7 Sulfur

Silvia Haneklaus, Elke Bloem, and Ewald Schnug
Institute of Plant Nutrition and Soil Science, Braunschweig, Germany

Luit J. de Kok and Ineke Stulen
University of Groningen, Haren, The Netherlands

CONTENTS

7.1 INTRODUCTION

Sulfur (S) is unique in having changed within just a few years, from being viewed as an undesired pollutant to being seen as a major nutrient limiting plant production in Western Europe. In East Asia, where, under current legislative restrictions, sulfur dioxide (SO_2) emissions are expected to increase further by 34% by 2030 (1), considerations of sulfur pollution are a major issue. Similarly in Europe, sulfur is still associated with its once detrimental effects on forests which peaked in the

1970s (2), and which gave this element the name 'yellow poison.' With Clean Air Acts coming into force at the start of the 1980s, atmospheric sulfur depositions were reduced drastically and rapidly in Western Europe, and declined further in the 1990s after the political transition of Eastern European countries. In arable production, sulfur deficiency can be retraced to the beginning of the 1980s (3). Since then, severe sulfur deficiency has become the main nutrient disorder of agricultural crops in Western Europe. It has been estimated that the worldwide sulfur fertilizer deficit will reach 11 million tons per year by 2012, with Asia (6 million tons) and the Americas (2.3 million tons) showing the highest shortage (4).

Severe sulfur deficiency not only reduces crop productivity and diminishes crop quality, but it also affects plant health and environmental quality (5). Yield and quality in relation to the sulfur nutritional status for numerous crops are well described in the literature. In comparison, research in the field of interactions between sulfur and pests and diseases is relatively new. Related studies indicate the significance of the sulfur nutritional status for both beneficial insects and pests.

Since the very early days of research on sulfur in the 1930s, significant advances have been made in the field of analysis of inorganic and organic sulfur compounds. By employing genetic approaches in life science research, significant advances in the field of sulfur nutrition, and in our understanding of the cross talk between metabolic pathways involving sulfur and interactions between sulfur nutrition and biotic and abiotic stresses, can be expected in the future.

This chapter summarizes the current status of sulfur research with special attention to physiological and agronomic aspects.

7.2 SULFUR IN PLANT PHYSIOLOGY

Sulfur is an essential element for growth and physiological functioning of plants. The total sulfur content in the vegetative parts of crops varies between 0.1 and 2% of the dry weight (0.03 to 0.6 mmol S g^{-1} dry weight). The uptake and assimilation of sulfur and nitrogen by plants are strongly interrelated and dependent upon each other, and at adequate levels of sulfur supply the organic N/S ratio is around 20:1 on a molar basis (6–9). In most plant species the major proportion of sulfur (up to 70% of the total S) is present in reduced form in the cysteine and methionine residues of proteins. Additionally, plants contain a large variety of other organic sulfur compounds such as thiols (glutathione; ~1 to 2% of the total S) and sulfolipids (~1 to 2% of the total S); some species contain the so-called secondary sulfur compounds such as alliins and glucosinolates (7,8,10,11). Sulfur compounds are of great significance in plant functioning, but are also of great importance for food quality and the production of phyto-pharmaceuticals (8,12).

In general, plants utilize sulfate (S^{6+}) taken up by the roots as a sulfur source for growth. 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 (13–15). In the chloroplasts of the shoot cells, sulfate is reduced to sulfide (S^{2-}) prior to its assimilation into organic sulfur compounds (16,17). Plants are also able to utilize foliarly absorbed sulfur gases; hence chronic atmospheric sulfur dioxide and hydrogen sulfide levels of 0.05 μL L^{-1} and higher, which occur in polluted areas, contribute substantially to the plant's sulfur nutrition (see below; 18–21).

The sulfur requirement varies strongly between species and it may fluctuate during plant growth. The sulfur requirement can be defined as 'the minimum rate of sulfur uptake and utilization that is sufficient to obtain the maximum yield, quality, and fitness,' which for crop plants is equivalent to 'the minimum content of sulfur in the plant associated with maximum yield' and is regularly expressed as kg S ha^{-1} in the harvested crop. In physiological terms the sulfur requirement is equivalent to the rate of sulfur uptake, reduction, and metabolism needed per gram plant biomass produced over time and can be expressed as mol S g^{-1} plant day^{-1}. The sulfur requirement of a crop at various stages of development under specific growth conditions may be predicted by upscaling the sulfur requirement in μmol S g^{-1} plant day^{-1} to mol S ha^{-1} day^{-1} by estimating the

crop biomass density per hectare (tons of plant biomass ha^{-1}). When a plant is in the vegetative growth period, the sulfur requirement ($S_{requirement}$, expressed as μmol S g^{-1} plant day^{-1}) can be calculated as follows (11):

$$S_{requirement} = S_{content} \times RGR$$

where $S_{content}$ represents the total sulfur concentration of the plant (μmol g^{-1} plant biomass) and RGR is the relative growth rate of the plant (g g^{-1} plant day^{-1}). The RGR can be calculated by using the following equation:

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

where W_1 and W_2 are the total plant weight (g) at time t_1 and t_2, respectively, and $t_2 - t_1$ the time interval (days) between harvests. In general, the sulfur requirement of different crop species grown at optimal nutrient supply and growth conditions ranges from 0.01 to 0.1 mmol g^{-1} plant dry weight day^{-1}. Generally, the major proportion of the sulfate taken up is reduced and metabolized into organic compounds, which are essential for structural growth. However, in some plant species, a large proportion of sulfur is present as sulfate and in these cases, for structural growth, the organic sulfur content may be a better parameter for the calculation of the sulfur requirement (see also Section 7.3.1.3).

7.2.1 Uptake, Transport, and Assimilation of Sulfate

The uptake and transport of sulfate in plants is mediated by sulfate transporter proteins and is energy-dependent (driven by a proton gradient generated by ATPases) through a proton–sulfate (presumably $3H^+/SO_4^{2-}$) co-transport (14). Several sulfate transporters have been isolated and their genes have been identified. Two classes of sulfate transporters have been identified: the so-called 'high- and low-affinity sulfate transporters,' which operate ideally at sulfate concentrations < 0.1 mM and ≥ 0.1 mM, respectively. According to their cellular and subcellular expression, and possible functioning, the sulfate transporter gene family has been classified into as many as five different groups (15,22–24). Some groups are expressed exclusively in the roots or shoots, or in both plant parts. Group 1 transporters are high-affinity sulfate transporters and are involved in the uptake of sulfate by the roots. 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 yet known. Regulation and expression of the majority of sulfate transporters are controlled by the sulfur nutritional status of the plants. A rapid decrease in root sulfate content upon sulfur deprivation is regularly accompanied by a strongly enhanced expression of most sulfate transporter genes (up to 100-fold), accompanied by a substantial enhanced sulfate uptake capacity. It is still questionable whether, and to what extent, sulfate itself or metabolic products of sulfur assimilation (viz *O*-acetylserine, 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 (15,22–24).

The major proportion of the sulfate taken up by the roots is reduced to sulfide and subsequently incorporated into cysteine, the precursor and the reduced sulfur donor for the synthesis of most other organic sulfur compounds in plants (16,17,25–27). Even though root plastids contain all sulfate reduction enzymes, reduction predominantly takes place in the chloroplasts of the shoot. The reduction of sulfate to sulfide occurs in three steps (Figure 7.1). First, sulfate is activated to adenosine 5′-phosphosulfate (APS) prior to its reduction, a reaction catalyzed by ATP sulfurylase. The affinity of this enzyme for sulfate is rather low (K_m ~1 mM) and the in situ sulfate concentration in the chloroplast may be rate-limiting for sulfur reduction (7). Second, the activated sulfate (APS) is reduced by APS reductase to sulfite, a reaction where glutathione (RSH; Figure 7.1) most likely functions as reductant (17,26). Third, sulfite is reduced to sulfide by sulfite reductase with reduced ferredoxin as reductant. Sulfide is

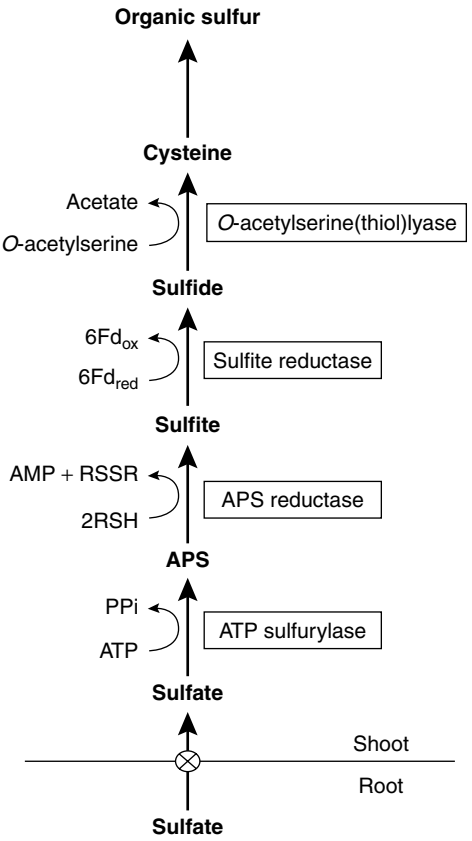

FIGURE 7.1 Sulfate reduction and assimilation in plants.

subsequently incorporated into cysteine, catalyzed by *O*-acetylserine(thiol)lyase, with *O*-acetylserine as substrate (Figure 7.1). The formation of *O*-acetylserine is catalyzed by serine acetyltransferase, and together with *O*-acetylserine(thiol)lyase it is associated as an enzyme complex named cysteine synthase (28,29). The synthesis of cysteine is a major reaction in the direct coupling between sulfur and nitrogen metabolism in the plant (6,9).

Sulfur reduction is highly regulated by the sulfur status of the plant. Adenosine phosphosulfate reductase is the primary regulation point in the sulfate reduction pathway, since its activity is generally the lowest of the enzymes of the assimilatory sulfate reduction pathway and this enzyme has a fast turnover rate (16,17,26,27). Regulation may occur both by allosteric inhibition and by metabolite activation or repression of expression of the genes encoding the APS reductase. Both the expression and activity of APS reductase change rapidly in response to sulfur starvation or exposure to reduced sulfur compounds. Sulfide, *O*-acetylserine, cysteine, or glutathione are likely regulators of APS reductase (9,16,17,26). The remaining sulfate in plant tissue is predominantly present in the vacuole, since the cytoplasmatic concentration of sulfate is kept rather constant. In general, the remobilization and redistribution of the vacuolar sulfate reserves is a rather slow process. Under temporary sulfur-limitation stress it may be even too low to keep pace with the growth of the plant, and therefore sulfur-deficient plants may still contain detectable levels of sulfate (13,15,22).

Cysteine is used as the reduced sulfur donor for the synthesis of methionine, the other major sulfur-containing amino acid present in plants, via the so-called trans-sulfurylation pathway (30,31). Cysteine is also the direct precursor for the synthesis of various other compounds such as glutathione, phytochelatins, and secondary sulfur compounds (12,32). The sulfide residue of the

cysteine moiety in proteins is furthermore of great importance in substrate binding of enzymes, in metal–sulfur clusters in proteins (e.g., ferredoxins), and in regulatory proteins (e.g., thioredoxins).

7.2.1.1 Foliar Uptake and Metabolism of Sulfurous Gases

In rural areas the atmosphere generally contains only trace levels of sulfur gases. In areas with volcanic activity and in the vicinity of industry or bioindustry, high levels of sulfurous air pollutants may occur. Sulfur dioxide (SO_2) is, in quantity and abundance, by far the most predominant sulfurous air pollutant, but locally the atmosphere may also be polluted with high levels of hydrogen sulfide (18,19,21). Occasionally the air may also be polluted with enhanced levels of organic sulfur gases, viz carbonyl sulfide, methyl mercaptan, carbon disulfide, and dimethyl sulfide (DMS).

