasmodiophora brassicae*, the causal organism of the clubroot disease. The reason for these contradictions is not understood and may be attributed to differences in the specificity of the pathogens involved.

Glucosinolates have been reported to have a significant allelopathic potential and are thought to be involved in the defense of ephemeral, unapparent plants or plant parts (Feeny, 1976). Several studies indicate that, in analogy to other ecological effects, this allelopathic impact is caused by isothiocyanates rather than by the intact glucosinolates (Brown and Morra, 1995; Bialy et al., 1990; Oleszek, 1995). In contrast, some studies suggest that neither glucosinolates nor isothiocyanates have significant allelopathic potential (Choesin and Boerner, 1991). These differences may be explained by the use of different plants species for the evaluation of the allelopathic potential.

Variations in the glucosinolate content

Like other secondary metabolites, also the concentration of glucosinolates accumulated varies in a wide range. These variations depends upon both genetic and environment. Individual variations are reported for a great number of species, e.g. *Brassica oleracea* (Kushad et al., 1999), *Brassica napus* (Li et al., 1999 ; Kraeling et al., 1990), *Arabidopsis thaliana* (Kliebenstein et al., 2001) *Tropaeolum majus* (Kleinwächter, 2002). Even within one single plant, the glucosinolate contents might vary drastically, depending on the developmental stage (Rangkadilok et al.; 2002, Brown et al., 2003) or on diurnal rhythms (Rosa et al., 1994; Rosa 1997).

Environmental influences on the accumulation of glucosinolates are described for nearly all factors known to influence plant metabolism, e.g. light and temperature (Rosa and Rodriguesl, 1998), climatic conditions (Ciska et al., 2000; Vallejo, 2003), water stress (Bouchereau et al., 1996) or the presence of high concentrations of heavy metals in the soil (Coolong et al, 2004). The most important factor to influence plant growth used by agronomists is the application of fertilizer. As well the application of nitrogen (e.g. Fismes et al., 2000; Bloem et al., 2001) as the application of sulfur significantly influences the amount of glucosinolates accumulated in the plants. As sulfur fertilization in nearly all cases so far analyzed results in a massive enhancement of the glucosinolate content (e.g. Kim et al., 2002, Bloem et al. 2001) it can be deduced that the sulfur available for the plants corresponds to a limiting factor in glucosinolate biosynthesis or accumulation, respectively.

Glucosinolates and nutrition

Many glucosinolate containing plants (e.g. cabbage, kale, broccoli, Brussels sprouts, cauliflower, and horse radish) are used by man as foods or spices. Thus, human metabolism often is affected by glucosinolates and their degradation products. These natural products are precursors of compounds with goitrogenic action in animals and humans. The active antithyroid compounds include isothiocyanates as direct products of glucosinolate hydrolysis, and thiocyanate ions as final decomposition products. As mentioned above, rhodanide affects thyroid functions (van Etten, 1969). Moreover, in some plants, the goitrogenic effects of glucosinolates are strongly enhanced by specific degradation products, such as oxazolidine-2-thiones (e.g., progoitrin, glucoconringin). These compounds inhibit the oxidation of iodate to iodine, which strongly affects thyroid function.

Based on their toxic properties and their pungent taste, glucosinolates are often classified as antinutritive compounds. However, the special taste of glucosinolates and their degradation products, respectively, is often desired by the consumer. Thus, numerous glucosinolate-containing plants are extensively consumed and represent important vegetables. Generally, glucosinolate levels in fresh plant parts (stems, leaves), based on fresh weight, are 0.1 % or less (van Etten et al., 1976). These moderate concentrations do no not create health problems when glucosinolate containing vegetables or cole crops are consumed.

In addition to the negative properties of glucosinolates and their degradation products on human nutrition, these compounds also seem to have positive effects. The consumption of glucosinolate-containing vegetables apparently reduces the risk of developing cancer. Most evidence concerning the anticarcinogenic effects of glucosinolate hydrolysis products comes from studies in animals (For review see Verhoeven et al., 1997; Jongen, 1996). However, epidemiological data concerning the cancer-preventive effects of *Brassica* vegetables, including cabbage, kale, broccoli, Brussels sprouts, and cauliflower, also support this assumption (Verhoeven et al., 1997). The exact mechanism by which glucosinolates and their degradation products, respectively, are involved in cancer prevention is not completely understood. The anti-carcinogenic effects of isothiocyanates appear to be mediated by tandem and co-operating mechanisms. First, carcinogen activation by cytochromes P450 is suppressed, probably by a combination of down-regulation of enzyme levels

and direct inhibition of their catalytic activities. These effects lower the levels of carcinogens ultimately formed. In addition, these compounds promote the induction of phase 2 enzymes, such as glutathione transferases and NAD(P)H: quinone reductase, enzymes that detoxify any residual electrophilic metabolites generated by phase I enzymes. In this manner, phase 2 enzymes destroy the ability of these residual compounds to damage DNA. (Zhang and Talalay, 1994; Zhang et al., 1994). 4-Methylsulfinylbutyl isothiocyanate (sulforaphane), isolated from broccoli, turned out to be a potent anticarcinogen. The isolated compound effectively induces phase II enzyme (Zhang et al., 1992). In contrast to a protective action, a few isothiocyanates apparently have mutagenetic potential in mammal cells and in bacteria (Verhoven et al., 1997). Nevertheless, as isothiocyanates block carcinogenesis by dual mechanisms and are present in substantial quantities in human diets, these agents are ideal candidates for the development of effective chemoprotection schemes for humans against cancer (Zhang and Talalay, 1994). Consequently, glucosinolate hydrolysis products are considered to be good candidates for creating "functional foods", designed to prevent cancer, e.g., by enhancement of the concentration of 4-methylsulfinylbutyl isothiocyanate in cole plants.

In contrast to green plant parts, the concentration of total glucosinolates in seeds may be much higher. Levels up to 10% dry weight have been reported (Josefsson, 1973, van Etten et al., 1974). Due to their general toxicity, these plant parts are not used for nutritional purposes. However, rapeseed meal, a side product of rapeseed oil production, is used as fodder for various animals. Strong efforts have been made, to breed *Brassica napus* varieties that contain small amounts of glucosinolates in the seeds. As classical breeding strategies have had only limited success, this goal may be achieved by gene technology, e.g., by knocking out the biosynthetic pathway.

Conclusions

In recent years, much scientific work has focused on the biochemistry of glucosinolates. Certainly, significant progress has been made in elucidating glucosinolate pathways and the steps of biosynthesis. However, due to the multiple enzyme systems present, i.e., both cytochrome P450 monooxygenases and flavine dependent oxygenases that produce glucosinolates in different plants by distinct routes, and the numerous mechanisms and modifications of pathways, precursors and products of these biosynthetic pathways many questions related to the biosynthesis of glucosinolates cannot be answered at present. In order to establish a solid basis for obtaining glucosinolate plants with desired properties, much basic research is still required. It seems feasible to increase the level of 4-methylsulfinylbutyl glucosinolate in order to increase the anticarcinogenic potential, and also to create seeds that only contain traces of glucosinolates. Unfortunately, and in contrast to the metabolism of cyanogenic glucosides, there is nearly no information on the *in vivo* metabolism of glucosinolates. Related knowledge about the accumulation, translocation, and turnover processes of glucosinolates is an important precondition for understanding those metabolic processes that will be modified in the corresponding transgenic plants. More knowledge about glucosinolates and their metabolism is required for successful biotechnological approaches.

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Regulation of glutathione (GSH) synthesis in plants: Novel insight from *Arabidopsis*

Andreas Wachter[1] and Thomas Rausch[1]

Abstract

During the past decade, cDNAs for the enzymes catalyzing GSH biosynthesis, GSH1 (γ-glutamyl-cysteine synthetase) and GSH2 (glutathione synthetase), have been cloned for several plant species. Mutant complementation and characterization of the recombinant GSH1 and GSH2 proteins have confirmed the predicted enzymatic functions. Gene expression analysis has indicated that higher plants respond to biotic and abiotic stress factors with an upgraded glutathione (GSH) synthesis, for which the induction of GSH1 appears to play a pivotal role. However, the expression of active GSH1 enzyme is regulated at multiple levels, including transcriptional, translational and post-translational controls. Here we summarize recent research on the subcellular compartmentation and regulated expression of the GSH1 enzyme in *A. thaliana.* Transgenic *A. thaliana* plants expressing GUS::EGFP fusions under the control of the *AtGSH1*-promoter revealed tissue- and cell type-specific differences in GSH1 expression, and a pronounced developmental modulation of the response to the stress hormone jasmonic acid. When the *AtGSH1* homolog from *Brassica juncea*, *BjGSH1-1*, was expressed in *A. thaliana* under the control of the 35S promoter, several lines showed a strong increase of GSH1 protein without a significant change of GSH content. Conversely, co-suppression lines were obtained which revealed strong decreases of *AtGSH1* and *BjGSH1-1* transcripts, GSH1 protein, and GSH content. In an attempt to selectively overexpress GSH1 protein in stomata, we transformed *A. thaliana* with a fusion between a strong, stomata-specific promoter (*PRP4*) and the *BjGSH1-1* coding sequence. While stomata-specific expression could be verified, *in situ* labeling of GSH with MCB did not reveal a significant increase in guard cell GSH content. We conclude that to engineer GSH1 activity in plants, the presence of multiple expression controls has to be taken into account.

Key words: Arabidopsis thaliana, glutathione synthesis, GSH1, GSH2, compartmentation, transcriptional/post transcriptional regulation, GSH1 overexpression, stomata

[1] Heidelberg Institute of Plant Sciences (HIP), Im Neuenheimer Feld 360, D-69120-Heidelberg, Germany

Introduction

Glutathione (GSH) is the predominant non-protein thiol compound in eukaryotic and prokaryotic cells. By its reversible oxidation to GSSG it represents a major cellular redox buffer. In higher plants, this buffer role is crucial for the cellular response to increased formation of reactive oxygen species (ROS) caused by abiotic or biotic stress (May et al., 1998; Noctor et al., 1998; Ruiz and Blumwald, 2002). Additional roles of GSH include its function i) as storage and long distance transport form for assimilated sulfur (Brunold and Rennenberg, 1997), ii) as electron donor for the APS reductase reaction (Bick et al., 1998), iii) as binding partner for GST-mediated conjugation of secondary plant metabolites and xenobiotics (Marrs, 1996; Alfenito et al., 1998; Wagner et al., 2002), and iv) as precursor for the heavy metal-binding phytochelatins (PCn) (Grill et al., 1985; Howden et al., 1995a, b; Cobbett et al., 1998; Cobbett, 1999; Ha et al., 1999; Cobbett, 2000). Furthermore, GSH also appears to act as important developmental signal as revealed by its influence on root meristem activity (Sanchez-Fernandez et al., 1997; Vernoux et al., 2000) and flowering (Ogawa et al., 2004).

GSH is synthesized in two ATP-dependent reactions, catalyzed by γ-glutamylcysteine synthetase (GSH1; EC 6.3.2.2.) and glutathione synthetase (GSH2; EC 6.3.2.3.; Figure 1). Higher plant *GSH1* and *GSH2* cDNAs have been cloned and functionally expressed (May and Leaver, 1994; Ullmann et al., 1996; Wang and Oliver, 1996). In *A. thaliana*, *GSH1* and *GSH2* are present as single genes (May and Leaver, 1994; Ullmann et al., 1996; The Arabidopsis Genome Initiative, 2000). The *in silico* analysis predicted plastidic transit peptides for both enzymes, but recent studies have indicated that only GSH1 is confined to the plastidic compartment, whereas the larger part of *GSH2* transcripts encode a cytosolic protein (Wachter and Rausch, 2004; Wachter et al, 2004).

Previous investigations support the notion that in response to several stress factors GSH1 (and to a lesser extent GSH2) expression is strongly up-regulated (Schäfer et al., 1998; Xiang and Oliver, 1998). As an upgraded synthesis of GSH has been

considered crucial for the cellular adaptation to oxidative stress (see above), several attempts have been made to increase the stress tolerance of higher plants by ectopic overexpression of GSH1. Initially, the *E.coli* GSH1 enzyme, with or without a plastidic transit peptide (Noctor et al., 1996, 1998), was expressed under the regulation of the 35S promoter. Different plant species differed in their response to ectopic GSH1 expression, ranging from increased stress tolerance (Zhu et al., 1999) to symptoms of oxidative stress due to a GSH/GSSG imbalance (Creissen et al., 1999).

In *A. thaliana*, ectopic overexpression of its own *GSH1* gene caused only a minor increase of GSH content, whereas in *antisense* plants GSH content was clearly reduced (Xiang et al., 2001). Previous studies on a redox-regulated 5'UTR binding factor indicated that in addition to transcriptional induction, the expression of GSH1 protein also appears to be under translational control (Xiang and Bertrand, 2000). Recently, Jez et al. (2004) described a post-translational redox control of GSH1 activity, adding an additional facet to the regulation of GSH1 activity. In this report, we present new data i) on the regulation of the *AtGSH1* promoter, ii) on the analysis of transgenic *A. thaliana* plants transformed with the *AtGSH1* homolog of *Brassica juncea*, *BjGSH1-1,* including *sense* transformants and co-suppression lines, and iii) on the targeted overexpression of *BjGSH1-1* in guard cells. The results strongly support a multiple control of GSH1 expression in plants.

Materials and Methods

Plant material

Arabidopsis thaliana, ecotype Columbia, was grown under greenhouse conditions (approx. 8 h light period). Plant tissues for protein and RNA extraction were immediately frozen in liquid nitrogen and stored at -80°C.

Gene constructs for plant transformation

A 1605 bp fragment containing sequences upstream of the predicted ATG start codon of *AtGSH1* was amplified by PCR using 5'Bamecs (5'-ATGCGGATCCATCGTATGTAACAATAATGG ATCTTGTAG-3') and 3'Bamecs (5'-ATGCG-GATCCGGTATATTAGCTCCTGCAATTATAAC AATTC-3') primers. The amplified promoter sequence was digested with *Bam*HI and cloned into appropriate site of the vector pBSK-LUC, containing the reportergene luciferase. The cassette of *AtGSH1* promoter and *LUC* was cut out with *Pvu*II and *Xho*I and ligated into *Eco*RI/*Sal*I sites of the vector pBinAR for plant transformation (Höfgen and Willmitzer, 1992). The *AtGSH1* promoter region was also amplified with 5'gatecs (5'GGGGACAAGTTTGTACAAAAAAGCAGGC TATCGATAT-GTAACACAATAAT-3') and 3'gatecs (5'GGGGACCACTTTGTACAAGAAAG-CTGGTGGTATATATAGCTCCTGCA-3') primers and cloned, by use of the Gateway™ (Invitrogen, Karlsruhe, Germany) recombination system, into the entry vector pDONR and subsequently into destination vector pKGWFS7 in front of a fusion of the reportergenes *EGFP* and *uidA*.

For overexpression of BjGSH1-1, a 1639 bp fragment, containing the long 5'UTR sequence and the full length coding sequence of BjGSH1-1, was amplified by PCR using 5'BamUTRECS1 (5'-ACTGGATCCAGCTCTCCACTGATAGGATTAT -3') and 3'SalECS1 (5'-TGACGTCGACTCAGT-AAAGCAGTTCCTGGAACACAGG-3') primers. This fragment was digested with *Bam*HI and *Sal*I and cloned into appropriate sites of vector pBinAR (Höfgen and Willmitzer, 1992). To analyze the tissue specificity of the promoter of the *AtPRP4* gene (at4g38770), 1552 bp upstream of the predicted start codon were amplified by PCR using primers 5'gatprp4 (5'-GGGGACAAGTTTGTAC-AAAAAAGCAGGCTAACACCTAGAACGCAGT CAGG-3') and 3'gatprp4 (5'-GGGGACCACTT-TGTACGAAAGCTGGGTTGGGATTCTCACCCT CTGAGA-3'). By use of the Gateway™ recombination system, the promoter sequence was cloned into the entry vector pDONR, and, subsequently, into destination vector pKGWFS7 in front of a fusion of the reportergenes *uidA* and *EGFP* for plant transformation. For guard cell specific overexpression of BjGSH1-1, the coding sequence was first amplified with primers 5'Bamecs1 (5'-ACTGGGATCCATGGCGTTATT-GTCTCAGGCAGGAGG-3') and 3'Salecs1 (5'-TGACGTCGACTCAGTAAAGCAGTTCCTGGA ACACAGG-3') and, after digestion with *Bam*HI and *Sal*I, cloned into appropriate sites of pBinAR (resulting in pBinAR-BjGSH1-1). The *AtPRP4* promoter was amplified using 5'Ncoprp4 (5'-ACTGCCATGGAACACCTAGAACGCAGTCAG G-3') and 3'Kpnprp4 (5'-ACTGGGTACCTGGGA-TTCTCACCCTCTGAGA-3') primers and subcloned into the pGEM-T (Promega) vector. The promoter sequence was released from pGEM-T by restriction with *Hinc*II and *Kpn*I and ligated into pBinAR-BjGSH1-1, which was digested with *Eco*RI and *Kpn*I and treated with Klenow fragment for filling of 3'recessed ends before.

Stable A. tumefaciens-mediated transformation of A. thaliana by floral dip

A. thaliana plants were transformed by the floral dip method according to Clough and Bent (1998). After transformation, seeds were screened on solid MS

medium containing 0.8 % agar and 50 µg ml^{-1} kanamycin under sterile conditions and transformants were transferred to soil after two weeks.

Quantitative determination of transcripts by Real-Time PCR

Total RNA was extracted from leaf tissue of *A. thaliana* and transcribed in cDNA as described before (Wolf et al., 2003). Real-Time PCR was performed using the Platinum Taq-DNA Polymerase (Invitrogen, Karlsruhe, Germany) and SYBR-Green as fluorescent reporter in the Biorad iCycler. Primers for the coding region of *AtGSH1* were 5'AtGSH1rt (5'-CAAGCTTGACGAATTTCAGG-AGC-3') and 3'AtGSH1rt (5'-ACGCCACCCGA-AACAACAG-3'). The *BjGSH1-1* transcripts were amplified with primers 5'BjGSH1rt (5'-AGTCGC-CGATCCGAACTTG-3') and 3'BjGSH1rt (5'-TTC-CGGTCCTGGAGCTTACG-3'). Primer sequences for actin (Act2/8) were reported previously (Ha et al., 1999). A serial dilution of cDNA was used as standard curve to calculate amplification efficiency for *AtGSH1* and actin primers. Each reaction was performed in triplicates, and specificity of amplification products was confirmed by melting curve and gel electrophoresis analysis. Relative abundance of *AtGSH1* and *BjGSH1-1* transcripts was calculated and normalized with respect to Act2/8 mRNA according to the method of Muller et al. (2002).

Immunoblot analysis

Total protein extraction and immunoblot analysis were performed as described in Bogs et al. (2003). The primary antiserum was used in a 1:10,000 dilution in 5% BSA.

Thiol analysis

For extraction of total thiols, 30 mg of deep-frozen grinded material was vortexed with 1 ml extraction buffer (0.1 N HCl, 1 mM EDTA, 4 % non soluble Polyvinylpyrrolidon) and centrifuged for 30 min at 15,000 g and 4 °C. 50 µl of the supernatant was mixed with 50 µl 500 mM CHES (2-(N-Cyclohexylamino)ethane sulfonic acid) pH 9.4, 10 µl 30 mM monobromobimane (MBB) and 10 µl 10 mM DTT and incubated for 15 min at room temperature in the dark for MBB labeling of thiols. The reaction was stopped by adding 400 µl 10 % acetic acid and thiols were analyzed by HPLC.

Quantitative analysis of luciferase (LUC) activity

LUC activities of leaf samples were determined as described by Lehr et al. (1999).

Histochemical analysis of β-glucuronidase (GUS) activity

For analysis of GUS activity, tissue samples were treated with GUS staining buffer (100 mM Na_2HPO_4/NaH_2PO_4, pH 7.0, 10 mM Na_2EDTA, 0.5 mM $K_3[Fe(CN)_6]$, 0.5 mM $K_4[Fe(CN)_6]$, and 0.08% X-GlucA (Duchefa, Haarlem, The Netherlands) for 16 h at 37°C. Green tissues were bleached with ethanol before examination.

In vivo labeling of glutathione and confocal laser scanning microscopy (CLSM) analysis

Monochlorobimane (MCB) *in vivo* labeling of glutathione was performed as described by Hartmann et al. (2003). For confocal analysis of MCB fluorescence, LSM410 (Zeiss, Jena) was used with the following settings: excitation 405 nm and emission longpass 420 nm, chlorophyll autofluorescence was detected in parallel using 560 nm longpass.

Results and discussion

AtGSH1 and AtGSH2 are differentially compartmentalized

The formation of reactive oxygen species (ROS) during abiotic or biotic stress exposure is not confined to a single compartment. Corroborating this notion, the ascorbic acid-GSH cycle, which eliminates ROS, is operative in different cellular compartments, including plastids, mitochondria, peroxisomes and the cytosol (Jiménez et al., 1997, 1998). Conversely, GSH1, the rate-limiting enzyme of GSH synthesis, was recently shown to be confined to the plastidic compartment, whereas the second enzyme, GSH2, appears to be largely cytosolic (Wachter and Rausch, 2004, Wachter et al, 2004) and only to a minor extent plastidic (Figure 1). These observations indicate that GSH and its dipeptide precursor γEC have to be transported between different cellular compartments. Only recently, the first plant GSH transporters have been identified (Bogs et al., 2003; Zhang et al., 2004), however, their intracellular localization has not yet been determined. Future research will have to address how these transport processes are regulated in an appropriate manner to meet the demands of the different cellular compartments for GSH, in particular after stress exposure.

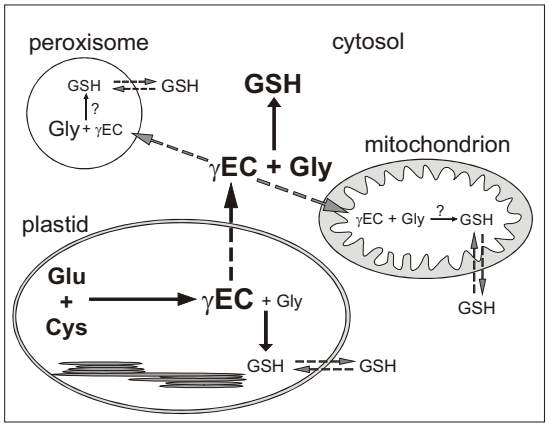

Figure 1:
Compartmentation of GSH synthesis in plant cells.The GSH1 enzyme is confined to the plastids, whereas the GSH2 enzyme is primarily localized in the cytosol, and, to a minor extent, in plastids (Wachter and Rausch, 2004). Indirect evidence suggests that, at least in some species, GSH2 may also be found in mitochondria and peroxisomes. Since GSH export from plastids appears to be slow or absent (Meyer and Fricker, 2002), it has been speculated that the product of the GSH1 reaction, γEC, may directly exit from the plastid and be the precursor for cytosolic GSH synthesis (Wachter, 2004; Wachter et al., 2004).

The AtGSH1 promoter shows a broad activity spectrum during plant development and an age-dependent response to the stress hormone jasmonic acid

Previous studies have shown that up-regulation of GSH synthesis is, at least under certain conditions, achieved by an increased transcription of the *GSH1* gene. We have generated transformed *A. thaliana* lines expressing a EGFP::GUS fusion under the control of the *AtGSH1* promoter. Histochemical analysis of several independent transformants has shown that despite a broad activity range in different organs and cell types, the *AtGSH1* promoter appears to be particularly active in vascular tissue, trichomes, stipules, flowers, embryos and root tips (Wachter, 2004). Our results confirm the notion that despite its wide activity window, the *AtGSH1* promoter shows a pronounced developmental component. As the stress hormone jasmonic acid has previously been shown to induce the expression of *GSH1* (Xiang and Oliver, 1998), we have analyzed the response of the *AtGSH1* promoter to JA in plants of different age. Surprisingly, we observed an induction or repression of promoter activity, depending on plant age (Table1). In leaves of rosette stage plants, JA caused a significant down-regulation of promoter activity as determined in transgenic *A. thaliana* plants transformed with a *AtGSH1* promoter-*LUC* (luciferase) construct. Conversely, in leaves of flowering plants this promoter was strongly activated. This conspicuous discrepancy indicates a differential sensitivity to JA and/or a change in endogenous JA content during plant development.

Table 1:
Quantitative analysis of promoter activity in *AtGSH1 promoter-LUC* transformants of different age. Leaves of 10-week-old (rosette stage) or 14-week-old (flowering stage) plants (several independent primary transformants) were fed with 50 µM jasmonic acid (or water) via the leaf base and incubated for 24 or 48 hrs. LUC activities before (arbitrarily set to 100%) and after jasmonic acid treatment were determined in total leaf extracts.