The impact of sulfurous air pollutants on crop plants appears to be ambiguous. Upon their foliar uptake, SO_2 and H_2S may be directly metabolized, and despite their potential toxicity used as a sulfur source for growth (18–21). However, there is no clear-cut transition in the level or rate of metabolism of the absorbed sulfur gases and their phytotoxicity, and the physiological basis for the wide variation in susceptibility between plants species and cultivars to atmospheric sulfur gases is still largely unclear (18–21). These paradoxical effects of atmospheric sulfur gases complicate the establishment of cause–effect relationships of these air pollutants and their acceptable atmospheric concentrations in agro-ecosystems.

The uptake of sulfurous gases predominantly proceeds via the stomata, since the cuticle is hardly permeable to these gases (33). The rate of uptake depends on the stomatal and the leaf interior (mesophyll) conductance toward these gases and their atmospheric concentration, and may be described by Fick's law for diffusion

$$J_{gas}\ (\text{pmol cm}^{-2}\ \text{s}^{-1}) = g_{gas}\ (\text{cm s}^{-1}) \times \Delta_{gas}\ (\text{pmol cm}^{-3})$$

where J_{gas} represents the gas uptake rate, g_{gas} the diffusive conductance of the foliage representing the resultant of the stomatal and mesophyll conductance to the gas, and Δ_{gas} the gas concentration gradient between the atmosphere and leaf interior (18,20,34). Over a wide range, there is a nearly linear relationship between the uptake of SO_2 and the atmospheric concentration. Stomatal conductance is generally the limiting factor for uptake of SO_2 by the foliage, whereas the mesophyll conductance toward SO_2 is very high (18,20,35). This high mesophyll conductance is mainly determined by chemical/physical factors, since the gas is highly soluble in the water of the mesophyll cells (in either apoplast or cytoplasm). Furthermore, the dissolved SO_2 is rapidly hydrated and dissociated, yielding bisulfite and sulfite ($SO_2 + H_2O \rightarrow H^+ + HSO_3^- \rightarrow 2H^+ + SO_3^{2-}$) (18,20). The latter compounds either directly enter the assimilatory sulfur reduction pathway (in the chloroplast) or are enzymatically or nonenzymatically oxidized to sulfate in either apoplast or cytoplasm (18,20). The sulfate formed may be reduced and subsequently assimilated or it is transferred to the vacuole. Even at relatively low atmospheric levels, SO_2 exposure may result in enhanced sulfur content of the foliage (18,20). The liberation of free H^+ ions upon hydration of SO_2 or the sulfate formed from its oxidation is the basis of a possible acidification of the water of the mesophyll cells, in case the buffering capacity is not sufficient. Definitely, the physical–biochemical background of the phytotoxicity of SO_2 can be ascribed to the negative consequences of acidification of tissue/cells upon the dissociation of the SO_2 in the aqueous phase of the mesophyll cells or the direct reaction of the (bi)sulfite formed with cellular constituents and metabolites (18,20).

The foliar uptake of H_2S even appears to be directly dependent on the rate of its metabolism into cysteine and subsequently into other sulfur compounds, a reaction catalyzed by *O*-acetylserine (thiol)lyase (19,21). The basis for the phytotoxicity of H_2S can be ascribed to a direct reaction of sulfide with cellular components; for instance, metallo-enzymes appear to be particularly susceptible to sulfide, in a reaction similar to that of cyanide (18,19,36).

The foliage of plants exposed to SO_2 and H_2S generally contains enhanced thiol levels, the accumulation of which depends on the atmospheric level, though it is generally higher upon exposure to H_2S than exposure to SO_2 at equal concentrations.

Changes in the size and composition of the thiol pool are likely the reflection of a slight overload of a reduced sulfur supply to the foliage. Apparently, the direct absorption of gaseous sulfur compounds bypasses the regulation of the uptake of sulfate by the root and its assimilation in the shoot so that the size and composition of the pool of thiol compounds is no longer strictly regulated.

7.2.2 Major Organic Sulfur Compounds

The sulfur-containing amino acids cysteine and methionine play a significant role in the structure, conformation, and function of proteins and enzymes in vegetative plant tissue, but high levels of these amino acids may also be present in seed storage proteins (37). Cysteine is the sole amino acid whose side-chain can form covalent bonds, and when incorporated into proteins, the thiol group of a cysteine residue can be oxidized, resulting in disulfide bridges with other cysteine side-chains (forming cystine) or linkage of polypeptides. Disulfide bridges make an important contribution to the structure of proteins. An impressive example for the relevance of disulfide bridges is the influence of the sulfur supply on the baking quality of bread-making wheat. Here, the elasticity and resistance to extensibility are related to the concentration of sulfur-containing amino acids and glutathione. First, it was shown in greenhouse studies that sulfur deficiency impairs the baking quality of wheat (38–41). Then, the analysis of wheat samples from variety trials in England and Germany revealed that decrease in the supply of sulfur affected the baking quality, before crop productivity was reduced (42,43). The sulfur content of the flour was directly related to the baking quality with each 0.1% of sulfur equalling 40 to 50 mL loaf volume. The data further revealed that a lack of either protein or sulfur could be partly compensated for by increased concentration of the other.

The crude protein of wheat can be separated into albumins and globulins, and gluten, which consist of gliadins and glutenins. The first, albumins and globulins, are concentrated under the bran and are thus present in higher concentrations in whole-grain flours. Their concentration is directly linked to the thousand grain weight. In the flour, gluten proteins are predominant and the gliadin/glutenin ratio influences the structure of the gluten, rheological features of the dough, and thus the baking volume (44). Gliadins are associated with the viscosity and extensibility, and glutenins with the elasticity and firmness of the dough (45). Here, the high-molecular-weight (HMW) glutenins give a higher proportion of the resistance of the gluten than low-molecular-weight (LMW) glutenins (46). Sulfur deficiency gives rise to distinctly firmer and less extensible doughs (Figure 7.2). Doughs from plants adequately supplied with sulfur show a significantly higher extensibility and lower resistance than do doughs made of flour with an insufficient sulfur supply (Figure 7.2). Sulfur-deficient wheat has a lower albumin content, but higher HMW-glutenin concentration and a higher HMW/LMW glutenin ratio (47).

Consequently the baking volume of sulfur-deficient wheat is reduced significantly. A comparison of British and German wheat varieties with similar characteristics for loaf volume and falling number is given in Table 7.1. In the German classification system, varieties C1 and C2 are used as feed or as a source for starch. Varieties B3, B4, and B5 are suitable for baking but are usually mixed with higher quality wheat. The highest bread-making qualities are in the A6–A9 varieties.

The results presented in Table 7.1 reveal that the quality of British and German varieties is similar. It is relevant in this context that the British varieties gave the same results in the baking experiment at lower protein concentrations than the German ones. The reason is that there was a higher sulfur concentration and thus a smaller N/S ratio in the British varieties. This means that higher sulfur concentrations can partially compensate for a lack of wheat protein and vice versa.

Sulfur supply has been recognized as a major factor influencing protein quality for a long time (48,49). Eppendorfer and Eggum (50,51), for instance, noted that the biological value of proteins in potatoes (*Solanum tuberosum* L.) was reduced from 94 to 55 by sulfur deficiency at high N supply, and from 65 to 40 and 70 to 61 in kale (*Brassica oleracea* var. *acephala* DC) and field beans

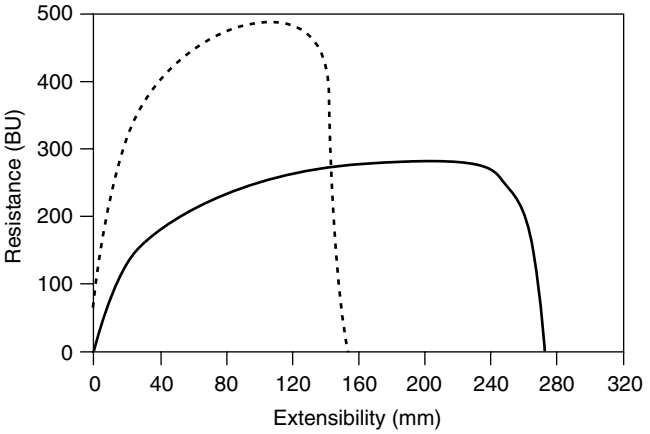

FIGURE 7.2 Extensographs for flour with average (continuous line) and low (broken line) sulfur content. +S flour: 0.146% S, 1.82% N, N:S = 12.5:1; −S flour: 0.089% S, 1.72% N, N:S = 19.3:1. (From Wrigley, C.W. et al., *J. Cereal Sci*., 2, 15–24, 1984.)

TABLE 7.1
Comparison of Quality Parameters of German and British Wheat Varieties

Parameter	British D	German B4	British B	German A6/A7
Loaf volume (ml)	612	612	717	713
Falling number (s)	215	276	247	381
Protein content (%)	10.8	13.1	12.6	14.3
S content (mg g^{-1})	1.38	1.25	1.46	1.35
N:S ratio	12.6	16.6	14.0	17.8

Source: From Haneklaus, S. et al., *Sulphur Agric*., 16, 31–35, 1992.

(*Vicia faba* L.), respectively. Whereas the essential amino acid concentrations declined due to sulfur deficiency, the content of amino acids of low nutritional value such as arginine, asparagine, and glutamic acid increased (50, 51). Figure 7.3 shows the relationship between sulfur supply to curly cabbage (*Brassica oleracea* var. *sabellica* L.), indicated by the total sulfur concentration in fully expanded younger leaves, and the cysteine and methionine concentration in leaf protein.

This example shows that a significant relationship between sulfur supply and sulfur-containing amino acids exists only under conditions of severe sulfur deficiency, where macroscopic symptoms are visible. The corresponding threshold is below leaf sulfur levels of 0.4% total sulfur in the dry matter of brassica species (52,53).

In comparison, sulfur fertilization of soybean significantly increased the cystine, cysteine, methionine, protein, and oil content of soybean grain (Table 7.2) (54).

The reason for these different responses of vegetative and generative plant tissue to an increased sulfur supply is that excess sulfur is accumulated in vegetative tissue as glutathione (see below) or as sulfate in vacuoles; the cysteine pool is maintained homeostatically because of its cytotoxicity (55). In comparison, the influence of sulfur supply on the seed protein content is related to the plant species. In oilseed rape, for instance, which produces small seeds, the total protein content is more or less not influenced by the sulfur supply (56). Species with larger seeds, which contain sulfur-rich proteins, such as soybean, respond accordingly to changes in the sulfur supply (5).

The most abundant plant sulfolipid, sulfoquinovosyl diacylglycerol, is predominantly present in leaves, where it comprises up to 3 to 6% of the total sulfur (10,57,58). This sulfolipid can occur in plastid membranes and is probably involved in chloroplast functioning. The route of biosynthesis

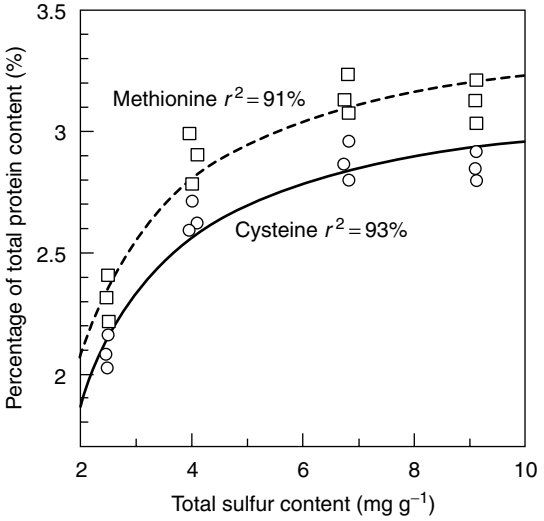

FIGURE 7.3 Relationship between the sulfur nutritional status of curly cabbage and the concentration of cysteine and methionine in the leaf protein. (From Schnug, E., in *Sulphur Metabolism in Higher Plants: Molecular, Ecophysiological and Nutritional Aspects*, Backhuys Publishers, Leiden, 1997, pp. 109–130.)

TABLE 7.2
Influence of Sulfur Fertilization on Sulfur-Containing Amino Acids, Total Protein, and Oil Content in Soybean Grains

	S-Containing Amino Acid (mg g^{-1})				
S Supply (mg kg^{-1})	Cystine	Cysteine	Methionine	Protein (%)	Oil (%)
0	1.9	1.2	7.6	40.3	19.6
40	2.4	1.6	10.5	41.0	21.0
80	2.9	1.9	13.9	41.6	20.6
120	2.9	2.0	16.4	42.2	20.8
$LSD_{5\%}$	0.14	0.10	1.13	0.99	0.19

Source: From Kumar, V. et al., *Plant Soil*, 59, 3–8, 1981.

of sulfoquinovosyl diacylglycerol is still under investigation; in particular, the sulfur precursor for the formation of the sulfoquinovose is not known, though from recent observations it is evident that sulfite is the likely candidate (58).

Cysteine is the precursor for the tripeptide glutathione (γGluCysGly; GSH), a thiol compound that is of great importance in plant functioning (32,59,60,61). Glutathione synthesis proceeds in a two-step reaction. First, γ-glutamylcysteine is synthesized from cysteine and glutamate in an ATP-dependent reaction catalyzed by γ-glutamylcysteine synthetase (Equation 7.1). Second, glutathione is formed in an ATP-dependent reaction from γ-glutamylcysteine and glycine (in glutathione homologs, β-alanine or serine) catalyzed by glutathione synthetase (Equation 7.2):

$$\text{Cys} + \text{Glu} + \text{ATP} \xrightarrow[\gamma\text{-glutamylcysteine synthetase}]{} \gamma\text{GluCys} + \text{ADP} + \text{Pi} \tag{7.1}$$

$$\gamma\text{GluCys} + \text{Gly} + \text{ATP} \xrightarrow[\text{glutathione synthetase}]{} \gamma\text{GluCysGly} + \text{ADP} + \text{Pi} \tag{7.2}$$

TABLE 7.3
Influence of Sulfur Fertilization on the Glutathione Content of the Vegetative Tissue of Different Crops

Crop Plant	Increase of Glutathione Concentration by S Supply	Reference
Asparagus spears	Field: 39–67 nmol g^{-1} (d.w.) per kg S[a] applied	62
Oilseed rape leaves	Field: 64 nmol g^{-1} (d.w.) per kg S[a] applied	63
	Pot: 3.9 nmol g^{-1} (d.w.) per mg S[b] applied	64
Spinach leaves	Pot: 656 nmol g^{-1} (f.w.) per µl l^{-1} H_2S[c]	65

[a]Maximum dose = 100 kg ha^{-1} S.
[b]Maximum dose = 250 mg pot^{-1} S.
[c]Maximum dose = 250 µl l^{-1} H_2S.

Glutathione and its homologs, for example, homoglutathione (γGluCysβAla) in Fabaceae and hydroxymethylglutathione (γGluCysβSer) in Poaceae, are widely distributed in plant tissues in concentrations ranging from 0.1 to 3 mM. The glutathione content is closely related to the sulfur nutritional status. In Table 7.3, the influence of the sulfur supply and sulfur status and the glutathione content is summarized for different crops. The possible significance of the glutathione content for plant health is discussed in Section 7.5.3.

Glutathione is maintained in the reduced form by an NADPH-dependent glutathione reductase, and the ratio of reduced glutathione (GSH) to oxidized glutathione (GSSG) generally exceeds a value of 7 (60–67). Glutathione fulfills various roles in plant functioning. In sulfur metabolism, glutathione functions as the reductant in the reduction of APS to sulfite (Figure 7.1). In crop plants, glutathione is the major transport form of reduced sulfur between shoot and roots, and in the remobilization of protein sulfur (e.g., during germination). Sulfate reduction occurs in the chloroplasts, and roots of crop plants mostly depend for their reduced sulfur supply on shoot–root transfer of glutathione via the phloem (59–61).

Selenium is present in most soils in various amounts, and its uptake, reduction, and assimilation strongly interact with that of sulfur in plants. Glutathione appears to be directly involved in the reduction and assimilation of selenite into selenocysteine (68). More detailed information about interactions between sulfur and other minerals is given in Section 7.2.4.

Glutathione provides plant protection against stress and a changing environment, viz air pollution, drought, heavy metals, herbicides, low temperature, and UV-B radiation, by depressing or scavenging the formation of toxic reactive oxygen species such as superoxide, hydrogen peroxide, and lipid hydroperoxides (61,69). The formation of free radicals is undoubtedly involved in the induction and consequences of the effects of oxidative and environmental stress on plants. The potential of glutathione to provide protection is related to the size of the glutathione pool, its oxidation–reduction state (GSH/GSSG ratio) and the activity of glutathione reductase.

Plants may suffer from an array of natural or synthetic substances (xenobiotics). In general, these have no direct nutritional value or significance in metabolism, but may, at too high levels, negatively affect plant functioning (70–72). These compounds may originate from either natural (fires, volcanic eruptions, soil or rock erosion, biodegradation) or anthropogenic (air and soil pollution, herbicides) sources. Depending on the source of pollution, namely air, water, or soil, plants have only limited possibilities to avoid their accumulation to diminish potential toxic effects. Xenobiotics (R-X) may be detoxified in conjugation reactions with glutathione (GSH) catalyzed by the enzyme glutathione *S*-transferase (70–72).

$$\text{R-X} + \text{GSH} \Rightarrow \text{R-SG} + \text{X-H}$$

The activity of glutathione *S*-transferase may be enhanced in the presence of various xenobiotics via induction of distinct isoforms of the enzyme. Glutathione *S*-transferases have great

significance in herbicide detoxification and tolerance in agriculture. The induction of the enzyme by herbicide antidotes, the so-called safeners, is the decisive step for the induction of herbicide tolerance in many crop plants. Under normal natural conditions, glutathione *S*-transferases are assumed to be involved in the detoxification of lipid hydroperoxides, in the conjugation of endogenous metabolites, hormones, and DNA degradation products, and in the transport of flavonoids. However, oxidative stress, plant-pathogen infections, and other reactions, which may induce the formation of hydroperoxides, also may induce glutathione *S*-transferases. For instance, lipid hydroperoxides (R-OOH) may be degraded by glutathione *S*-transferases:

$$\text{R-OOH} + 2\text{GSH} \Rightarrow \text{R-OH} + \text{GSSG} + \text{H}_2\text{O}$$

Plants need minor quantities of essential heavy metals (zinc, copper, and nickel) for growth. However, plants may suffer from exposure to high toxic levels of these metals or other heavy metals, for example, cadmium, copper, lead, and mercury. Heavy metals elicit the formation of heavy-metal-binding ligands. Among the various classes of metal-binding ligands, the cysteine-rich metallothioneins and phytochelatins are best characterized; the latter are the most abundant ligands in plants (73–78). The metallothioneins are short gene-encoded polypeptides and may function in copper homeostasis and plant tolerance. Phytochelatins are synthesized enzymatically by a constitutive phytochelatin synthase enzyme and they may play a role in heavy metal homeostasis and detoxification by buffering the cytoplasmatic concentration of essential heavy metals, but direct evidence is lacking so far. Upon formation, the phytochelatins only sequester a few heavy metals, for instance cadmium. It is assumed that the cadmium–phytochelatin complex is transported into the vacuole to immobilize the potentially toxic cadmium (79). The enzymatic synthesis of phytochelatins involves a sequence of transpeptidation reactions with glutathione as the donor of γ-glutamyl-cysteine (γGluCys) residues according to the following equation:

$$(\gamma\text{GluCys})_n\text{Gly} + (\gamma\text{GluCys})_n\text{Gly} \Rightarrow (\gamma\text{GluCys})_{n+1}\text{Gly} + (\gamma\text{GluCys})_{n-1}\text{Gly}$$

The number of γ-glutamyl-cysteine residues $(\gamma\text{GluCys})_n$ in phytochelatins ranges from 2 to 5, though it may be as high as 11. In species containing glutathione homologs (see above), the C-terminal amino acid glycine is replaced by β-alanine or serine (73–78). During phytochelatin synthesis, the sulfur demand is enhanced (80) so that it may be speculated that the sulfur supply is linked to heavy metal uptake, translocation of phytochelatins into root cell vacuoles, and finally transport to the shoot and expression of toxicity symptoms. The sulfur/metal ratio is obviously related to the length of the phytochelatin (81), which might offer a possibility to adapt to varying sulfur nutritional conditions. Hence, increasing cadmium stress (10 μmol Cd in the nutrient solution) yielded an enhanced sulfate uptake by maize roots of 100%, whereby this effect was associated with decreased sulfate and glutathione contents and increased phytochelatin concentrations (81). The studies of Raab et al. (82) revealed that 13% of arsenic was bound in phytochelatin complexes, whereas the rest occurred as nonbound inorganic compounds.

7.2.3 Secondary Sulfur Compounds

There are more than 100,000 known secondary plant compounds, and for only a limited number of them are the biochemical pathways, functions, and nutritional and medicinal significance known (84). Detailed overviews of the biochemical pathways involved in the synthesis of the sulfur-containing secondary metabolites, glucosinolates and alliins, are provided by Halkier (84) and Lancaster and Boland (85). Bioactive secondary plant compounds comprise various substances such as carotenoids, phytosterols, glucosinolates, flavonoids, phenolic acids, protease inhibitors, monoterpenes, phyto-estrogens, sulfides, chlorophylls, and roughages (87). Often, secondary metabolites are accumulated in plant tissues and concentrations of 1 to 3% dry weight have been determined (88). Secondary compounds in plants usually have a pharmacological effect on humans (87). Therefore, secondary metabolites contribute significantly to food quality, either as nutritives or

antinutritives. Plants synthesize a great array of secondary metabolites as they are physically immobile (88), and the presence of secondary compounds may give either repellent or attractant properties.

The bioactive components in medicinal plants comprise the whole range of secondary metabolites and crop-specific cultivation strategies, which include fertilization, harvesting, and processing techniques, and which are required for producing a consistently high level of bioactive constituents. Ensuring a consistently high quality of the raw materials can be a problem, particularly if the active agent is unstable and decomposes after harvesting of the plant material, as is true for many secondary metabolites such as the sulfur-containing alliins and glucosinolates (89).

Glucosinolates are characteristic compounds of at least 15 dicotyledonous families. Of these, the Brassicaceae are the most important agricultural crops. Glucosinolates act as attractants, repellents, insecticides, fungicides, and antimicrobial protectors. The principal structure of a glucosinolate is given in Figure 7.4.

There are about 80 different glucosinolates, which consist of glucose, a sulfur-containing group with an aglucon rest, and a sulfate group (87). Alkenyl glucosinolates such as progoitrin and gluconapin have an aliphatic aglucon rest, whereas indole glucosinolates such as glucobrassicin and 4-hydroxyglucobrassicin in rape (*Brassica napus* L.) have an aromatic aglucon rest (Figure 7.4). Additional information about the characteristics of glucosinolate side-chains is given by Underhill (91), Larsen (92), and Bjerg et al. (93).

Glucosinolates are generally hydrolyzed by the enzyme myrosinase, which is present in all glucosinolate-containing plant parts. Bones and Rossiter (94) provided basic information about the biochemistry of the myrosinase–glucosinolate system. A proposed pathway for the recyclization of sulfur (and N) under conditions of severe sulfur deficiency is described by Schnug and Haneklaus (53).