Plant individual	LUC activity 24 hrs after JA-treatment	LUC activity 48 hrs after JA-treatment
	10-week-old plants	
at16-1	6%	3%
at16-2	59%	97%
at16-3	93%	42%
at12-1	25%	23%
at12-2	67%	24%
	14-week-old plants	
at6-9	520%	960%
at6-19	1820%	5410%
at6-20	360%	12360%
at6-21	690%	4530%

In BjGSH1-1 sense transformants of A. thaliana, the total cellular GSH content remains largely unaffected, whereas co-suppression lines show reduced contents of GSH, GSH1 protein, and AtGSH1 transcripts

In an attempt to manipulate the expression of GSH1 protein by ectopic expression of a transgene, we have transformed *A. thaliana* with a full length *GSH1* cDNA from *Brassica juncea* (BjGSH1-1; AJ563921), expressed under the control of the 35S promoter; note that for this transformation we included the full 5'UTR sequence. A larger number of independent transformants were isolated and analyzed for GSH1 protein content (Figure 2), endogenous *AtGSH1* transcripts and *BjGSH1-1* transcripts (Figure 3), and for their GSH and cysteine contents (Figure 4). The immunoblot analysis (Figure 2) revealed six GSH1-overexpressing lines, whereas seven lines showed a clear decrease of GSH1 protein as compared with wildtype plants. Note that in the overexpressing

lines the size of the ectopically expressed BjGSH1-1 protein was identical with the predicted size of the mature protein after removal of the transit peptide, indicating import into plastids and correct processing. Selected overexpressing and putative co-suppression lines were analyzed for their *AtGSH1* and *BjGSH1-1* transcript amounts by Real-Time PCR (Figure 3). Co-suppression lines showed a drastic decrease of the endogenous *AtGSH1* transcript and only 3 % of the *BjGSH1-1* transgene expression level of the overexpression lines. The amount of endogenous *AtGSH1* transcript was significantly lowered in overexpression lines as compared to wildtype plants.

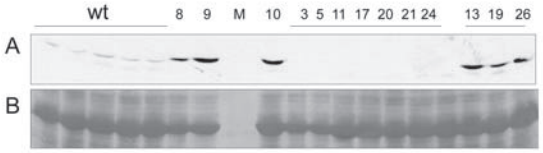

Figure 2:
Immunological analysis of *BjGSH1-1* transformants with an GSH1 antiserum reveals overexpression and co-suppression lines. A, immunoblot analysis of GSH1 expression in mature leaves of *BjGSH1-1* transformants and wildtype plants (wt). B, amidoblack staining of the membrane shows equal loading of total protein. 20 µg of total protein per sample were separated by SDS-PAGE (10% gel), followed by immunoblot analysis.

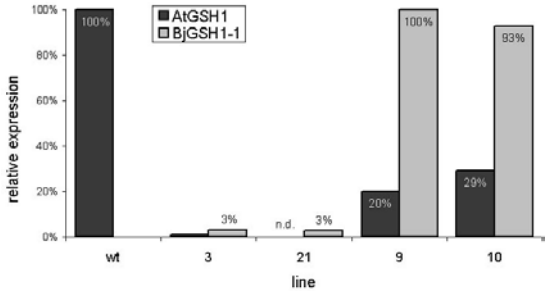

Figure 3:
Real-Time PCR analysis of expression of *AtGSH1* (and *BjGSH1-1*) in leaves of wildtype plants and four independent *BjGSH1-1* transformant lines.Data are normalized with respect to actin expression. For *AtGSH1*, data are standardized relative to wildtype values (=100%), for *BjGSH1-1* relative to expression of line 9 (=100%).

With respect to their GSH and cysteine contents, the co-suppression lines showed a strongly reduced GSH content, with a concomitant minor increase in cysteine content (Fig. 4). Conversely, transgenic lines exhibiting a strong increase of GSH1 protein (Figure 2, lines 9, 10 & 13) showed only a minor increase (less than 20%) of GSH, whereas their cysteine contents resembled that of wildtype plants. Thus, despite a strong increase of correctly processed GSH1 protein the GSH content was barely affected. A cysteine limitation cannot *a priori* be excluded, however, another possible explanation could be the formation of enzymatically inactive GSH1 protein.

Recently, Jez et al. (2004) have demonstrated a redox-mediated post-translational regulation of GSH1 activity, operating via an intramolecular disulfide formation. It is noteworthy that the *E. coli* GSH1 enzyme previously used to boost GSH synthesis in plants does not show this type of regulation.

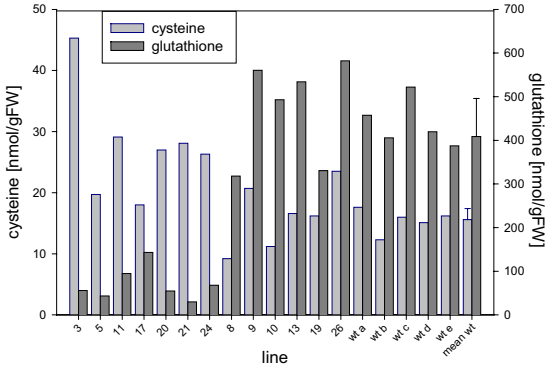

Figure 4:
Cysteine and glutathione contents in mature leaves of *A. thaliana* transformants expressing BjGSH1-1 (including long 5'UTR) under control of the CaMV-35S promoter. Thiol contents were determined for several independent transformant lines (including overexpression and co-suppression lines; see Figs.4&5) and five different wildtype (wt a to wt f) samples.

Guard cell-specific expression of BjGSH1-1 protein does not affect the cytosolic GSH content

In previous attempts to increase the stress tolerance of plants by ectopic overexpression of *GSH1*, the transgene was expressed under the regulation of the 35S promoter, which conveys a more or less constitutive expression. Recently it was shown that stress-induced stomatal closure is mediated by ROS, and a particular role could be assigned to dehydroascorbic acid dehydrogenase (DHAR), a key enzyme of the ascorbic acid-GSH cycle (Chen and Gallie, 2004). This enzyme uses GSH as electron donor for ascorbic acid reduction. Based on the hypothesis that a change in stomatal GSH synthesis capacity could equally affect stomatal regulation, we have developed a strategy to selectively overexpress genes of GSH synthesis (or the ascorbic acid-GSH cycle) in guard cells. For this

purpose we have as a first step cloned an expression cassette in which the transgene is expressed under the regulation of the *AtPRP4* promoter. This promoter was chosen as the corresponding ortholog of *Nicotiana glauca* shows a very high expression in guard cells as opposed to most other plant tissues (Smart et al., 2000). We have used this cassette to drive the expression of the reportergene *GUS* or *BjGSH1-1* in transgenic *A. thaliana*plants. Histochemical GUS staining confirmed the highly selective *AtPRP* promoter activity in guard cells (Wachter, 2004). As expected, the analysis of the *AtPRP4* promoter-*BjGSH1-1* transformants revealed no significant change in total leaf GSH content (Table 2). To detect a possible GSH increase in guard cells we have labeled the GSH content *in vivo* with MCB (data not shown). At this degree of resolution, we did not observe a significant increase of MCB labeling. However, it should be pointed out that this method labels only the cytosolic GSH pool. A physiological study of the transpiration rates of the *AtPRP4* promoter-*BjGSH1-1* transformants under different stress conditions is currently under way in our lab.

Table 2:
Total glutathione content in rosette leaves from *A. thaliana* transformants expressing *BjGSH1-1* under control of the *AtPRP*4 promoter and wildtype plants.

Line	GSH (nmol g^{-1} FW)
PRP4-BjGSH1-1	197 ± 43
wildtype	242 ± 31

Conclusions

Recent advances in our understanding of GSH function and the regulation of its synthesis and transport have shed new light on the involved molecular mechanisms. In particular, the various roles of GSH for plant development AND stress tolerance highlight its central role as an important S-metabolite with multiple functions. Consequently, attempts have been made to improve its *in planta* function by genetic engineering of its synthesis and/or redox state. These attempts have as yet met only moderate success, one of the reasons certainly being the multiple ways by which GSH synthesis is regulated *in vivo*, including transcriptional and post-transcriptional mechanisms. The data presented here underline the complex regulation of GSH synthesis

Acknowledgements

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Ecological significance of H_2S emissions by plants - a literature review

Pia Wickenhäuser[1], Elke Bloem[1], Silvia Haneklaus[1] and Ewald Schnug[1]

Abstract

The emission of several volatile reduced sulfur gases (H_2S, COS, DMS, CS_2 and methylmercaptan) from various plant species was determined in various experiments. From these volatile substances H_2S is one of the most important sulfur gases emitted by higher plants in response to an excess of sulfur. So far, a correlation between soil applied sulfur fertilization and H_2S emission of agricultural crops was not proven, but it was shown in field experiments that sulfur fertilization and the sulfur nutritional status, respectively had a significant effect on fungal infections in oilseed rape. These findings underline the concept of sulfur-induced resistance (SIR) of plants. H_2S is highly fungi toxic and therefore a relationship between increasing hydrogen sulfide emissions of plants and a higher resistance of crops against pests and diseases can be assumed. A better understanding of the natural defense system of domesticated plants based on the release of H_2S may contribute to a significant reduction of the input of fungicides in agriculture and thus to more sustainability in crop production. In organic farming, sulfur induced resistance may play a major role for maintaining plant health. From environmental point of view the degradation of toxic surface ozone concentrations by plant-released H_2S is another process of ecological relevance.

Key words: hydrogen sulfide, sulfur induced resistance, SIR, surface ozone

Sulfur induced resistance – release of H_2S

The significance of sulfur (S) for the resistance of crops against pests and diseases became evident at the end of the 1980's. At this time macroscopic S deficiency became a widespread nutrient disorder because of the desulfurization of industrial emissions in Western Europe (Booth et al., 1991). At the same time infections of oilseed rape with *Pyrenopeziza brassicae* spread out in regions which where never infected before (Schnug and Ceynowa 1990; Schnug et al., 1995a).

It has been known since long time that S has protective effects against pests and diseases. Most of this knowledge is, however, restricted to the effects of foliar-applied elemental S (Jolivet, 1993). In comparison, little is known about soil-applied S in sulfate form, which may have a strong influence on plant resistance by directly stimulating biochemical processes in the primary and secondary metabolism (Schnug, 1997). In fertilizer experiments under field conditions it could be shown that soil-applied S fertilization significantly reduced fungal infections of oilseed rape with light leaf spot (*Pyrenopeziza brassicae*), grapes with powdery mildew (*Uncinula necator*) and potato tubers with stem cancer (*Rhizoctonia solani*) (Schnug et al., 1995a; Bourbos et al., 2000; Klikocka et al., 2004). The results of these experiments indicate that different S metabolites are involved in disease resistance, which were induced by S fertilization and thus underpinning the concept of sulfur induced resistance (SIR) (Schnug et al., 1995a; Haneklaus et al., 2004). An improved understanding of how S is involved in the stress resistance of plants together with efficient fertilizer strategies are a challenge for future agricultural production techniques. The aim of S fertilizer strategies will be to maximize the inherent potential stress resistance, which otherwise would not be expressed due to an insufficient S supply, whilst maintaining an environmentally and economically sustainable farming (Schnug, 1997).

The mechanisms of SIR are not yet fully understood. Mechanisms to tackle with biotic stress, which are provided by the S metabolism involve among others glutathione, phytoalexins and glucosinolates (Haneklaus et al., 2004). The release of volatile S compounds is putatively an important mechanism in SIR, too. The emission of several volatile reduced S gases (H_2S, COS, DMS, CS_2 and methylmercaptan) from various plant species was determined (Schröder, 1993). Under growth conditions with an excessive S supply significant amounts of gaseous S compounds are released into the atmosphere from which H_2S is the most abundant gas emitted (Rennenberg, 1991). The release of H_2S is thought to be actively regulated by the plant metabolism rather than being a metabolic side-product. An indication for the first hypothesis is that H_2S emissions could be observed also under field conditions with a moderate sulfur supply (Rennenberg, 1991). Anyway, an excess S supply by atmosphere and pedosphere induces the emission of volatile S compounds by plants. The release of H_2S occurs when the influx of S compounds via leaf or root in the form of cysteine, sulfate, SO_2 or COS exceeds the conversion of these S sources into protein, glu-

[1] Institute of Plant Nutrition and Soil Science, Federal Agricultural Research Centre Braunschweig-Völkenrode, Bundesallee 50, D-38116 Braunschweig, Germany

tathione, methionine and other S containing compounds (Rennenberg, 1991). The emission of H_2S is comparable with a pressure valve for the plant to dispose of excess S (Filner et al., 1984). It has been suggested that the release of H_2S regulates, homeostatically the size of the cysteine pool and thus maintains it at a low level because of its cytotoxicity. H_2S may be released prior or after cysteine formation (Giovanelli, 1990), but the question is still open which enzymes catalyze the release of H_2S. Another possible mechanism, which induces the H_2S emission by plants could be the involvement in the natural defense system of crop plants against fungal infections (Haneklaus et al., 2004).

Conditions determining the H_2S emission by plants are physiological factors such as the growth stage (Seykia et al., 1982a; Rennenberg and Filner 1983; Filner et al., 1984; Lakkineni et al., 2003) and metabolic activity of the plant tissue, but also nutritional and environmental factors (Fall et al., 1988; Rennenberg 1991; Schröder 1993; Lakkineni et al., 2003). Generally, the emission of S gases increases with temperature and illumination (Lamb et al., 1987; Seykiya et al., 1982b; Fall et al., 1988). The strategy to dispose of excess S depends on a concentration gradient for H_2S between plant and atmosphere. The presence of high atmospheric H_2S concentrations prevents H_2S emission, so that it is not surprising that H_2S fumigation resulted in a rapid accumulation of thiols, including cysteine in the plant tissue (Rennenberg, 1991; De Kok et al., 1998).

Data for the natural release of gaseous S-compounds reported in literature vary over a wide range (Seykia et al., 1982a, b, c; Rennenberg and Filner 1982, 1983, 1984; Filner et al., 1984; Fall et al., 1988; Schröder 1993; Collins 1996; Lakkineni et al., 2003). Filner et al., (1984) calculated a worldwide S emission from plants of 7.4 Tg S yr^{-1}, while Winner et al. (1981) came to a value of 54 Tg S yr^{-1}. Globally, Crutzen (1983) calculated the annual S emissions of H_2S, DMS and methylmercaptan from agricultural fields to be in the range of < 4 Tg S yr^{-1}. One reason for the large discrepancies observed for S emissions are analytical problems. H_2S measurements are difficult to conduct if emissions are low, because analytical systems need to be extremely sensitive so that there is only a few data available that provides information about the release of gaseous S compounds in the low range (Wilson et al., 1978; Seykia et al., 1982b; Lakkineni et al., 1990; Bloem et al., 2004a). Another problem of H_2S measurements is that most experiments were conducted under artificial conditions, e.g. with cut plant parts that were fed with concentrated S solutions (Wilson et al., 1978; Seykia et al., 1982b; Rennenberg and Filner 1983). Therefore such estimates need to be treated carefully. The metabolism of living crops and cut plant parts reacts completely different, and consequently higher H_2S emissions were measured from detached leaves and leaf discs than from whole plants. Extrapolation of H_2S emissions, which were measured from detached leaves or plant parts will therefore lead to an overestimation of the H_2S emission by the crop (Bloem et al., 2004a). In the laboratory it was possible to stimulate leaves to emit H_2S at 1000 times higher rates than under field conditions (Filner et al., 1984). When sulfate was fed to intact roots of whole plants, the increase in the H_2S emission was usually much lower (Rennenberg and Filner, 1982, 1983; Filner et al., 1984). Apparently, the root system constitutes a barrier for the influx of sulfate into the plant, and hence prevents an immediate release of H_2S from excessive sulfate in the soil (Rennenberg and Lamoureux, 1990). In some experiments the H_2S emissions were stimulated by injuring the roots, but for the same reason as in case of the cut leave these results are also not suitable to calculate the H_2S emissions by plants under natural conditions.

Although it is generally assumed that H_2S can be reliably determined using cryogenic trapping with gas chromatographic analysis, slight variations of the analytical procedure may result in significant losses of H_2S (Rennenberg, 1991). Despite these analytical problems that have to be overcome, the determination of H_2S emissions from intact plants in dependence on the S supply and infections with fungal diseases will be a milestone for addressing key metabolites involved in SIR. The role of S nutrition and fungal infections for the potential release of H_2S emissions was shown in field experiments with *Brassica napus* L. (Bloem et al., 2004b). For instance, the activity of the H_2S releasing enzyme L-cysteine desulfhydrase significantly increased in infected plant tissue and, to a lower extent in plants with a higher S nutritional status. (Bloem et al., 2004b).

Surface ozone concentrations

H_2S emissions by plants may degrade toxic surface ozone and thus be of high ecological significance (Schnug 1997). Surface ozone concentrations increased in rural areas over the last decade on an average by 1.8 g m^{-3} yr^{-1} (Figure 1). At the same time plant S concentrations declined at a constant rate of 0.45 mg yr^{-1} (Figure 1; Schnug, 1993, Schnug, 1997).

Assuming that: a) H_2S emissions from plants decline linearly together with the S supply (Collins 1996, Rennenberg 1984) at a rate of 0.57 nmol m^{-2} h^{-1} (calculated from the data of Schnug and Haneklaus 1994); b) crops have an average leaf area index of 1; c) crops assimilate and reduce S during an av-

erage of 100 days a year and 10 h a day; and d) H_2S degrades O_3 in a 1:1 ratio; then up to 75% of the observed increase of surface ozone could be attributed to the decrease in the total amount of S-turnover in the "green part" of the ecosystem (Schnug, 1997).

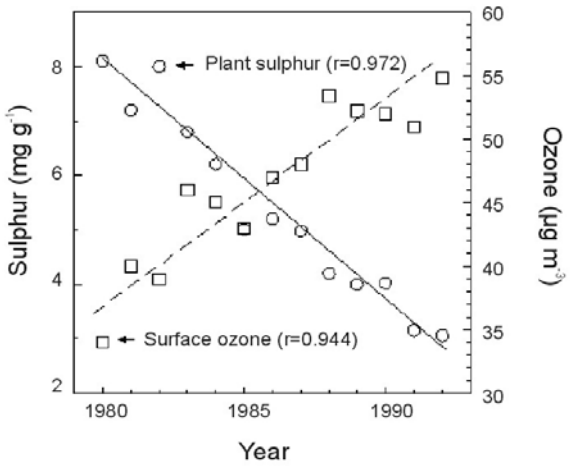

Figure 1:
Atmospherical surface ozone concentrations and total sulfur in younger, fully developed leaves of field grown *Brassica napus* varieties in northern Germany from 1980-1992 (Schnug 1993).

These figures here are only an estimate and may change depending on the actual input parameters, but they still outline the important function of S assimilation and reduction in ecosystems. Despite the significance of these findings for air quality, higher S inputs in the past century enabled plants to adapt to increasing environmental stress caused by higher surface ozone concentrations and, vice versa, the decline of the S supply within only one decade (Schnug, 1991, 1993) may have serious consequences for the stability of recent ecosystems. For example, S deficiency is thought to be one of the reasons why 50% of all forests are damaged. These damages are caused by a reduced resistance against abiotic stress because of a continuously declining S supply on the one hand, and steadily increasing environmental stress on the other hand (Williams, 1982, Zhao, 1996).

Conclusions

So far there is no scientific proof for a correlation between the rate of soil-applied S and the amount of H_2S released by plants. In the case of other secondary S compounds such as glutathione and glucosinolates significant positive relationships were found (De Kok et al., 1998; De Kok and Stulen, 1993, Schnug et al., 1995b; Haneklaus et al., 1999; Bloem et al., 2004). H_2S is highly fungi-toxic (Pavlista, 1995) and therefore a relationship between increasing H_2S emissions and the resistance of crops against pests and diseases is likely (Seykia et al., 1982c; Beauchamp et al., 1984; Schröder 1993). All these findings clearly show that extensive field measurements are required to evaluate the impact of different nutritional conditions and fungal diseases on the emission of H_2S. It is the aim of a joint research project financed by the DFG (German Research Foundation) to determine the release of H_2S in relation to the S nutritional status of agricultural crops and to answer the question whether such relationship is involved in SIR. The identification of the mechanisms causing SIR will be an important milestone for a sustainable agricultural production as the input of fungicides could be minimized or completely waived (Haneklaus et al., 2004). Consumers are increasingly concerned about the contamination of foodstuff with pesticide residues and consequently markets for plant production from farming systems avoiding such contaminations are expanding (Schnug, 1997). Thus, SIR may become an important strategy to efficiently combat pathogens in sustainable farming systems, favorably organic farming. An important advantage of SIR compared to pesticides is that the resistance will not be rapidly broken by new pathotypes (Haneklaus et al., 2004). And an indirect effect of an increased release of H_2S could be the detoxification of toxic surface ozone concentrations by which oxidative stress would be lowered outside the organism (Schnug, 1993, 1997).

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Sulfur status of Chinese soils and response of Chinese cabbage to sulfur fertilization in the Beijing area

Liping Yang[1,2], Ineke Stulen[2] and Luit J. De Kok[2]

Abstract

During recent years sulfur deficiency has become a major problem in agricultural crops throughout China, due to an imbalance of sulfur in relation to N, P and K in the fertilizers. One-fourth of the tested Chinese soils appeared to be sulfur deficient. Pot experiments at locations in the Beijing area showed that shoot biomass production of Chinese cabbage was significantly enhanced upon sulfur fertilization of the soil. A level of fertilization of 15-30 kg S ha^{-1} was sufficient to get optimum yield. However, the level of fertilization in other regions in China might have to be adjusted to the level of local atmospheric sulfur deposition.

Key words: Chinese cabbage, plant nitrogen, plant nutrients, N/S ratio, plant sulfur, available soil sulfur, sulfur deficiency, sulfur dioxide, sulfur nutrition

Introduction

Sulfur is the fourth major nutrient after N, P and K for agricultural crops and is essential for growth and physiological functioning of plants. Sulfur is needed for the synthesis of the amino acids cysteine and methionine, which are of great significance in the structure, conformation and function of proteins and enzymes. Furthermore, it is incorporated into several other metabolites, as thiols (glutathione), sulfolipids and secondary sulfur compounds (alliins, glucosinolates, phytochelatins), which play an important role in the physiology of plants and in the protection and adaptation of plants against stress and pests (De Kok et al., this issue). Sulfur fertilization is not always optimal, which might negatively affect both crop yield and quality. It has been recognized that currently sulfur deficiency is one of the major plant nutrient stresses in crops throughout the world (Schnug, 1991; McGrath and Zhao, 1996; Schnug and Haneklaus, 1998; Zhao et al., 1999). In China sulfur deficiency has also become apparent and now occurs frequently (Wang et al., 2001; Cui and Wang, 2003; Zhao et al., 2003; Li and Liu, 2004; Meng et al., 2004). The use of high yielding varieties, increased cultivation intensity, and an overall improvement of cultural management practices has resulted in a sustained increase in crop production in the last decades. This had led to increased removal of nutrients from agricultural ecosystems. The sulfur input to soil has decreased due to the use of low sulfur-containing fertilizers. In the past fertilizers such as ammonium sulfate, single superphosphate, potassium sulfate and farmyard manure were used. At present these fertilizers are often replaced by low sulfur or sulfur-free fertilizers such as complex fertilizers (like N15P15K15), DAP (diammonium phosphate) and urea. For example, the share of ammonium sulfate production in the total nitrogen fertilizers production in China dropped from 100% in the 1950s to 44.9% in the 1960s, 6% in the 1970s and 0.7% in the 1990s. The N/S ratio in the fertilizers used in China increased from 1.0 in 1960 to 8.8 in 1990 (Liu, 1995). Recently, China has improved the balance of N, P and K in fertilizers, however, the importance of S and other micronutrients is often ignored. As a consequence in several regions, sulfur has become a limiting factor for optimal yield and quality of crops. In order to get insight into the sulfur status of Chinese agricultural soils, more than 18,000 samples from all over the country and about 900 samples from the Beijing and Tianjin areas were analyzed.

Chinese cabbage is a common and widely grown vegetable throughout the country, especially in northern China, since it has a high yield and relatively short growing period. For instance, winter Chinese cabbage usually has a yield of 100-120 ton ha^{-1} in the Beijing and Tianjin areas. With the current high production levels, an adequate supply of nutrients must be available for optimum plant growth and production. However, Chinese farmers tend to apply more nitrogen fertilizer than is needed for optimal yield, whereas often insufficient phosphate and potassium are applied. In addition, the significance of the secondary nutrients and micronutrients are ignored, resulting in loss of potential yield and income from production of this vegetable. Responses to sulfur fertilization were reported for some leaf vegetables and Chinese cabbage in China (Chen et al., 2000; Liu et al., 2003).