The degradation of glucosinolates results in the so-called mustard oils, which are responsible for smell, taste, and biological effect. Glucosinolates are vacuolar defense compounds (95) of qualitative value (96) and are effective against generalist insects at low tissue concentrations (97). Isothiocyanates, the breakdown products after enzymatic cleavage of glucosinolates, may retard multiplication of spores but do not hamper growth of fungal mycelium (98), and fungi may overcome the glucosinolate–myrosinase system efficiently (99,100).

The influence of the sulfur nutritional status on the content of glucosinolates and other sulfur-containing secondary metabolites, which are related to nutritional and pharmaceutical quality, is shown in Table 7.4.

Generally, nitrogen fertilization reduces the glucosinolate content (104). However, under field conditions the effect of nitrogen fertilization on glucosinolate content varies substantially between seasons (105). Schnug (103) noted a distinct interaction between nitrogen and sulfur fertilization when nitrogen was supplied insufficiently, whereby the alkenyl, but not the indole, glucosinolate content in seeds of rape increased at higher nitrogen and sulfur rates. Kim et al. (106) also showed that nitrogen fertilization increased the alkenyl-glucosinolates, gluconapin, and glucobrassicanapin in particular, in rape.

More than 80% of the total sulfur in *Allium* species is present in secondary compounds. *Allium* species contain four *S*-alk(en)yl-L-cysteine sulfoxides, namely *S*-1-propenyl-, *S*-2-propenyl-,

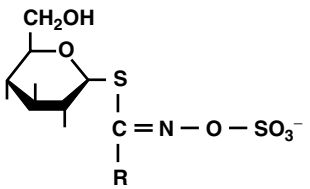

FIGURE 7.4 Basic structure of glucosinolates. (From Schnug, E., in *Sulfur Nutrition and Sulfur Assimilation in Higher Plants*, SPB Academic Publishing, The Hague, 1990, pp. 97–106.)

TABLE 7.4
Influence of Sulfur Fertilization on the Concentration of Sulfur-Containing Secondary Metabolites in Vegetative and Generative Tissues of Different Crops

Crop	Plant Part	S Metabolite	Influence of S Supply on Secondary Compound	Reference
Garlic	Leaves	Alliin	2.4 μmol g^{-1} (d.w.) per 10 mg S[a]	101
	Bulbs	Alliin	0.7 μmol g^{-1} (d.w.) per 10 mg S[a]	101
Mustard	Seeds	Glucosinolates	0.7 μmol g^{-1} per 10 kg S[b]	102
Nasturtium	Whole plant	Glucotropaeolin	3.4 μmol g^{-1} (d.w.) per 10 kg S[c]	89
	Leaves		4.3 μmol g^{-1} (d.w.) per 10 kg S[c]	89
	Stems		1.1 μmol g^{-1} (d.w.) per 10 kg S[c]	89
	Seeds		2.3 μmol g^{-1} per 10 kg S[c]	89
Oilseed rape	Leaves	Glucosinolates	0.04–1.5 μmol g^{-1} (d.w.) per 10 kg S[d]	63
	Seeds	Glucosinolates	0.3–0.6 μmol g^{-1} per 10 kg S[d]	63
			2.1 μmol g^{-1} per 10 kg S[e]	
			0.8 μmol g^{-1} per 10 kg S[f]	103
Onion	Leaves	(Iso)alliin	0.7 μmol g^{-1} (d.w.) per 10 mg S[a]	101
	Bulbs		0.4 μmol g^{-1} (d.w.) per 10 mg S[a]	101

[a]Maximum dose = 250 mg pot^{-1} S and 500 mg pot^{-1} N.
[b]Maximum dose = 185 kg ha^{-1} S.
[c]Maximum dose = 50 kg ha^{-1} S.
[d]Maximum dose = 100 and 150 kg ha^{-1} S.
[e]Severe S deficiency.
[f]Moderate S deficiency.

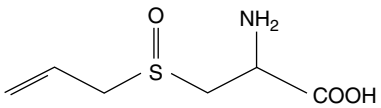

FIGURE 7.5 Chemical structure of alliin. (From Watzl, B., *Bioaktive Substanzen in Lebensmitteln*, Hippokrates Verlag, Stuttgart, Germany, 1999.)

S-methyl- and *S*-propyl-L-cysteine sulfoxides (107). Iso-alliin is the main form in onions, whereas alliin is the predominant form in garlic (108) (Figure 7.5). Alliins supposedly contribute to the defense of plants against pests and diseases. In vitro and in vivo experiments revealed a bactericidal effect against various plant pathogens (109).

The characteristic flavor of *Allium* species is caused after the enzyme alliinase hydrolyzes cysteine sulfoxides to form pyruvate, ammonia, and sulfur-containing volatiles. In the intact cell, alliin and related cysteine sulfoxides are located in the cytoplasm, whereas the C-S lyase enzyme alliinase is localized in the vacuole (110). Disruption of the cell releases the enzyme, which causes subsequent α,β-elimination of the sulfoxides, ultimately giving rise to volatile and odorous LMW organosulfur compounds (111). The cysteine sulfoxide content of *Allium* species is an important quality parameter with regard to sensory features, since it determines the taste and sharpness.

Alliin acts as an antioxidant by activating glutathione enzymes and is regarded as having an anticarcinogenic and antimicrobial effect (86). On average, 21% of sulfur, but only 0.9% of nitrogen, are present as (iso)alliin in onion bulbs at the start of bulb growth (101). The ratio between protein-S and sulfur in secondary metabolites of the *Allium* species is, at between 1:4 and 1:6, much wider than in members of the *Brassica* family (between 1:0.3 and 1:2). The reason for this

difference is supposedly the fact that glucosinolates may be reutilized under conditions of sulfur deficiency whereas alliins are inert end products. Interactions between nitrogen and sulfur supply exist in such a way that nitrogen and sulfur fertilization has been shown to decrease total sulfur and nitrogen concentration, respectively, in onion (101).

7.2.4 Interactions between Sulfur and Other Minerals

Interactions between sulfur and other minerals may significantly influence crop quality parameters (5,113,114). Sulfur and nitrogen show strong interactions in their nutritional effects on crop growth and quality due to their mutual occurrence in amino acids and proteins (see Section 7.2.3). Further examples of nitrogen–sulfur interactions that are not mentioned in previous sections of this chapter are shown below.

7.2.4.1 Nitrogen–Sulfur Interactions

Under conditions of sulfur starvation, sulfur deficiency symptoms are expressed moderately at low nitrogen levels but extremely with a high nitrogen supply. This effect explains the enhancement of sulfur deficiency symptoms in the field after nitrogen dressings (114). The question of why sulfur deficiency symptoms are more pronounced at high nitrogen levels is, however, still unanswered. For experimentation, these results are relevant as the adjustment of the nitrogen and sulfur nutritional status of plants is essential before any hypothesis on the effect of a nitrogen or sulfur treatment on plant parameters can be stated or proved.

The use of the nitrogen/sulfur ratio as a diagnostic criterion is problematic because the same ratio can be obtained at totally different concentration levels in the tissue. Surplus of one element may therefore be interpreted falsely as a deficiency of the other (see Section 7.3.1.3). Clear relationships between nitrogen/sulfur ratios and yield occur only in ranges of extreme ratios. Such ratios may be produced in pot trials but do not occur under field conditions. The effect of increasing nitrogen and sulfur supply on crop seed yield with increasing nitrogen supply is more pronounced with protein than with carbohydrate crops (Table 7.5).

TABLE 7.5
Seed Yield of Single (NIKLAS) and Double Low (TOPAS) Oilseed Rape Varieties in Relation to the Nitrogen and Sulfur Supply in a Glasshouse Experiment

	Seed Yield (g pot^{-1})			
	500 mg N		1000 mg N	
	NIKLAS	TOPAS	NIKLAS	TOPAS
Control	0 a	0 a	0 a	0 a
25 mg S	2.10 b	0.9 b	0 a	0 a
50 mg S	3.15 c	2.85 c	1.25 b	0.35 b
75 mg S	2.55 b	2.65 c	5.30 c	5.85 c
100 mg S	3.05 c	2.50 c	6.70 d	7.50 d

Note: Different characters after figures indicate statistically significant differences of means by Duncan's Multiple Range Test.

Source: From Schnug, E., Quantitative und Qualitative Aspekte der Diagnose und Therapie der Schwefelversorgung von Raps (*Brassica napus* L.) unter besonderer Berücksichtigung glucosinolatarmer Sorten. Habilitationsschrift, D.Sc. thesis, Kiel University, 1988.

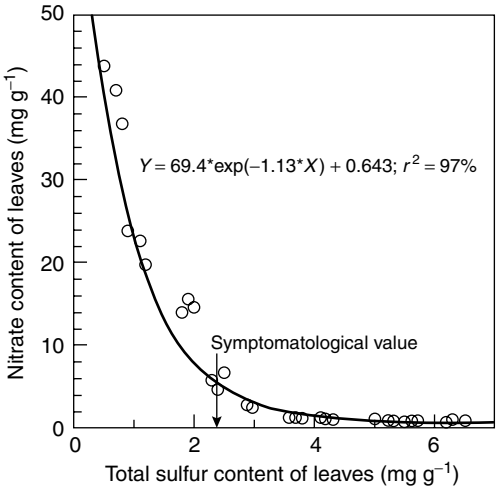

FIGURE 7.6 Nitrate concentrations in the dry matter of lettuce in relation to the sulfur nutritional status of the plants. (From Schnug, E., in *Sulphur Metabolism in Higher Plants: Molecular, Ecophysiological and Nutritional Aspects*, Backhuys Publishers, Leiden, 1997, pp. 109–130.)

Changes in the nitrogen supply affect the sulfur demand of plants and vice versa. Under conditions of sulfur deficiency, the utilization of nitrogen will be reduced and consequently nonprotein nitrogen compounds, including nitrate, accumulate in the plant tissue (Figure 7.6) (5,112).

The antagonistic relationship between sulfur supply and nitrate content exists in the range of severe sulfur deficiency, when macroscopic symptoms are visible. The higher the nitrogen level in the plants, the stronger the effect on the nitrate content will be. Thus, an adequate sulfur supply is vital for minimizing undesired enrichment with nitrate.

Photosynthesis and growth of pecan (*Carya illinoinensis* Koch) increased with N supply in relation to the nitrogen/sulfur ratio in pecan leaves (115). Both parameters were, however, reduced when combined leaf nitrogen and sulfur concentrations of <35 mg g^{-1} nitrogen and 3.7 mg g^{-1} sulfur were noted (115).

The initial supply of a crop with nitrogen and sulfur is decisive for its influence on the glucosinolate content, probably due to physiological or root-morphological reasons (103). Nitrogen fertilization to oilseed rape insufficiently supplied with nitrogen and sulfur will lead to decreasing glucosinolate concentrations because the demand of an increasing sink due to increasing numbers of seeds will not be met by the limited sulfur source. Only if the rooting depth or density is enhanced by the nitrogen supply, which increases the plant-available sulfur pool in the soil, does the glucosinolate content increase too. Higher glucosinolate concentrations in seeds can also be expected after nitrogen applications to crops with a demand for nitrogen but adequate sulfur supply due to the increased biosynthesis of sulfur-containing amino acids, which are precursors of glucosinolates. In the case of a crop already sufficiently supplied with nitrogen, there is no evidence for any specific nitrogen–sulfur interactions on the glucosinolate content (5,116).