In general, Chinese cabbage is grown in the vicinity of cities and here yield and quality might be negatively affected by air pollution (Zheng et al., 1996). Coal is still the principle source of energy in China and its combustion results in high levels of the air pollutants SO_2, NO_x, and acid

[1] Soil and Fertilizer Institute, Chinese Academy of Agricultural Sciences (CAAS), 100081, Beijing, China
[2] Laboratory of Plant Physiology, University of Groningen, P.O. Box 14, 9750 AA Haren, The Netherlands

Table 1:
Levels of available nutrients in fluviogenic soil from Changping County, Beijing, China and the critical levels for the different nutrients.

	pH	Organic matter (%)	Nutrients (mg l^{-1})										
			Ca	Mg	K	N	P	S	B	Cu	Fe	Mn	Zn
Soil test results	8.1	1.09	2204	244	53	12	15	0.5	0.46	2.5	8.0	4.1	1.7
Critical levels			400	121	78	50	12	12	0.20	1.0	10	5.0	2.0

rain. The impact of acid deposition on agricultural crops and forests in southern China has been reviewed by Feng (2000). Despite the potential toxicity of sulfurous air pollutants they also may contribute to the plants´ sulfur fertilization. For instance, one of the primary causes of sulfur deficiency in North America and Western Europe is attributed to the ongoing reduction of atmospheric sulfur deposits as the consequence of strict regulations on industrial sulfur emissions (Schnug, 1991; McGrath et al., 1996). This is supported by laboratory experiments, which have shown that dependent on the atmospheric level and the pedospheric sulfur suply of plants, SO_2 may act both as toxin and nutrient (De Kok et al., 1998, 2000; De Kok and Tausz, 2001; Yang et al., 2003). It remains to be questioned to what extent SO_2 pollution in the vicinity of Chinese cities is toxic or contributes to sulfur fertilization of Chinese cabbage. The current paper presents results of pilot experiments with two cultivars of Chinese cabbage, which were grown in pots with local soil with and without additional sulfur fertilization at two sites in the Beijing area.

Material and methods

Soil testing

ASI Soil Analysis Methods (PPI/PPIC Beijing Office, 1992; Portch and Hunter, 2003) for available soil sulfur test was adopted. Available soil sulfur was extracted by 0.08 M calcium phosphate and measured by the turbidimetric procedure for SO_4^{2-}-S in the PPIC-CAAS Corporative Soil and Plant Analysis Laboratory. If the level of available soil sulfur is lower than 12 mg l^{-1}, the soils are considered to be sulfur deficient. Soils containing sulfur levels ranging from 12 to 24 mg l^{-1} are potentially sulfur deficient. At these soil sulfur levels supplemental sulfur fertilization is required to obtain optimal crop yield and quality. If available sulfur is higher than 24 mg l^{-1}, the soils are considered to be sulfur sufficient.

Response of Chinese cabbage to sulfur fertilization at two sites in the Beijing area

Two experimental sites were selected; one at central Beijing inside the 3rd Ring Road (site A) and one at the outskirts of Beijing outside the 6th Ring Road (site B). The data of atmospheric SO_2 concentrations in Beijing were provided by the Beijing Environmental Protection Bureau and were also measured by the national standard method (GB/T 15262: Ambient air – Determination of sulfur dioxide – Formaldehyde absorbing – Pararosaniline spectrophotometry).

For the experiments a fluviogenic soil was taken from Changping County, Beijing; it is the main soil type in the Beijing and Tianjin areas. The soil was air-dried for a few days and sieved through a 2 mm screen. Available nutrients and adsorption characteristics were determined by ASI Soil Analysis Methods. From the obtained data it was evident that the soil had a high pH, high levels of plant available Ca, Mg and Cu and low levels of plant available N, P, K, S, Fe, Mn and Zn (Table 1). Two cultivars of Chinese cabbage (*Brassica pekinensis*, cv. Kasumi F1, Nickerson-Zwaan, the Netherlands and cv. Beijing 3, China) were used in the experiments.

In the summer of 2002 the response of Chinese cabbage to sulfur fertilization was tested at the two experimental sites. Plants were fertilized with nutrients at levels more than adequate for maximum growth but much less than those considered to be toxic or out of balance with other plant nutrients and conditions. The levels of the various nutrients were added to the soil according to "a Systematic Approach to Soil Fertility Evaluation and Improvement", and were based on soil test results and sorption studies (data not shown). The nutrients were added as follows: 50 mg N l^{-1} soil, 234 mg K l^{-1} soil, 55 mg P l^{-1} soil, 0.4 mg B l^{-1} soil, 20 mg Fe l^{-1} soil, 28 mg Mn l^{-1} soil, 5 mg Zn l^{-1} soil and 66 mg S l^{-1} soil. The latter represents an equivalent to a level of sulfur fertilization of approx. 130 kg ha^{-1} and is referred to in the figures as +S. In part of the pots no sulfur was added; referred to as -S. The nutrients were added as a solution and mixed thoroughly with the soil. The soil was watered to field capacity and 15-20 seeds were sown in each pot (with 800 ml air-dried soil), and then thinned to 4 plants per pot after emergence. All treatments were irrigated by a system of capillary irrigation (1.5 g NH_4NO_3 per 5 liters of de-ionized water) at the bottom of the pot in order to maintain a soil moist content close to

field capacity. The plants in pots were placed under a plastic transparent foil in order to provide protection against heavy rainfall in summer. After 20 days the first harvest of the plants was carried out and two plants in the diagonal corner in each pot were harvested. The second harvest was carried out after 28 days.

In the summer of 2003 the response of Chinese cabbage to various levels of sulfur fertilization was tested at one of the experimental sites (site A). The same cultivars of Chinese cabbage were used. The same soil as used in the first experiment and the basal nutrients at the optimum levels were added, except S (see above). Sulfur was applied as K_2SO_4 at levels of 0, 15, 30, 60, 90 and 120 kg S ha^{-1} which was calculated by 20 cm cultivated layer and 1.2 g cm^{-3} soil bulk density of this soil (so the applied rate was 0.0, 6.3 12.5, 25.0, 37.5, 50.0 mg S kg^{-1} soil in the pot experiments). The soil was watered to field capacity and 20 seeds were sown in each pot (containing 1 kg air-dried soil) and thinned to 2 plants per pot after emergence. During the experiment period all pots were watered with the same amount (50-100 ml) of NH_4NO_3 solution (2.0 g NH_4NO_3 per 5 liters of deionized water) every day. There were 5 replicates in each treatment of the 6 fertilization levels of sulfur. The pots were put under a plastic shed, which provided the plants protection against heavy rainfall in summer. The plants were harvested after 28 days.

The fresh and dry (80 °C, 24 hours) weight of shoots was measured after harvest. Total nitrogen was determined with the Kjeldahl method according to Barneix et al. (1988). Analysis of the total S content was carried out as described by Durenkamp and De Kok (2002). Sulfate was determined after HPLC separation according to Tausz et al. (1996). The content of P, K, Zn, Mn, Fe, Ca and Mg of the shoots were determined after H_2SO_4-H_2O_2 digestion (Lu, 1999).

Results and discussion

The status of available soil sulfur

During recent years a total of 18,183 soil samples from China (and 923 samples from Beijing and Tianjin) were analyzed. From the data on available soil sulfur it is obvious that 24% (27% in Beijing and Tianjin) of the soils tested were S deficient, with available sulfur levels less than 12 mg l^{-1} (the critical level), while 18% (14% in Beijing and Tianjin) of the soils contained available sulfur levels ranging from 12 to 24 mg l^{-1}, which might be considered to be potentially sulfur deficient (Table 2). The data demonstrated that sulfur deficiency of soils is a widespread problem in China and that in these areas additional sulfur fertilization is required for optimal crop yield and quality.

SO_2 pollution levels in Beijing

The atmospheric SO_2 concentration in Beijing has substantially decreased during recent years. This can be ascribed to the great effort to reduce air pollution levels in the city. The change in use of coal to natural gas as energy source and a stricter regulation of pollutant emissions have resulted in a strong decrease of SO_2 emission over the period of 1998 to 2002 (Figure 1). The natural gas supply in the city was more than 1.8 billion m^3 in 2002, which was about 6 times higher than in 1998. The use of high quality and lower-sulfur coals was 8 million ton in 2002, which was 4-fold higher than in 1998. SO_2 annual mean concentration has decreased from 120 µg m^{-3} in 1998 to 67 µg m^{-3} in 2002. During 2002 and 2003, the atmospheric SO_2 levels were monitored at the experimental sites during the experimental period and the daily mean concentrations in Beijing are shown in Table 3 and Figure 2. SO_2 concentrations in Beijing in the summer time were about 20 µg m^{-3}.

Impact of sulfur fertilization on Chinese cabbage

Sulfur fertilization of the fluviogenic soil from the Beijing and Tianjin areas had a substantial impact on Chinese cabbage and resulted in a significant increase of the shoot fresh weight production of two cultivars of Chinese cabbage (Figure 3). The fresh weight of the shoot of Beijing 3 was significantly higher upon sulfur fertilization at both harvests. However, an increase in shoot weight of Kasumi F1 upon sulfur fertilization was only observed at day 28. This indicated that the local cultivar Beijing 3 had a higher sulfur demand than Kasumi F1 (Figure 3). There were no differences in plant growth within the same treatment for either harvesting day or experimental site.

Table 2:
The status of available sulfur (mg l^{-1}) in the selected soil.

	Min.	Mean	Max.	<12	12-24	24-48	>48	Number of samples
				% of total selected samples				
China	0	40	820	24	18	28	30	18,183
Beijing and Tianjin	0	55	262	27	14	16	43	923

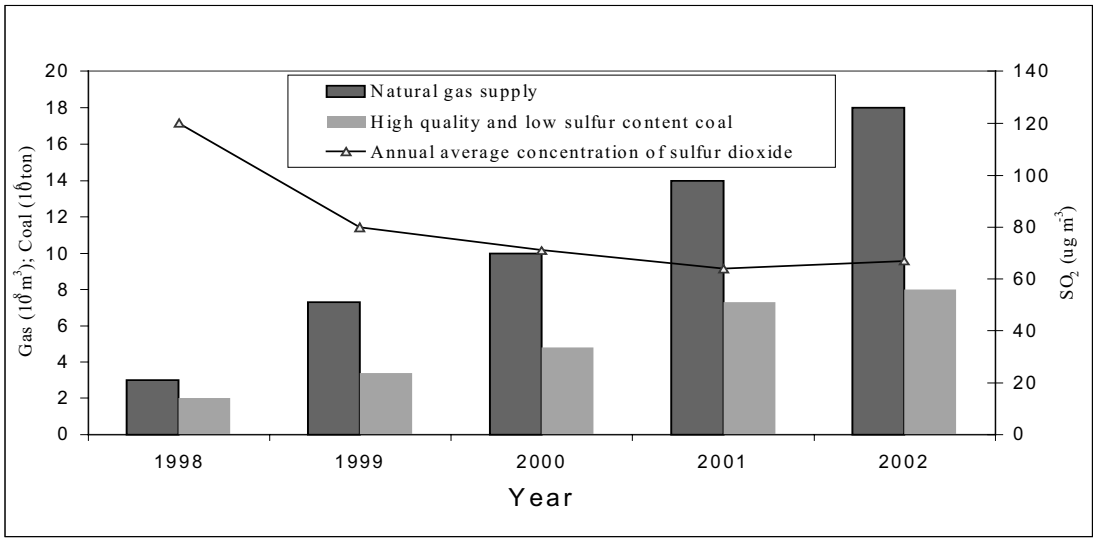

Figure 1:
Energy supply and SO_2 concentration change in recent years. Data from Beijing Environment Monitoring Station.

Sulfur fertilization resulted in an increase of the total sulfur, which was mainly due to a higher sulfate content of the plants (Figure 3). The organic sulfur content was also increased upon sulfur fertilization for both harvests at the different sites.

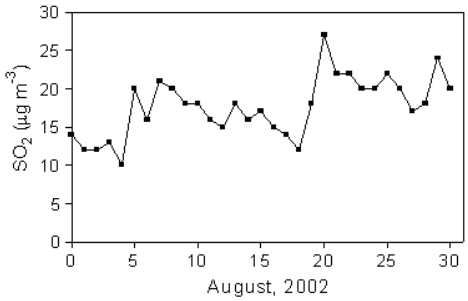

Figure 2:
SO_2 concentrations (daily mean) in Beijing during the experimental period. Data from Beijing Environment Monitoring Station.