In general, no significant influence of nitrogen fertilization on the alliin content has been found for onions (*Allium cepa* L.) and garlic (*Allium sativum* L.), but there is a tendency that a higher nitrogen supply results in a decreased alliin content (101). In comparison, an increasing sulfur supply has been related to an increasing alliin content in leaves and bulbs of both crops. There were also interactions between nitrogen and sulfur in such a way that the total sulfur content of onion leaves was correlated highly with nitrogen fertilization: the sulfur concentration of leaves decreased with increasing N fertilization, and the total nitrogen concentration of onion bulbs decreased with increasing sulfur fertilization. The same observations were made by Freeman and Mossadeghi (117) for garlic plants, where the nitrogen concentration decreased from 4.05 to 2.93% with sulfur fertilization,

and by Randle et al. (118), who reported decreasing total bulb sulfur concentrations in response to increasing nitrogen fertilization.

7.2.4.2 Interactions between Sulfur and Micronutrients

Owing to antagonistic effects, sulfur fertilization reduces the uptake of boron and molybdenum. In soils with a marginal plant-available concentration of these two plant nutrients, sulfur fertilization may induce boron or molybdenum deficiency, particularly on coarse-textured sites where brassica crops are grown intensely in the crop rotation (119). In comparison, sulfur fertilization is an efficient tool to reduce the selenium, molybdenum, arsenic, bromine, and antimony uptake on contaminated sites. The influence of elemental sulfur applications on the concentration of trace elements of fully developed leaves of nasturtium (*Tropaeolum majus* L.) was tested on two sites in northern Germany (120). The results of this study reveal a significantly increased uptake of copper, manganese, cobalt, nickel, and cadmium, with increasing levels of sulfur. This increased uptake was caused by a higher availability of these elements due to the acidifying effect of elemental sulfur. At the same time, antagonistic effects were noted for arsenic, boron, selenium, and molybdenum in relation to the soil type.

The enzyme sulfite oxidase is a molybdo-enzyme, which converts sulfite into sulfate (121) and is thus important for sulfate reduction and assimilation in plants (see Figure 7.1). Stout and Meagher (122) have shown that the sulfate supply influences molybdenum uptake. Sulfate–molybdate antagonism can be observed at the soil–root interface and within the plant, as an increasing sulfur supply results in lower molybdenum concentrations in the tissues (123). The significance of sulfate–molybdate antagonism in agriculture is described comprehensively by Macleod et al. (124).

Selenium, like molybdenum, is chemically similar to sulfur. Comprehensive reviews about interactions between sulfate transporters and sulfur assimilation enzymes, and selenium–molybdenum uptake and metabolism, are given by Terry et al. (125) and Kaiser et al. (126). Accumulation of glutathione due to elevated levels of sulfate in the soil and SO_2/H_2S in the air was reduced drastically in spinach (*Spinacia oleracea* L.) leaf discs by selenate amendments (127). In those studies the uptake of sulfur was not influenced by the selenate treatment. Bosma et al. (128) suggested that selenate decreases sulfate reduction due to antagonistic effects during plant uptake, in combination with a rapid turnover of glutathione. An increasing sulfate supply gives higher sulfate concentrations in the plant tissue, so that the competition between sulfur and selenium for the enzymes of the sulfur assimilation pathway will finally result in less synthesis of selenoamino acids (129).

This antagonistic effect is of no practical significance on seleniferous soils, but it could be relevant on deficient and marginal sites (130). Field experiments with combined sulfur and selenium applications to grass-clover pastures, on selenium-deficient and high-selenium sites revealed that selenium concentrations in the different botanical species showed distinct differences in relation to the site (130).

On the high-selenium site, sulfur fertilization significantly decreased the selenium concentration in pasture. Spencer (130) attributed this action to a dilution effect, as the total selenium content remained constant. Studies on the pungency of onion bulbs in relation to the sulfur supply revealed that although sulfur content was increased at elevated selenium levels, the pungency was reduced (131). Kopsell and Randell (131) proposed that selenium had an impact on the biosynthetic pathway of flavor precursors.

A synergistic effect of sulfur and selenium on the shoot sulfur concentration was noted for hydroponically grown barley (*Hordeum vulgare* L.) and rice (*Oryza sativa* L.). With increasing selenium concentrations in the solution, a steep increase in the sulfur concentration of the shoots occurred even with a low sulfur supply (132).

Sulfur and phosphorus interactions in plants are closely related to plant species, because of the different root morphologies and nutrient demands of different species (133). A synergistic effect of sulfur and phosphorus on crop yield occurred for sorghum (*Sorghum vulgare* Pers.), maize (*Zea mays* L.), wheat (*Triticum aestivum* L.), and mustard (*Brassica* spp. L.) (134–137). A synergistic relationship

between sulfur and potassium, which enhances crop productivity and quality, was determined in several studies (138–140).

7.3 SULFUR IN PLANT NUTRITION

7.3.1 Diagnosis of Sulfur Nutritional Status

7.3.1.1 Symptomatology of Single Plants

Visual diagnosis of sulfur deficiency in production fields requires adequate expertise and needs to involve soil or plant analysis (141). The literature describes symptoms of sulfur deficiency as being less specific and more difficult to identify than other nutrient deficiency symptoms (142–145). The symptomatology of sulfur deficiency is very complex and shows some very unique features. In this section, the basic differences in sulfur deficiency symptoms of species in the Gramineae representative of monocotyledonous, and species in the Cruciferae and Chenopodiaceae representative of dicotyledonous crops will be given for individual plants and on a field scale.

When grown side by side and under conditions of sulfur starvation, crops begin to develop sulfur deficiency symptoms in the order of oilseed rape (canola), followed by potato, sugar beet (*Beta vulgaris* L.), beans (*Phaseolus vulgaris* L.), peas (*Pisum sativum* L.), cereals, and finally maize. The total sulfur concentration in tissue corresponding to the first appearance of deficiency symptoms is highest in oilseed rape (3.5 mg g^{-1} S), and lowest in the Gramineae (1.2 mg g^{-1} S). Potato and sugar beet show symptoms at higher concentrations (2.1 to 1.7 mg g^{-1} S) than beans or peas (1 to 1.2 mg g^{-1} S).

Brassica species, such as oilseed rape, develop the most distinctive expression of symptoms of any crop deficient in sulfur. The symptoms are very specific and thus are a reliable guide to sulfur deficiency. There is no difference in the symptomatology of sulfur deficiency in high and low glucosinolate-containing varieties (103). The symptomatology of sulfur deficiency in brassica crops is characteristic during the whole vegetation period and is described below for specific growth stages according to the BBCH scale (146). Symptoms generally apply to dicotyledonous plants, except when specific variations are mentioned in the text. Colored guides of sulfur deficiency symptoms are provided by Bergmann (143) and Schnug and Haneklaus (53,114,147).

Even before winter, during the early growth of oilseed rape, leaves may start to develop visible symptoms of sulfur deficiency. As sulfur is fairly immobile within the plant (13), symptoms always show up in the youngest leaves. Though the plants are still small, symptoms can cover the entire plant. Sulfur fertilization before or at sowing will ensure a sufficient sulfur supply, particularly on light, sandy soils, and will promote the natural resistance of plants against fungal diseases (148).

Oilseed rape plants suffering from severe sulfur deficiency show a characteristic marbling of the leaves. Leaves begin to develop chlorosis (149–154), which starts from one edge of the leaves and spreads over intercostal areas; however, the zones along the veins always remain green (103,155). The reason for the green areas around the veins is most likely the reduced intercellular space in that part of the leaf tissue, resulting in shorter transport distances and a more effective transport of sulfate. Sulfur-deficient potato leaves show the same typical color pattern and veining as oilseed rape, whereas sugar beet, peas, and beans simply begin to develop chlorosis evenly spread over the leaf without any veining (156,157). A comparative evaluation of crop-specific, severe sulfur deficiency symptoms is given in Figure 7.7.

Chlorosis very rarely turns into necrosis (103,157) as it does with nitrogen and magnesium deficiencies, and is an important criterion for differential diagnosis. Even under conditions of extreme sulfur deficiency, an oilseed rape plant will not wither. The intensity of sulfur deficiency symptoms of leaves depends on the nitrogen supply of the plants (see Section 7.2.4.1). In general, a high nitrogen supply promotes the expression of sulfur deficiency symptoms and vice versa (158).

FIGURE 7.7 Macroscopic sulfur deficiency symptoms of oil seed rape (*Brassica napus* L.), cereals, and sugar beet (*Beta vulgaris* L.) at stem extension and row closing, respectively (from left to right). (For a color presentation of this figure, see the accompanying compact disc.)

FIGURE 7.8 Marbling, spoon-like leaf deformations and anthocyanin enrichments of sulfur-deficient oilseed rape plants (*Brassica napus* L.) (from left to right). (For a color presentation of this figure, see the accompanying compact disc.)

A characteristic secondary symptom of severe sulfur deficiency is a reddish-purple color due to the enrichment of anthocyanins in the chlorotic parts of brassica leaves (Figure 7.8). Under field conditions, the formation of anthocyanins starts 4 to 7 days after chlorosis. The phenomenon is initialized by the enrichment of carbohydrates in the cells after the inhibition of protein metabolism. Plants detoxify the accumulated carbohydrates as anthocyanates, which result from the reaction with cell-borne flavonols to avoid physiological disorders (159–165). Many other nutrient deficiencies are also accompanied by formation of anthocyanins, which therefore is a less specific indicator for sulfur deficiency.

In particular, leaves which are not fully expanded produce spoon-like deformations when struck by sulfur deficiency (Figure 7.8). 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 sulfur-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 sulfur deficiency. Marbling, deformations, and anthocyanin accumulation can be detected up to the most recently developed small leaves inserted in forks of branches (Figure 7.8).

FIGURE 7.9 White flowering (left) and morphological changes of petals (right) of sulfur-deficient oilseed rape (*Brassica napus* L.). (For a color presentation of this figure, see the accompanying compact disc.)

The higher succulence of sulfur-deficient plants (143,166) was suspected to be caused by enhanced chloride uptake due to an insufficient sulfate supply (159). However, with an increase of chloride concentrations by 0.4 mg Cl g^{-1} on account of a decrease of sulfur concentrations by 1 mg g^{-1} in leaves, this effect seems to be too small to justify the hypothesis (103). More likely, the above-explained mechanical effects of distortion, together with cell wall thickening, cause the appearance of increased succulence due to the accumulation of starch and hemicellulose (167).

During flowering of oilseed rape, sulfur deficiency causes one of the most impressive symptoms of nutrient deficiency: the 'white blooming' of oilseed rape (Figure 7.9). The white color presumably develops from an overload of carbohydrates in the cells of the petals caused by disorders in protein metabolism, which finally ends up in the formation of colorless leuco-anthocyanins (168). As with anthocyanins in leaves, the symptoms develop most strongly during periods of high photosynthetic activity. Beside the remarkable modification in color, size, and shape of oilseed rape, the petals change too (Figure 7.9). The petals of sulfur-deficient oilseed rape flowers are smaller and oval shaped, compared with the larger and rounder shape of plants without sulfur-deficiency symptoms (169). The degree of morphological changes, form, and color, are reinforced by the strength and duration of severe sulfur deficiency (53). The fertility of flowers of sulfur-deficient oilseed rape plants is not inhibited. However, the ability to attract honeybees may be diminished and can be of great importance for the yield of nonrestored hybrids, which need pollination by insect vectors (169).

The strongest yield component affected by sulfur deficiency in oilseed rape is the number of seeds per pod, which is significantly reduced (103). 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 (Figure 7.10). Extremely low numbers of seeds per pod, in some cases even seedless 'rubber pods,' are characteristic symptoms of extreme sulfur deficiency (Figure 7.10).

7.3.1.2 Symptomatology of Monocots

The symptoms in gramineous crops such as cereals and corn are less specific than in cruciferous crops. In early growth stages, plants remain smaller and stunted and show a lighter color than plants without symptoms (170). The general chlorosis is often accompanied by light green stripes along the veins (Figure 7.11) (170–172). Leaves become narrower and shorter than normal (173).