Table 3:
SO_2 concentrations at two experimental sites in the Beijing area in 2002. SO_2 concentration was measured every day at site A and twice a week at site B during the experiment period (for information on sites see Material and methods).

	SO_2 (µg m^{-3})	
	Mean	Range
Site A	17	2-34
Site B	17	9-32

Sulfur fertilization only slightly increased total nitrogen content of Beijing 3 for both harvests, whereas that of Kasumi F1 was hardly affected (Figure 3). The N/S ratio in non-sulfur fertilized plants was much higher than that of the sulfur-fertilized plants in both cultivars especially after 28 days when shoot growth was reduced (Figure 3). The ratio of N/S was between 15 to 20 in the sulfur-fertilized plants.

Table 4:
Effect of sulfur fertilization on P, K, Ca, Mg, Fe, Zn and Mn content of shoots of two cultivars of Chinese cabbage. Plants were grown at site A for 28 days. Data represent the mean of 3 measurements with 8 plants in each (± SD). Different letters (a, b) indicate significant differences at $p \leq 0.05$ between different treatments.

		P	K	Ca	Mg	Fe	Zn	Mn
		(%)	(%)	(%)	(%)	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})
Beijing 3	-S	0.42 ±0.04a	2.67 ±0.22a	2.9 ± 0.2a	0.33 ±0.02a	294 ± 53a	47 ± 7a	51 ± 4a
	+S	0.39 ±0.06a	3.81 ±0.53b	2.5 ± 0.2a	0.34 ±0.02a	302 ± 21a	64 ±13b	72 ±13b
Kasumi F1	-S	0.42 ±0.01a	2.48 ±0.05a	2.8 ± 0.1a	0.32 ±0.03a	293 ± 53a	40 ± 6a	45 ± 8a
	+S	0.53 ±0.05b	3.32 ±0.16b	3.2 ± 0.2b	0.38 ±0.02b	376 ± 60b	65 ±10b	65 ± 6b

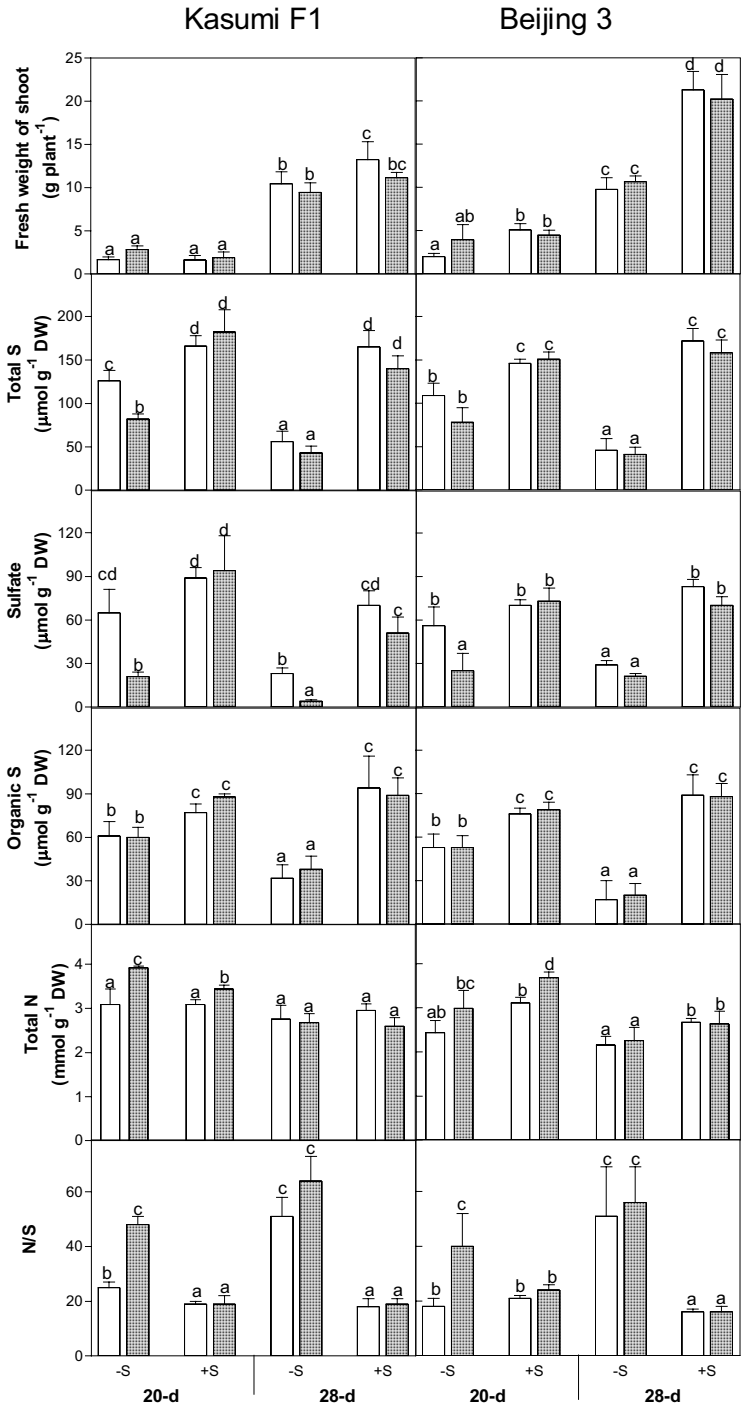

Figure 3:
Response of growth, sulfur and nitrogen metabolites of two cultivars of Chinese cabbage to sulfur fertilization at two sites in the Beijing area. Plants were grown in the fluviogenic soil for 20 and 28 days at site A (open bars) and site B (dotted bar, see Material and methods). Without sulfur fertilization (-S) and with 66 mg SO_4^{2-}-S l^{-1} soil (+S). The fresh weight of shoots (g) represents the mean of 12 measurements with 2 plants in each (± SD). Total S, total N, and sulfate content (µmol g^{-1} DW) of the shoot represent the mean of 3 measurements with 8 plants in each (±SD) at day 20 and the mean of 4 measurements with 6 plants in each (± SD) at day 28. The organic sulfur content was derived by subtracting the sulfate content from that of the total S content. Different letters indicate significant differences at $p \leq 0.05$ between (+S) and (-S) treatments.

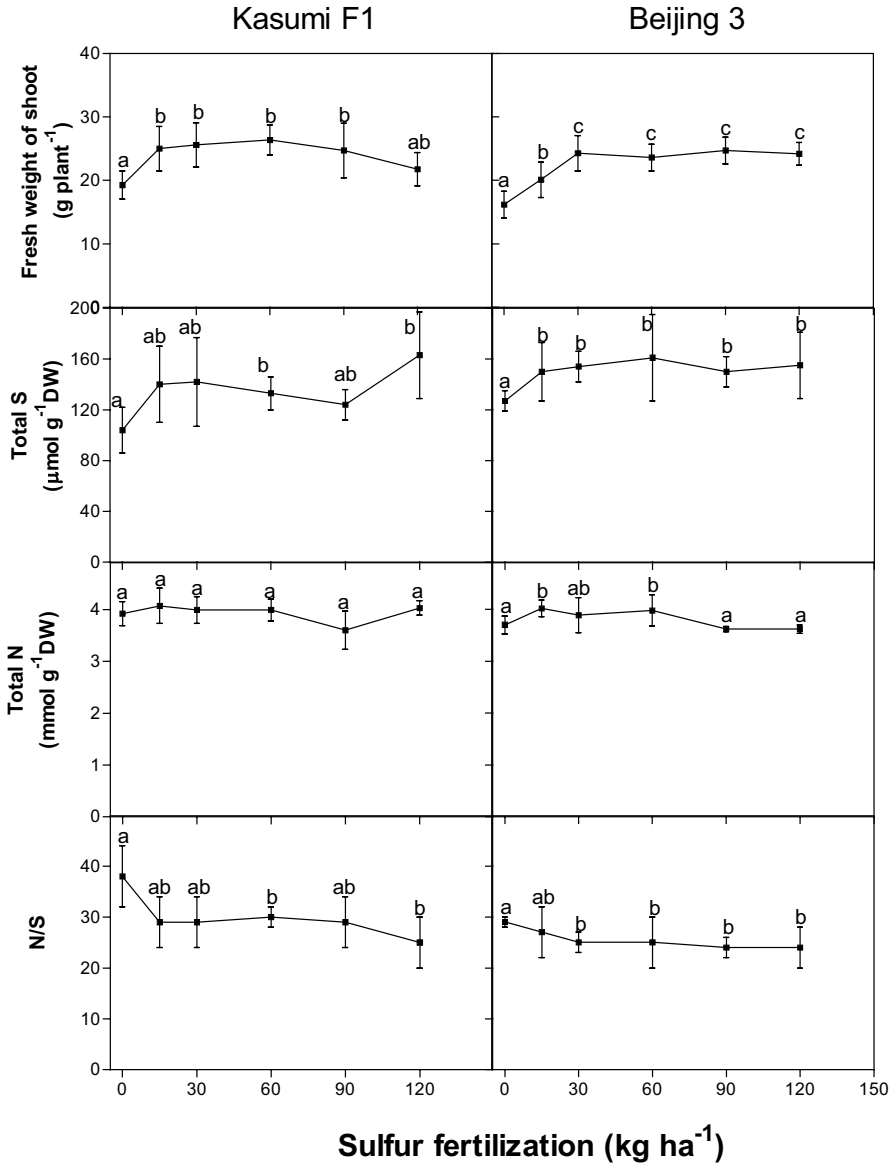

Figure 4:
Effect of different levels of sulfur fertilization on growth, total S, total N and N/S ratio of two cultivars of Chinese cabbage. Sulfur was applied as K_2SO_4 at levels of 0, 15, 30, 60, 90 and 120 kg S ha^{-1} which was calculated by 20 cm cultivated layer and 1.2 g cm^{-3} soil bulk density of this soil (so the applied rate was 0.0, 6.3 12.5, 25.0, 37.5, 50.0 mg S kg^{-1} soil in the pot experiments, see Material and methods). Data of the fresh weight of shoot represent the mean of 5 measurements with 2 plants in each (± SD). Total S and total N content of the shoot represent the mean of 3 measurements with 2 plants in each (± SD). Different letters indicate significant differences at $p \leq 0.05$ between different treatments.

Upon 28 days of sulfur fertilization the levels of other plant nutrients in shoots was also affected (Table 4). The levels of P, K, Fe, Mg, Zn, Ca and Mn in shoots of Chinese cabbage cv. Kasumi F1 were slightly enhanced upon sulfur fertilization. In cv. Beijing 3 sulfur fertilization only resulted in an enhancement of the levels of K, Zn, Mn.

Optimizing of sulfur fertilization for Chinese cabbage

It was evident from the previous results that the levels of sulfur in the fluviogenic soil from Beijing and Tianjin were not sufficient for optimal growth of Chinese cabbage. In order to assess optimal sulfur fertilization plants were grown on

soil fertilized with 0, 30, 60, 90 and 120 kg S ha^{-1} (Figure 4). The shoot fresh weight increased by 50 % when 30 kg S ha^{-1} sulfur fertilizer was applied for Beijing 3 and was not further affected at higher levels of sulfur fertilization. There were no differences in shoot biomass production at 30, 60, 90 and 120 kg S ha^{-1}. For Kasumi F1, sulfur fertilization at 15 kg ha^{-1} was sufficient for optimal shoot biomass production.

Sulfur fertilization resulted in a slight increase of the total S content in both cultivars of Chinese cabbage and an increase of the total N content in Beijing 3 (Figure 4). As a consequence the N/S ratio in shoots of Kasumi F1 decreased from 38 in the non-fertilized to 29 in the fertilized plants (Figure 4). Likewise, the N/S ratio of Beijing 3 decreased from 29 to 24-25.

It has been suggested that the N/S ratio could be used as a diagnostic tool to determine plant sulfur deficiency, based on an assumed direct interaction between nitrogen and sulfur assimilation in plants (Zhao et al., 1996; Thomas et al., 2000; Blake-Kalff et al., 2002; Randall et al., 2003). However, one should be cautious in the use of the N/S ratio for sulfur diagnosis, since it may also be strongly affected by the level of nitrogen fertilization. A high N/S ratio could be due to the oversupply of nitrogen even though sulfur was sufficient. For instance, the two experiments showed different N/S ratios in sulfur-sufficient plants. It was 15-20 for both cultivars in the first year, while it was 24-25 for Beijing 3 and 29 for Kasumi F1 in the second year, since the level of nitrogen fertilization was somewhat higher. The total S and total N content of non-sulfur fertilized plants were higher in the second year (Figure 4) than in the first year (Figure 3). This may be ascribed to the different irrigating regimes of the pots. During the first year plants in pots were irrigated by a system of capillary irrigation (irrigation water contained 1.5 g NH_4NO_3 per 5 liters of de-ionized water) and the water content of the soil was maintained close to "field capacity". During the second year pots were watered daily with 50-100 ml irrigation water containing 2.0 g NH_4NO_3 per 5 liters of de-ionized water. As a consequence there was more variation in the soil water content.

There is no doubt that plants are able to utilize foliarly absorbed sulfurous air pollutants as a sulfur source for growth (De Kok et al., 1998, 2000; De Kok and Tausz, 2001; Yang et al., 2003). It has been demonstrated that levels of ≥ 0.06 µl l^{-1} SO_2 (≅170 µg m^{-3} SO_2) are sufficient to cover the sulfur need of Chinese cabbage for growth (Yang et al., 2003). It remains to be questioned to what extent atmospheric SO_2 deposition has contributed to the sulfur fertilization of Chinese cabbage at the different sites in the Beijing area, although the ambient SO_2 levels were relatively low (Table 3).

Conclusions

Sulfur fertilization of soils is necessary to obtain optimal yield in various areas in China. For instance the present data showed that in the Beijing area a level of sulfur fertilization of 15-30 kg S ha^{-1} was needed to get optimal biomass production of Chinese cabbage. However, the level of fertilization in other regions in China might have to be adjusted to the level of local atmospheric sulfur deposition.

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The role of sulfur fertilizers in balanced fertilization

Yiming Zhou[1], Defang Wang[1], Jinghua Zhu[1], Qingshan Liu[2] and Ming Xian Fan[3]

Abstract

A survey on the nutrient content of soils in the Tianjin area in the north of China, showed that 30% of total farmland area was sulfur and potassium deficient. Deficiency occurred mainly in cinnamon soil, chao cinnamon soil and partly chao soil in Ji and Baodi counties. Results collected from field trials on rice, wheat and corn showed that deficiency of S or K caused a reduction in grain yield ranging from 6 - 24%. Combined S and K fertilization resulted in substantial increases in crop production. Fertilization of the soils of 12 trials with Chinese cabbage, garlic, scallion, chili, green turnips and carrot with NPK, adding 60 - 120 kg ha^{-1} sulfur, increased yields by 16.0 - 36.4% with a large of value/cost ratio of 12.3 - 28.7, and high vegetable quality. In nutrient management, S combined with other nutrients has to become a common fertilizer practice to guarantee optimal crop production.

Keywords: Sulfur, potassium, grain, vegetables

Introduction

The Tianjin area is located in North China, which is facing the Bohai Sea eastwards and backing Yanshan Range northwards. It comprises a total land area of 11,920 square km^2 and 4,893 km^2 of it is used as farmland. The area has total population of over 10,000,000 distributed over 12 districts and counties. The staple grain crops cultivated in the Tianjin area are wheat, maize and rice. The area is also an important basis of vegetable production. In addition to farmland, there are forests, various fruit trees and cash crops planted in the Tianjin area.

In addition to the soil potassium depletion, since the 1980s sulfur deficiency of soils has become a major problem due to a replacement of high-sulfur-containing fertilizers by sulfur-free or low-sulfur-containing fertilizers, a decrease in organic manure fertilization and the use of high yield crops (Liu Chongqun and Hu Sinong, 1993; Zhou Yimin and Jing Haichun, 1995).

Developing high quality crop and vegetable production and ensuring the supply to urban consumption are the foremost tasks facing the urban-suburban type agricultural production in metropolis. In order to achieve the goal of agricultural production with high yield, improving crop quality and increasing farmer's income, balanced fertilization is necessary. Therefore we arranged the following studies.

Material and methods

The selection of experiment fields and the condition of soil nutrients

We arranged field experiments mainly in Ji and Baodi counties in cinnamon and Chao soils where soil sulfur and potassium contents are lower than other districts (Figure 1 and 2).

Analysis methods

$Ca(H_2PO_4).H_2O$ extraction and $BaSO_4$ contrast turbid ratio method was used for analyzing available sulfur content in soil, and other nutrients analysis by using Systematic Approach of Soil Nutrient Status (Dowel and Porch, 1988). The commix of $HNO_3$and $HCLO_3$ was used to digest plant and $BaSO_4$ contrast turbid ratio method was used for analyzing plant total S EDTA titration was used for SO_4^{2-} in irrigation water.

Treatment and fertilizer application

Winter wheat, corn, cotton and vegetables sensitive to sulfur fertilizer including cabbage, Chinese cabbage and three pungent crops (garlic, scallion and chili) were selected as testing crops in field trials. With various S application rates (60 - 120 kg ha^{-1}), the effect of combination of S and K or other nutrient on crops yield was examined with the treatments of NP (or NPS), NPK, and NPKS etc. Urea, DAP and potassium chloride were used as nitrogen, phosphorus and potassium source respectively. All of the P, S and K were applied as basal at seeding time. For nitrogen, 40 percent was applied as basal dressing and the rest was applied as top dressing at two times. Crop yield was measured on each plot. Soils samples were taken by soil auger from the cultivable layer (0 - 20 cm).

[1] Tianjin Soil and Fertilizer Institute, Tianjin 300192, China,
[2]Agri-Technical Demon Station of Ji county, China
[3]The Sulphur Institute, Washington DC, USA

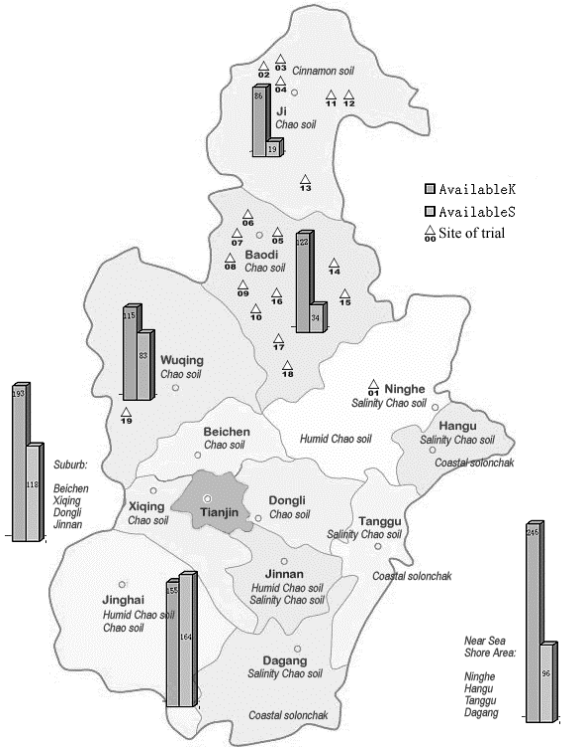

Figure 1:
The main soil types, the average contents of available S and K, and the number of trials in different districts of Tianjin, China.

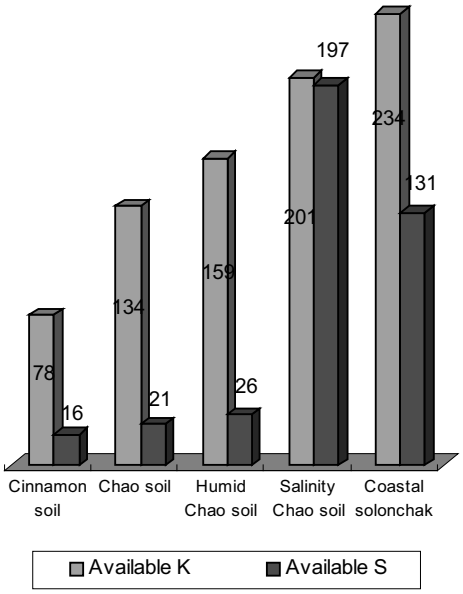

Figure 2: The mean contents of available S and K in the different soil types.

Results and discussion

The role of sulfur fertilizers in balanced fertilization of staple crops

From 1994 to 2000, we conducted the field trials on rice, wheat and corn with various combinations of NPKS. The results are shown in Table 1. It indicated that in most cases, the deficiency of S or K resulted in yield reduction in certain degrees. Sulfur deficiency led to 6 -16% yield reduction. The combination use of S and K produced the best results. Therefore, in fertilizer nutrient management, S combined with other nutrients has become one of the necessary measures in sound crop production.

Effect of sulfur fertilizers on yield of Chinese cabbage

Chinese cabbage is the major vegetable grown in Tianjin. Due to its high yield and quality for food, it has become one of the important vegetables in North China, and has been growing in large areas in Tianjing. Chinese cabbage has also a high demand for both sulfur and potassium.

Seven field trials on Chinese cabbage were arranged at Baodi and Ji County respectively, from 2000 though 2003. The results are presented in Table 2. It was shown that compared with farmer's routine treatment (only NP) the treatment of NPK (in 2000, 2003) increased yield by 7.9 - 13.7%. Based on NPK, adding 60 - 120 kg ha^{-1} sulfur, Chinese cabbage yield were increased by 16.9 - 26.4%. Clearly, sulfur fertilization promoted potassium use efficiency. It also increased the effect of phosphorus on Chinese cabbage yield (in 2001). In Figure 3 it is shown that adding 104 kg ha^{-1} phosphorus the yield of Chinese cabbage increased by 18% compared to no P treatment with a value : cost ratio of 16; based on NPK treatment, adding S 60 kg ha^{-1}, though the Chinese cabbage yield increased by 5%, but value : cost ratio was boosted to 19. It means a small amount of sulfur fertilizer input produced high economic return to farmers.

Table 1:
Effect of S and K fertilization on yield of corn, rice and wheat.

No.	Sites	Crop	Treatment	Mean yield (kg ha^{-1})	Relative yield (%)
1	Maozhuang, NingHe County	Rice	$N_{275}P_{215}S_{60}$	7878	89
			$N_{275}P_{215}K_{150}$	8231	93
			$N_{275}P_{215}K_{150}S_{60}$	8885	100
2	Mongeying, Ji County	Wheat	$N_{225}P_{173}S_{60}$	6120	83
			$N_{225}P_{173}K_{135}$	7380	100
			$N_{225}P_{173}K_{135}S_{60}$	7380	100
3	Dongerying, Ji County	Corn(Shendan 7)	$N_{102}P_{69}S_{60}$	3462	83
			$N_{102}P_{69}K_{90}$	3509	84
			$N_{102}P_{69}K_{90}S_{60}$	4166	100
4	Dongerying, Ji County	Corn (Yedan13)	$N_{135}P_{1380}S_{60}$	6270	88
			$N_{135}P_{138}K_{150}$	6495	91
			$N_{135}P_{138}K_{150}S_{60}$	7110	100
5	Haogezhuang, Baodi County	Corn (Shendan 7)	$N_{205}P_{138}S_{60}$	7823	88
			$N_{205}P_{138}K_{90}$	8327	94
			$N_{205}P_{138}K_{90}S_{60}$	8867	100
6	Haogezhuang, Baodi County	Corn (Yedan 13)	$N_{205}P_{138}S_{60}$	5670	76
			$N_{205}P_{138}K_{90}$	6405	86
			$N_{205}P_{138}K_{90}S_{60}$	7455	100

Table 2:
Effect of S, K and P fertilization on yield of Chinese cabbage in 2000-2003.