There is no morphological deformation to observe, and usually no accumulation of anthocyanins either. Although the symptoms are very unspecific and are easily mistaken for symptoms of nitrogen deficiency, their specific pattern in fields provides good evidence for sulfur deficiency. Owing to an

FIGURE 7.10 Enrichment of anthocyanins during ripening of oilseed rape (*Brassica napus* L.) (left) and reduction of number of seeds per pod (right). (For a color presentation of this figure, see the accompanying compact disc.)

FIGURE 7.11 Macroscopic sulfur deficiency symptoms of winter wheat (*Triticum aestivum* L.) at stem extension. (For a color presentation of this figure, see the accompanying compact disc.)

early reduction of fertile flowers per head, sulfur-deficient cereals are characterized by a reduced number of kernels per head, which alone, however, is not conclusive evidence for sulfur deficiency (174).

7.3.1.3 Sulfur Deficiency Symptoms on a Field Scale

Some characteristic features in the appearance of fields can provide early evidence of sulfur deficiency. Sulfur deficiency develops first on the light-textured sections of a field. From above, these areas appear in an early oilseed rape crop as irregularly shaped plots with a lighter green color

FIGURE 7.12 Chlorotic patches in a field (left) and resultant effects on mature plants (right), indicating severe sulfur deficiency symptoms in relation to soil characteristics. (For a color presentation of this figure, see the accompanying compact disc.)

(wash outs). The irregular shape distinguishes the phenomenon from the regular shape of areas caused by nitrogen deficiency, which usually originates from inaccurate fertilizer application (Figure 7.12). Owing to frequent soil compaction and limited root growth, sulfur deficiency develops first along the headlands and tramlines or otherwise compacted areas of a field.

The appearance of sulfur-deficient oilseed rape fields is more obvious at the beginning of blooming; white flowers of oilseed rape are distinctively smaller and therefore much more of the green undercover of the crop shines through the canopy of the crop. Another very characteristic indicator of a sulfur-deficient site is the so-called second flowering of the oilseed rape crop. Even if a sulfur-deficient crop has finished flowering, it may come back to full bloom if sufficient sulfur is supplied. The typical situation for this action comes when a wet and rainy spring season up until the end of blooming is followed suddenly by warm and dry weather. During the wet period precipitation, water, which has only one-hundredth to one-tenth the sulfur concentrations of the entire soil solution, dilutes or leaches the sulfate from the rooting area of the plants, so that finally plants are under the condition of sulfur starvation. With the beginning of warmer weather, evaporation increases and sulfur-rich subsoil water becomes available to the plants and causes the second flowering of the crop. During maturity, sulfur deficiency in oilseed rape crops is revealed by a sparse, upright-standing crop.

Similarly, in cereals, sulfur deficiency develops first on light-textured parts of the field, yielding irregularly shaped 'wash-out' areas in images from above. Nitrogen fertilization promotes the expression of these irregularly distributed deficiency symptoms, such as uneven height and color. The irregular shape distinguishes these symptoms from areas caused by faulty nitrogen fertilizer application. In the field, these particular zones can be identified by a green yellowish glow in the backlight before sunset. Later, vegetation in these areas resembles a crop that is affected by drought. Owing to an inferior natural resistance (see also Section 7.5.2), the heads in sulfur-deficient areas can be infected more severely by fungal disease (e.g., *Septoria* species), which gives these areas a darker color as the crop matures.

7.4 SOIL ANALYSIS

A close relationship between the plant-available sulfur content of the soil and yield is a prerequisite for a reliable soil method. Such a significant correlation was verified in pot trials under controlled growth conditions (103,175–178). Several investigations have shown, however, that the relationship between inorganic soil sulfate and crop yield is only weak, or even nonexistent, under field conditions (103,179–181). Such missing or poor correlations are the major reason for the large number of different methods of soil testing, and they justify ongoing research for new methods (114,182–185). Soil analytical methods for plant-available sulfate differ in the preparation of the soil samples, concentration and type of extractant, duration of the extraction procedure, the soil-to-extractant ratio, the

conditions of extraction, and the method that is used for the determination of sulfur or sulfate-S in the extract. A serious problem with regard to all laboratory methods is the treatment and preservation of soil samples prior to analysis. Increased temperature and aeration of the sample during storage increase the amount of extractable sulfur by oxidizing labile organic sulfur fractions, and occasionally mobilize reduced inorganic sulfur (186–188).

Besides water, potassium or calcium dihydrogenphosphate solutions are the most commonly used solvents to extract plant-available sulfate from soils (189,190). Soils with a high sulfate adsorption capacity are low in pH, so that phosphate-containing extractants extract more sulfate than other salt solutions because of ion-exchange processes. Sodium chloride is also used in countries where soils are frequently analyzed for available nitrate (183,191,192). Less frequently, magnesium chloride (193) or acetate solutions are employed (194,195). Other methodical approaches involve, for instance, anion-exchange resins (196,197) and perfusion systems (198).

In aerated agricultural soils, the organic matter is the soil-inherent storage and backup for buffering sulfate in the soil solution (199–201), and methods are described which focus on capturing organic sulfur fractions that might be mineralized during the vegetation period and thus contribute to the sulfate pool in soils (183,202–204). Such special treatments are, for example, the heating of the samples or employing alkaline conditions or incubation studies, which allow the measurement of either the easily mineralized organic sulfur pool or the rapidly mineralized organic sulfur. Most methods, however, extract easily soluble, plant-available sulfate.

The practical detection limit of sulfur determined by ICP-AES was 0.5 mg S L^{-1}, corresponding to 3.3 mg S kg^{-1} (205) in the soil. On sulfur-deficient sites, however, sulfate-S concentrations of only 2 mg S kg^{-1} were measured regularly in the topsoil by ion chromatography (206). Ion chromatography is much more sensitive, with a practical detection limit of 0.1 mg SO_4-S L^{-1} (corresponding to 0.67 mg S kg^{-1}), allowing sulfate-S to be determined at low concentrations in soils. Additionally, this fact explains why soil sulfate-S measured by ICP-AES is usually below the detection limit. No matter which method is applied, and on which soils or crops the method is used, there is an astonishing agreement in the literature for approximately 10 mg SO_4-S kg^{-1} as the critical value for available sulfur in soils (68,192,207). With the most common methods for the determination of sulfur (ICP and the formation of $BaSO_4$), values of < 10 mg S kg^{-1} will identify a sulfur-deficient soil with a high probability.

As expected, comparisons of different extractants and methods revealed that under the same conditions, all of these methods extract more or less the same amount of sulfate from the soil (178,182,183,185,198,203,207–209). Occasionally observed differences among methods were more likely to be caused by interferences due to the extractant itself (183) rather than by the method of sulfate-S determination (186,187).

As there is virtually no physicochemical interaction between the soil matrix and sulfate, the amount that is present and extractable from the soil is the main indicator commonly used to describe the sulfur nutritional status of a soil. Opinions in the literature on whether or not soil testing is a suitable tool for determining the sulfur status of soils vary from high acceptance (210–215) down to full denial (179,216–220).

Conclusions leading to high acceptance were always drawn from pot trials, which usually yield high correlation coefficients between soil analytical data, and give sulfur content or sulfur uptake of plants as the target value (103,178,183,185,192,194,198,212,221–223,225). Pot trials are always prone to deliver very high correlations between soil, and plant data or yield, as there is no uncontrolled nutrient influx and efflux. However, in the case of field surveys involving a greater range of sites and environmental factors, correlations are poor or fail to reach significance (103,180). For the relationship between available sulfur in soils and foliar sulfur, larger surveys employing a wide range of available sulfur in soils (5 to 250 mg S kg^{-1}), and plants (0.8 to 2.1 g S kg^{-1}), reported correlation coefficients for a total of 1701 wheat and 1870 corn samples of $r = 0.292$ ($P \leq 0.001$) and $r = 0.398$ ($P \leq 0.001$), respectively (195). Timmermann and coworkers (225) determined a correlation coefficient of $r = 0.396$ ($P < 0.05$) for 93 oilseed rape samples. In the field surveys conducted

by Schnug (103), a significant relationship could not be verified for 489 oilseed rape samples ($r = 0.102$, $P > 0.05$) or for 398 cereal samples ($r = 0.098$, $P > 0.05$).

These results imply that a maximum of 16% of the variability of the sulfur concentrations in leaves can be explained by the variability of available sulfur in soils. However, Timmermann et al. (225) were able to improve the relationship between soil and plant data by using the ratio of available sulfur and nitrogen in soils (N_{min}/S_{min}) instead of just sulfur. This application gave a value of $r = -0.605$ ($P \leq 0.01$), which still explains less than one third of the variability.

The key problem of soil analysis for plant-available sulfur is that it is a static procedure that aims at reflecting the dynamic transfer of nutrient species among different chemical and biological pools in the soil. This concept is appropriate if the sample covers the total soil volume to which active plant roots have access and if no significant vertical and lateral nutrient fluxes occur to and from this specific volume. Sulfate, however, has an enormously high mobility in soils and can be delivered from sources such as subsoil or shallow groundwater, and sulfur has virtually no buffer fraction in the soil. Thus, the availability of sulfate is a question of the transfer among pools in terms of space and time rather than among biological or chemical reserves. Under field conditions sulfate moves easily in or out of the root zones so that close correlations with the plant sulfur status can hardly be expected. Attempts have been made to take subsoil sulfate into account by increasing the sampling depth (103,226–230), but the rapid vertical and lateral mobility of sulfate influences subsoils too. Thus, this procedure did not yield an improvement of the expressiveness of soil analytical data (103,225).

The soil sulfur cycle is driven by biological and physicochemical processes which affect flora and fauna. The variability of sulfate-S contents in the soil over short distances is caused by the high mobility of sulfate-S. Sulfate is an easily soluble anion, and it follows soil water movements. Significant amounts of adsorbed sulfate are found only in clay and sesquioxide-rich soil horizons with pH values < 5, which is far below the usual pH of northern European agricultural soils. Seasonal variations in mineralization, leaching, capillary rise, and plant uptake cause temporal variations in the sulfate-S content of the soil (205). The high spatiotemporal variation of sulfate in soils is the reason for the inadequacy of soil analysis in predicting the nutritional status of sulfur in soils. Thus, under humid conditions, the sulfur status of an agricultural site is difficult to assess (231). An overview of the factors of time and soil depth in relation to the variability of sulfate-S contents is given in Figure 7.13. The highest variability of sulfate-S could be observed on two sites in soil samples collected in April (Figure 7.13). On a sandy soil, the variability was distinctly higher at the second and third dates of sampling in comparison with a loamy soil, but time-dependent changes were significant only in the deeper soil layers. Though the range of sulfate-S contents measured was smaller on the loamy soil than on the sandy soil, the differences proved to be significant in all soil layers between the first and third and second and third dates of sampling respectively (Figure 7.13).

Sources and sinks commonly included in a sulfur balance are inputs by depositions from atmosphere, fertilizers, plant residues, and mineralization, and outputs by losses due to leaching. A frequent problem when establishing such simple sulfur balances is that the budget does not correspond to the actual sulfur supply. The reason is that under temperate conditions it is the spatiotemporal variation of hydrological soil properties that controls the plant-available sulfate-S content. A more promising way to give a prognosis of the sulfur supply is a site-specific sulfur budget, which includes information about geomorphology, texture, climatic data, and crop type and characteristics of the local soil water regime (Figure 7.14).