No.	Sites	Year	Variety	Treatment	Average ton ha^{-1}	Increase in yield (%)	Increase income (10^3 $ ha^{-1})
7	Bangjun, Baodi district	2000	Beijing3	$N_{120}P_{104}$	74.2	0	2.6
				$N_{120}P_{104}K_{90}$	84.4	13.7	3.0
				$N_{120}P_{104}K_{90}S_{60}$	93.8	26.4	3.3
8	Niudaokou, Baodi district		Tianjin55	$N_{160}P_{52}$	74.5	0	2.6
				$N_{160}P_{52}K_{90}$	80.3	7.9	2.8
				$N_{160}P_{52}K_{90}S_{60}$	87.1	16.9	3.1
9	Shiqiao, Baodi district	2001	Qiulv75	$N_{450}K_{135}S_{60}$	74.4	0	1.6
				$N_{450}P_{104}K_{135}$	83.3	5.1	1.8
				$N_{450}P_{104}K_{135}S_{60}$	87.6	17.8	1.9
10	Anding, Baodi district	2002	Beijing3	$N_{300}P_{173}S_{120}$	107.6	0	2.0
				$N_{300}P_{173}K_{90}$	119.0	10.6	2.5
				$N_{300}P_{173}K_{90}S_{120}$	137.3	27.6	2.8
11	Liangsq, Ji county		Beijing3	$N_{360}P_{200}S_{120}$	96.6	0	1.9
				$N_{360}P_{200}K_{150}$	111.1	14.9	2.2
				$N_{360}P_{200}K_{150}S_{120}$	127.1	31.6	2.8
12	Mongq1, Ji county	2003	Qiulv75	$N_{360}P_{225}$	113	0	5.3
				$N_{360}P_{225}K_{300}$	123	8	5.6
				$N_{360}P_{225}K_{300}S_{120}$	128	19	5.9
13	Mongq2, Ji county		Beijing3	$N_{360}P_{225}$	87	0	3.6
				$N_{360}P_{225}K_{300}$	97	8.9	3.9
				$N_{360}P_{225}K_{300}S_{120}$	114	25.2	4.6

At harvest time we investigated the effect of K mixed with S on yield and quality of Chinese cabbage. The results (See Table 3) showed that effect of NPK, NPKS on Chinese cabbage plant height, stem thickness, and plant weight improved clearly over farmer's routine fertilization, with 2 - 3.3 cm, 3.2 - 3.3 cm, and 0.9 - 1kg respectively.

A field trial was conducted to compare two Chi-

Table 3:
Effect of S and K fertilization on Chinese cabbage cv. Beijing3 (n = 18).

Treatment	Plant height (cm)	Stem thickness (cm)	Plant weight (kg)
$N_{120}P_{104}$	42.5	38.0	2.9
$N_{120}P_{104}K_{90}$	43.8	40.2	3.6
$N_{120}P_{104}K_{90}S_{60}$	44.6	41.3	3.9

Table 4:
Effect of S and K fertilization on garlic.

Treatment	Plant high (with garlic shooting) (cm)	Fresh weight (no garlic shooting/a plant) (g)	Diameter of a garlic head (cm)	Weight of a garlic head (g)
$N_{150}P_{120}$	42	32.4	3.87	20.1
$N_{150}P_{120}S_{60}$	48	39.3	4.11	22.9
$N_{150}P_{120}$ K_{112}	47	34.2	4.04	21.9
$N_{150}P_{120}$ $K_{1120}S_{60}$	51	44.3	4.25	23.7

Table 5:
Effect of S and K fertilization on scallion.

Treatment	Plant height (cm)	Length of white stem (cm)	Fresh weight of a plant (g)	Diameter of stem (cm)
$N_{180}P_{110}$	83.3	26.8	80	1.9
$N_{180}P_{110}$ K_{90}	88.1	27.4	112	1.9
$N_{180}P_{110}$ $K_{90}S_{60}$	87.3	27.7	129	2.2

Table 6:
Effect of S and K fertilization on scallion yield.

Treatment	Scallion yield (kg ha^{-1})			Average (kg ha^{-1})	Yield increase (%)
	1	2	3		
$N_{180}P_{110}$	35445	36645	36870	36320	0
$N_{180}P_{110}$ K_{90}	40800	41460	39240	40500	11.5
$N_{180}P_{110}$ $K_{90}S_{60}$	45525	41010	44955	43830	20.7

nese cabbage varieties response to sulfur fertilizer in 2003. The result showed that applying the same quantity of S fertilizer increased Chinese cabbage Qiulv75 yield by 16.3%, but by 10.6% on Beijing 3 compared to NPK treatment. This result illuminated the differences between Chinese cabbage varieties in response to sulfur. It indicates that when applying S fertilizer we should concern with the varieties sensitivity to S to get higher benefit from S fertilization.

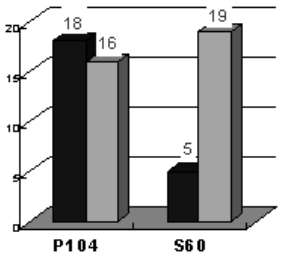

Figure 3:
Effect of P and S fertilization on Chinese cabbage yield (black bars) and value cost ratio (gray bars)

Effect of S fertilizer, and combination of S and K on garlic, scallion and chili yields and quality

Garlic, scallion and chili are sensitive crops to sulfur, and have high sulphur demand. In order to evaluate the response of these crops to sulfur fertilizer, five field trials were conducted from 2000 through 2003 in Baodi county (site no.14,15,16,17, respectively). The results of garlic were shown in Table 4 and Figure 3. The data in Figure 3 illustrate that comparing with NP treatment, adding S increased garlic yield by 16%, resulted in a high value : cost ratio of 25. But for K, the yield increase and VCR was 7% and 6.3, respectively. It means garlic is more sensitive to S than K in this region. The treatment of combined application of NPKS resulted in the highest VCR on garlic with impressive yield increasing. It indicated that NPKS balanced fertilization is the way to get high yield, improve crop quality and increase farmer's income

The results of scallion and chili were showed in Table 5, 6, 7, 8. The scallion yield in NPK treatment was 4185 kg ha^{-1} higher than NP treatment, or

increasing by 11.5%. However, the NPKS treatment produced 7515 kg/ha more than NPK treatment, and increasing production by 20.7% (seeing Table 6).

Chili was harvested in Oct. 2000, when the price of 1st class dry chili was 1.82$ kg^{-1}. The output of NPS treatment was 1879 kg/ha, 367 kg ha^{-1} higher than NP treatment. The output of NPKS was 2063 kg/ha, 552 kg ha^{-1} higher than NP treatment. Using the local first class dry chili price, the treatment of NPS increased income by 665 $ ha^{-1}, but treatment of NPKS increased more than 1000 $ ha^{1}, compared with NP. With the NPKS balanced fertilization on scallion and chili, high quality, output, and income are obtained.

In order to evaluate the effects of combined K and S on scallion seed yield, we arranged field trials at Shiqiao Village, Baodi District. The result is shown in Table 9. Comparing with normal farmers' practice (CK), the K application increased scallion seed yield by 15.0%, for the S2 application the maximal yield reached 717kg ha^{-1}, an increase of 39.6% in yield and 1438 $ha^{-1} in income. It showed that treatment of NPKS2 have the highest volume in plant weight, weight of head, and diameter of head in four treatments, which produced the highest output. It indicated that in this region the proper amount of application sulfur fertilizer should be more than 100 kg ha^{-1} for scallion seed production.

Effects of combined of K and S application on green turnips and carrot yield

The green turnips field trials were arranged at Caijiafang Village, Wuqing (site no. 18). From the field survey that was conducted in the mid-growth period, potassium and sulfur deficiency had affected growth of green turnips. The leaves of green turnips showed different degrees of nutrient deficiency symptoms. Based on the results collected from the field trials at harvesting time (Table 10), adding 60 kg S ha^{-1} increased yield by 28.7% over NPK treatment, with the higest value : cost ratio of 28.7. While adding K increased yield by 24.9% over NPS, with value to cost ratio of 27.1.

The carrot field trials were arranged at Shengrenzhuang Village, Baodi District (site no. 19) and the result was shown in Table 11. The carrot biologic character evaluation on 40 carrot plants showed that the treatment of NPKS have the higher values in total fresh weight/per pant, fresh weight/per carrot, the carrot length and thickness than treatment of either NP or NPK, resulting in the highest yield in all treatments. Compared with farmer's routine treatment (NP) applying 90 kg ha^{-1} K_2O increased carrot yield by 9.5%; adding 120 kg ha^{-1} S increased carrot yield to 24.7 ton ha^{-1}, with income of 1048 $ ha^{-1} and value : cost ratio of 12.3.

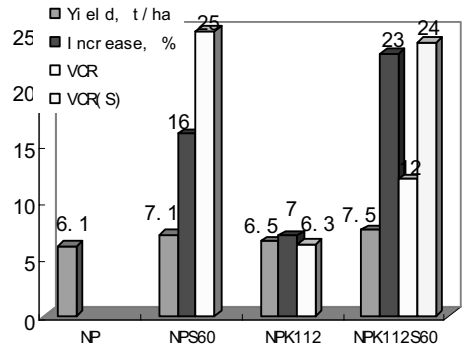

Figure 4:
Effect of S and K fertilization on garlic yield.

Conclusions

Based on the results collected from field trials conducted in Tianjing from 2000 to 2003, it can be concluded that: sulfur and potassium deficiencies were identified in about 30% of total farmland area, and mainly distributed in cinnamon soil, chao cinnamon soil and partly chao soil in Ji and Baodi county. Deficiency of S or K cause grain yields reduction by 6% - 24% in rice, wheat and corn productions in these areas, combination use of S and K produced highest yield.

Adding 60 - 120 kg ha^{-1} sulfur to NPK recommendation increased Chinese cabbage yields by 16.9 - 26.4% in seven field trials. Results of garlic, scallion, and chili also showed that adding S60kg/ha increased yields by 16 - 19.5% over NP treatment, and NPKS combined application increased yields by 20. - 36.4% over NPK treatment, with high vegetable quality. Results of green turnips and carrot field trials showed that comparing with NPK treatment, adding S 60-120 kg ha^{-1} increased yield by 17.6 - 28.8% with maximum value : cost ratio of 12.3 - 28.7.

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Table 7:
Effect of combined of S and K fertilization on chili shape.

Treatment	Plant height (cm)	Plant weight (kg)	Number of chili per plant	Chili weight (g)	Number of ill chili per plant	Number of green chili per plant
$N_{150}P_{90}$	81.6	0.173	40.1	1.90	8.1	5.4
$N_{150}P_{90}S_{60}$	82.8	0.181	44.7	2.08	7.4	4.4
$N_{150}P_{90}K_{90}$	79.0	0.196	42.6	2.05	5.2	3.5
$N_{150}P_{90}K_{90}S_{60}$	82.4	0.216	50.8	2.11	4.5	3.4

Table 8:
Effect of S fertilization on yield of first class chili.

Treatment	Yield of first class chili (kg ha^{-1})	Increase by S fertilization rate (%)
$N_{150}P_{90}$	1512	0
$N_{150}P_{90}S_{60}$	1879	19.5
$N_{150}P_{90}K_{90}S_{60}$	2063	36.4

Table 9:
Effect of combined of K and S fertilization on scallion seed production (kg ha^{-1}) in 2001.

Treatment	Repeat				Increasing amount	Increasing income ($ ha^{-1})	Increasing %	Response to K %	Response to S %
	I	II	III	Aver-age					
$N_{180}P_{104}$	490	520	530	513	0	0	0	0	-
$N_{180}P_{104}K_{90}$	590	570	610	590	77	531	15.0**	15	0
$N_{180}P_{104}K_{90}S_{60}$	670	690	650	670	157	1105	30.5**	-	13.6**
$N_{180}P_{104}K_{90}S_{120}$	721	713	716	717	203	1438	39.6**	-	21.5**

LSD 0.05 = 35.3*; LSD 0.01 = 53.5**

Table 10:
Effect of combined K and S fertilization on yield of green turnips (ton ha^{-1}).

Treatment	Repeat				Average	Response to K (%)	Response to S (%)	Value cost ratio
	I	II	III	IV				
$N_{206}P_{173}S_{60}$	53.0	54.7	49.9	52.8	52.6	0	-	27.1
$N_{206}P_{173}S_{60}K_{112}$	66.7	67.0	63.7	65.5	65.7	24.9**	28.8**	28.7
$N_{206}P_{173}K_{112}$	50.8	52.9	49.0	51.4	51.0	-	0	23.3

LSD 0.05 = 2.97*; LSD0.01 = 4.49**

Table 11:
Effect of combine K and S fertilization on yield of carrots.

Treatment	Repeat (kg 667 m^{-2})					Yield increase (%)	Income in-crease ($ ha^{-1})	Value cost ratio
	I	II		IV	Average			
$N_{180}P_{90}$	21.3	19.5	926	21.3	21.0	0	926	9.0
$N_{180}P_{90}K_{150}$	23.4	23.0	981	23.1	23.0	9.5*	981	11.9
$N_{180}P_{90}K_{150}S_{120}$	23.4	24.2	1048	24.5	24.7	17.6**	1048	12.3

LSD 0.05 = 1.79*; LSD 0.01 = 2.71**

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