The results presented in Figure 7.14 reveal that plant sulfur status is distinctly higher on sites with access to groundwater than on sandy soils not influenced by groundwater. The significance of plant-available soil water as a source and storage for sulfur has been disregarded or underestimated so far. However, especially under humid growth conditions, plant-available soil water is the largest contributor to the sulfur balance (205). Leaching and import from subsoil or shallow groundwater sources (184,205) can change the amount of plant-available sulfate within a very short time. Groundwater is a large pool for sulfur, because sulfur concentrations of 5 to 100 mg S L^{-1} are common

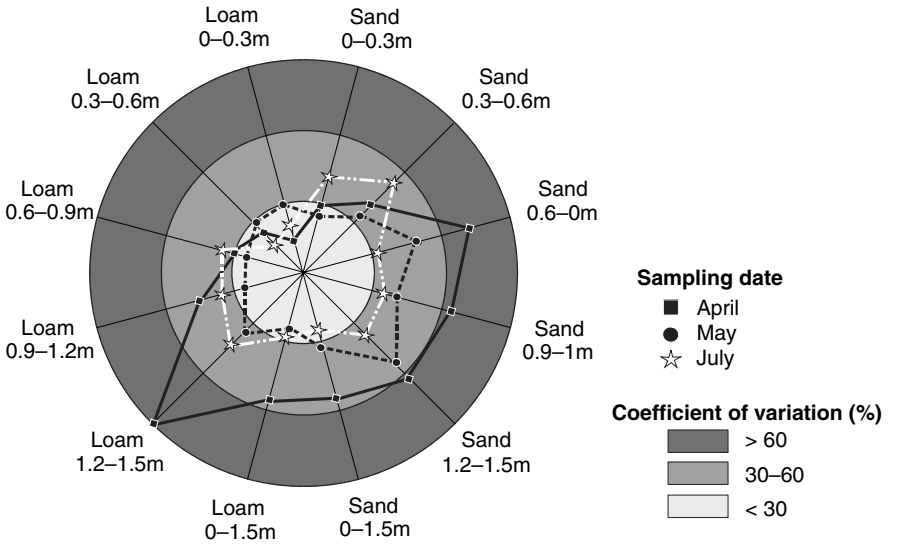

FIGURE 7.13 Spatiotemporal variability of the sulfate contents of different soil layers in two soil types. (From Bloem, E. et al., *Commun. Soil Sci. Plant Anal*., 32, 1391–1403, 2001.)

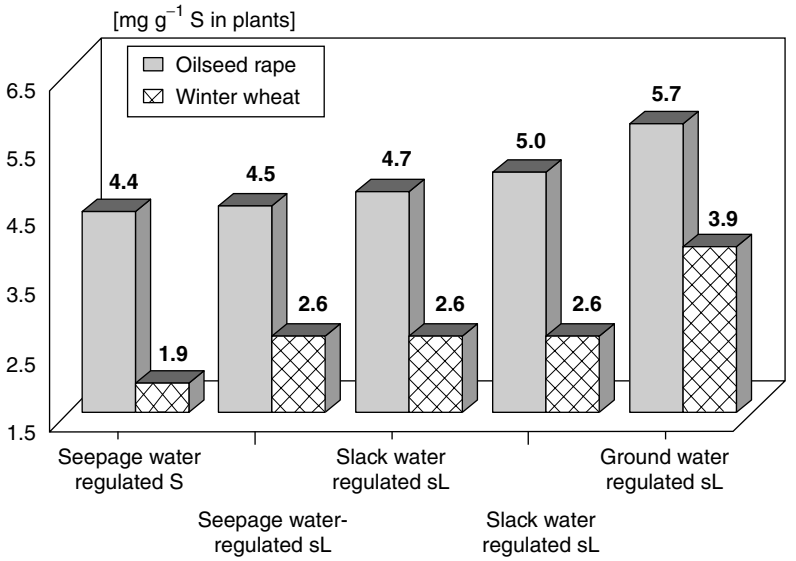

FIGURE 7.14 Total sulfur content of young leaves of oilseed rape and total aboveground material of winter wheat at stem extension in relation to soil hydrological parameters and soil texture (S=Sand; sL=sandy Loam) on the Isle of Ruegen. (From Bloem, E., Schwefel-Bilanz von Agraroekosystemen unter besonderer Beruecksichtigung hydrologischer und bodenphysikalischer Standorteigenschaften, Ph.D. thesis, TU-Braunschweig, Germany, 1998.)

in surfaces near groundwater (205,232). There are three ways in which groundwater contributes to the sulfur nutrition of plants. First, there is a direct sulfur input if the groundwater level is only 1 to 2 m below the surface, which is sufficient to cover the sulfur requirement of most crops as plants can utilize the sulfate in the groundwater directly by their root systems. Second, groundwater, which is used for irrigation, can supply up to 100 kg S ha^{-1} to the crop (205,233–235), but irrigation water will contribute significantly to the sulfur supply only if applied at the start of the main growth period

of the crop. Third, the capillary rise of groundwater under conditions of a water-saturation deficit in the upper soil layers leads to a sulfur input. This process is closely related to climatic conditions. The sulfur supply of a crop increases with the amount of plant-available water or shallow groundwater. The higher the water storage capacity of a soil, the less likely are losses of water and sulfate-S by leaching and the greater is the pool of porous water and also the more likely is an enrichment of sulfate just by subsequent evaporation. Thus, heavy soils have a higher charging capacity for sulfate-S than light ones.

7.5 PLANT ANALYSIS

Plant families and species show great variabilities in sulfur concentrations. In general, gramineous species have lower sulfur levels than dicotyledonous crops (see Section 7.3.2). Within each genus, however, species producing S-containing secondary metabolites accumulate more sulfur than those without this capacity. The ratios of sulfur concentrations in photosynthetically active tissue of cereals, sugar beet, onion, and oilseed rape are approximately 1:1.5:2:3 (114,236). Thus plants with a higher tendency to accumulate sulfur, such as brassica species, are very suitable as monitor crops to evaluate differences between sites and environments, or for quick growing tests (176). Generative material is less suited for diagnostic purposes (237), because the sulfur concentration in seeds is determined much more by genetic factors (43,103,116). During plant growth, morphological changes occur and there is translocation of nutrients within the plant. Thus, changes in the nutrient concentration are not only related to fluctuations in its supply, but also to the plant part and plant age. These factors need to be taken into account when interpreting and comparing results of plant analysis (216,238–243). Basically, noting the time of sampling and analyzed plant part is simply a convention, but there are some practical reasons for it that should be considered: (a) photosynthetically active leaves show the highest sulfur concentrations of all plant organs, and as sulfur has a restricted mobility in plants sulfur concentrations in young tissues will respond first to changes in the sulfur supply; (b) sampling early in the vegetative state of a crop allows more time to correct sulfur deficiency by fertilization. It is relevant in this context that plant analysis is a reliable tool to evaluate the sulfur nutritional status, but usually it is not applicable as a diagnostic tool on production fields because of the shortcomings mentioned above.

In dicotyledonous crops, young, fully expanded leaves are the strongest sinks for sulfur, and they are available during vegetative growth. Therefore, they are preferable for tissue analysis (88,103,244). Oilseed rape, for instance, delivers suitable leaves for tissue analysis until 1 week after flowering, and sugar beet gives suitable leaves until the canopy covers the ground and the storage roots start to extend (103).

For the analysis of gramineous crops, either whole plants (1 cm above the ground) after the appearance of the first and before the appearance of the second node, or flag leaves are best suited for providing samples for analysis (142,143,245–249).

In all cases, care has to be taken to avoid contamination of tissue samples with sulfur from foliar fertilizers or sulfur-containing pesticides. Care is also needed when cleaning samples, because water used for washing may contain significant amounts of sulfate. Paper used for sample drying and storage contains distinct amounts of sulfate, originating from the manufacturing process. As sulfate bound in paper is more or less insoluble, the risk of contamination when washing plants is low, but adherent paper particles may significantly influence the results obtained.

7.5.1 Analytical Methods

Sulfur occurs in plants in different chemical forms (250), and nearly all of them have been tested as indicators for sulfur nutritional status. The parameters analyzed by laboratory methods for the purpose of diagnostics can be divided into three general classes: biological, chemical, and composed parameters.

Biological parameters are the sulfate and glutathione content. Many authors proposed the sulfate-S content as the most suitable diagnostic criterion for the sulfur supply of plants (241,242,251–255). They justify their opinion by referring to the role of sulfate as the major transport and storage form of sulfur in plants (256,257). Other authors, however, attribute this function also to glutathione (55,258,259). Based on this concept, Zhao et al. (260) investigated the glutathione content as a diagnostic parameter for sulfur deficiency.

Although indeed directly depending on the sulfur supply of the plant (64,103), neither of the compounds is a very reliable indicator for the sulfur status because their concentrations are governed by many other parameters, such as the actual physiological activity, the supply of other mineral nutrients, and the influence of biotic and abiotic factors (5,63,256,261). Biotic stress, for instance, increased the glutathione content by 24% (63). Amino acid synthesis is influenced by the deficiency of any nutrient and thus may indirectly cause an increase in sulfate or glutathione in the tissue. An example for this action is the increase in sulfate following nitrogen deficiency (103,262,263). Significant amounts of sulfate may also be physically immobilized in vacuoles (see Section 7.2.1).

In plant species synthesizing glucosinolates, sulfate concentrations can also be increased by the release of sulfate during the enzymatic cleavage of these compounds after sampling (103). As enzymatically released sulfate can amount to the total physiological level required, this type of post-sampling interference can be a significant source of error, yielding up to 10% higher sulfate concentrations (63,103). It is probably also the reason for some extraordinarily high critical values for sulfate concentrations reported for brassica species (220,264). The preference for sulfate analysis as a diagnostic criterion may also come from its easier analytical determination compared to any other sulfur compound or to the total sulfur concentration (265).

Hydrogen iodide (HI)-reducible S, acid-soluble sulfur, and total sulfur are chemical parameters used to describe the sulfur status of plants. None of them is related to a single physiological sulfur-containing compound. The HI-reducible sulfur or acid-soluble sulfur estimate approximately the same amount of the total sulfur in plant tissue (~50%). The acid-soluble sulfur is the sulfur extracted from plant tissue by a mixture of acetic, phosphoric, and hydrochloric acids according to Sinclair (167), who described this extractant originally for the determination of sulfate. Schnug (103) found in tissue samples from more than 500 field-grown oilseed rape and cereal plants that the acid-soluble sulfur content (y) is very closely correlated with the total sulfur content (x). The slope of the correlations is identical, but the intercept is specific for species with or without S-containing secondary metabolites:

$$\text{oilseed rape: } y = 0.58x - 1.25;\ r = 0.946 \qquad \text{cereals: } y = 0.58x - 0.39;\ r = 0.915$$

As the total sulfur content in Sinclair's (167) solution is easy to analyze by ICP, this extraction method seems to be a promising substitute for wet digestion with concentrated acids or using x-ray fluorescence spectroscopy for total sulfur determination (53,103,266–268).

The total sulfur content is most frequently used for the evaluation of the sulfur nutritional status (see Section 7.5.3). Precision and accuracy of the analytical method employed for the determination of the total sulfur content are crucial. In proficiency tests, X-ray fluorescence spectroscopy proved to be fast and precise (269,270). Critical values for total sulfur differ in relation to the growth stage (242,261), but this problem is also true for all the other parameters and can be overcome only by a strict dedication of critical values to defined plant organs and development stages (103). If this procedure is followed strictly, the total sulfur content of plants has the advantage of being less influenced by short-term physiological changes that easily affect fractions such as sulfate or glutathione.

Composed parameters are the nitrogen/sulfur (N:S) ratio, the percentage of sulfate-S from the total sulfur concentration, and the sulfate/malate ratio. The concept of the N/S ratio is based on the fact that plants require sulfur and nitrogen in proportional quantities for the biosynthesis of amino acids (271–273). Therefore, deviations from the typical N/S ratio were proposed as an indicator for sulfur deficiency (239,274–281). Calculated on the basis of the composition of amino acids in oilseed rape leaf protein, the optimum N/S ratio for this crop should theoretically be 12:1 (103,282), but

empirically maximum yields were achieved at N/S ratios of 6:1 to 8:1 (216,242,253,283). Distinct relationships between N/S ratio and yield occur only in the range of extreme N/S ratios. Such N/S ratios may be produced in pot trials but do not occur under field conditions (see Figure 7.16).

There is no doubt that balanced nutrient ratios in plant tissues are essential for crop productivity, quality, and plant health, but the strongest argument against using the N/S ratio to assess the nutritional status is that it can result from totally different N and sulfur concentrations in the plant tissue. Surplus of one element may therefore falsely be interpreted as a deficiency of the other (284). The suitability of N/S ratios as a diagnostic criterion also implies a constancy (273,285–288), which is at least not true for species with a significant secondary metabolism of S-containing compounds such as *Brassica* and *Allium* species (289,290). Additionally, it requires the determination of two elements and thus is more laborious and costly.

The percentage of sulfate-S of the total sulfur content has been proposed as a diagnostic criterion (240–242,251–255). Except for laboratories operating x-ray fluorescence spectroscopy, which allows the simultaneous determination of sulfate-S and total sulfur (291,292), this determination doubles the analytical efforts without particular benefit. The sulfate/malate ratio is another example of a composed parameter (293). Though both parameters can be analyzed by ion chromatography in one run, the basic objection made with regard to sulfate (see above), namely its high variability, also applies to malate.

7.5.2 Assessment of Critical Nutrient Values

Critical values are indispensable for evaluating the nutritional status of a crop. Important threshold markers are: (a) the symptomatological value, which reflects the sulfur concentration below which deficiency symptoms become visible (see Section 7.3.1); (b) the critical nutrient value, which stands for the sulfur concentration above which the plant is sufficiently supplied with sulfur for achieving the maximum potential yield or yield reduced by 5, 10, or 20% (294); and (c) the toxicological value, which indicates the sulfur concentration above which toxicity symptoms can be observed. However, there is no one exclusive critical nutrient value for any crop, as it depends on the growth conditions, the developmental stage of the plant at sampling, the collected plant part, the determined sulfur species, the targeted yield, and the mathematical approach for calculating it. Smith and Loneragan (295) provided a comprehensive, general overview of the significance of relevant factors influencing the derivation of critical values. Numerous, differing critical sulfur values and ranges exist for each crop and have been compiled, for instance by Reuter and Robinson (294), for all essential plant nutrients and cultivated plants including forest plantations. In this section, an attempt was made to compile and categorize, from the literature, available individual data based on studies with varying experimental conditions of the variables, total sulfur and sulfate concentrations, and N/S ratios in relation to different groups of crops for facilitating an easy and appropriate evaluation of sulfur supply. Plant groups were assembled by morphogenetic and physiological features. Because of the wide heterogeneity of results for similar classes of sulfur supply and for a better comparability of results, concentrations were agglomerated into three major categories: deficient, adequate, and high, irrespective of the sampled plant part during vegetative growth (Table 7.6). A prior-made subdivision, which took these relevant criteria into consideration (see Section 7.3.1) next to additional characteristics of the sulfur supply (symptomatological and critical values of total S, sulfate, and N/S ratio), did not prove to be feasible as the variation of results was so high that no clear ranges, let alone threshold values, could be assigned for individual classes and crops, or crop groups. Smith and Loneragan (295) stressed that in addition to various biotic and abiotic factors, experimental conditions, plant age, and plant part, all influence the nutrient status; the procedure to derive a critical value itself has a significant impact, so that it is possible to define only ranges for different nutritional levels. This finding also implies that it is more or less impossible to compare results from different experiments. The integration of individual studies, which imply extreme values, are not suitable for a generalization of an affiliation to a certain class of sulfur supply and, more importantly, such interpretation may even yield an erroneous evaluation of the sulfur supply. In comparison, the compilation

TABLE 7.6
Mean Critical Values and Ranges of Sulfur Nutrition for Different Groups of Agricultural Crops

S Nutritional Status			
Deficient	**Adequate**	**High**	**Parameter**
Poaceae: barley (*Hordeum vulgare*), corn (*Zea mays*), oats (*Avena sativa*), rice (*Oryza sativa*), sorghum (*Sorghum vulgare*), sugarcane (*Saccharum* ssp.), wheat (*Triticum aestivum*; *Triticum durum*)			
			S_{tot} (mg g^{-1})
0.94	1.7	4.7	Median
0.6	1.4	4.0	25% quartile
1.2	2.5	6.0	75% quartile
0.1–2.0	0.3–8.9	3.3–10.0	Range
41	145	18	(*n*)
			N/S ratio
24	16.0	—	Median
19.5	10.7	—	25% quartile
29.3	19.0	—	75% quartile
11.9–55	7–38	—	Range
15	45	—	(*n*)
			Sulfate (mg kg^{-1})
60	150	5400	Median
36.5	82.5	1500	25% quartile
235	1030	8300	75% quartile
23–400	30–6400	1200–11200	Range
4	20	5	(*n*)
Oil crops I: Mustard (*Brassica juncea*), oilseed rape, spring and winter varieties (*Brassica napus; Brassica campestris*)			
			S_{tot} (mg g^{-1})
1.6	4.8	—	Median
2.3	3.2	—	25% quartile
3.3	6.7	—	75% quartile
1.1–5.8	1.7–10.4	—	Range
8	54	—	(*n*)
			N:S ratio
—	6–7	—	Median
—	—	—	25% quartile
—	—	—	75% quartile
—	—	—	Range
—	1	—	(*n*)
			Sulfate (mg kg^{-1})
—	—	—	Median
—	—	—	25% quartile
—	—	—	75% quartile
—	—	—	Range
—	—	—	(*n*)
Oil crops II: Cotton (*Gossypium hirsutum*), linseed (*Linum usitatissimum*), peanut (*Arachis hypogaea*), soybean (*Glycine max*), sunflower (*Helianthus annuus*)			
			S_{tot} (mg g^{-1})
1.7	2.3	3	Median
0.9	2.0	—	25% quartile

Continued

TABLE 7.6 (*Continued*)

S Nutritional Status			
Deficient	**Adequate**	**High**	**Parameter**
2.0	3.1	—	75% quartile
0.8–2.9	1.1–9.9	—	Range
19	108	2	(*n*)
			N:S ratio
—	15.8	—	Median
—	13	—	25% quartile
—	20	—	75% quartile
—	12–25	—	Range
—	8	—	(*n*)
			Sulfate (mg kg^{-1})
10	360	—	Median
10	190	—	25% quartile
20	475	—	75% quartile
3–100	100–700	—	Range
6	5	—	(*n*)
Legumes: Chickpea (*Cicer arietinum*), Faba bean (*Vicia faba*), (field) pea (*Pisum sativum*), lentil (*Lens culinaris*), navy, bush, snap, green, dwarf, french beans (*Phaseolus vulgaris*), lupin (*Lupinus angustifolius, Lupinus albus, Lupinus cosentinii*), black gram (*Vigna mungo*), cowpea (*Vigna unguiculata*), pigeon pea (*Cajanus cajan*)			
			S_{tot} (mg g^{-1})
1.1	2.7	—	Median
0.7	2.0	—	25% quartile
1.5	3.6	—	75% quartile
0.7–3.0	0.7–6.5	—	Range
7	62	—	(*n*)
			N:S ratio
—	15.5	—	Median
—	—	—	25% quartile
—	—	—	75% quartile
—	—	—	Range
—	2	—	(*n*)
			Sulfate (mg kg^{-1})
—	1600	11200	Median
—	500	—	25% quartile
—	3400	—	75% quartile
—	200–6400	—	Range
—	5	1	(*n*)
Root crops: Carrot *(Daucus* carota), cassava *(Manihot esculentum),* potato (*Solanum tuberosum*), sugar beet, fodder beet, beetroot *(Beta vulgaris),* sweet potato (*Ipomoea batatas*)			
			S_{tot} (mg g^{-1})
1.4	3.0	3	Median
0.8	2.0	—	25% quartile
2.2	3.7	—	75% quartile
0.4–3.0	0.75–6.3	—	Range
8	45	1	(*n*)
			N:S ratio
—	11	—	Median
—	—	—	25% quartile
—	—	—	75% quartile

TABLE 7.6 (*Continued*)

S Nutritional Status			
Deficient	**Adequate**	**High**	**Parameter**
—	—	—	Range
—	1	—	(*n*)
			Sulfate (mg kg^{-1})
150	400	2800	Median
50	250	—	25% quartile
200	3880	—	75% quartile
50–200	250–14000	—	Range
6	5	1	(*n*)

Fodder crops/pastures: Alfalfa (*Medicago sativa*), annual ryegrass (*Lolium rigidum*), Bahia grass (*Paspalum notatum*), Balansa cover (*Trifolium balansae*), barley grass (*Hordeum leporinum*), barrel medic (*Medicago truncatula*), Bermuda grass (*Cynodon dactylon*), Berseem clover (*Trifolium alexandrinum*), black medic (*Medicago lupulina*), Buffel grass (*Cechrus ciliaris*), burr/annual medic (*Medicago polymorpha*), Caribbean Stylo (*Stylosanthes hamata*), Centro (*Centrosema pubescens*), Cluster clover (*Trifolium glomeratum*), cocksfoot (*Dactylis glomerata*), dallis grass (*Paspalum dilatatum*), *Digitaria eriantha*, *Dolichos lablab* (*Lablab purpu*reus), glycine (*Neonotonia wightii*), *Glycine tabacina*, Great brome grass (*Bromus diandrus*), greenleaf desmodium (*Desmodium intortum*), Guinea grass (*Panicum maximum*), Kentucky bluegrass (*Poa pratensis*), Kenya white clover (*Trifolium semipilosum*), Kikuyu grass (*Pennisetum clandestinum*), Leucaena (*Leucaena leucocephala*), Lotonis (*Lotonis bainesii*), Murex medic (*Medicago murex*), Phalaris (*Phalaris aquatica*), perennial ryegrass (*Lolium perenne*), phasey bean (*Macroptilium lathroides*), purple bean (*Macroptilium atropurpureum*), Rhodes grass (*Chloris gayana*), Setaria (*Setaria sphacelata*), Shrubby Stylo (*Stylosanthes scabra*), silver leaf desmodium (*Desmodium uncinatum*), Sorghum-sudangrass (*Sorghum bicolor x S. sudanese*), Sticky Stylo (*Stylosanthes viscosa*), Stylo (*Stylosanthes guianensis*), subterranean clover (*Trifolium subterraneum*), Townsville Stylo (*Stylosanthes humilis*), white clover (*Trifolium repens*), wooly burr medic (*Medicago minima*)

			S_{tot} (mg g^{-1})
1.5	2.1	3.2	Median
1.1	1.7	3	25% quartile
3	2.7	5.6	75% quartile
0.6–3.1	0.7–6.5	2.3–7.5	Range
68	297	13	(*n*)
			N:S ratio
15	20	—	Median
—	16.3	—	25% quartile
—	20	—	75% quartile
—	10–29	—	Range
1	23	—	(*n*)
			Sulfate (mg kg^{-1})
109	500	10850	Median
98	209	—	25% quartile
146.5	1350	—	75% quartile
20–1300	20–3900	—	Range
16	64	2	(*n*)

Brassica vegetables: Broccoli (*Brassica oleracea* var. *italica*), brussels sprouts (*Brassica oleracea* var. *gemmifera*), cabbage (*Brassica oleracea*), cauliflower (*Brassica oleracea* var. *botrytis*), Chinese kale (*Brassica oleracea* var. *alboglabra*), Chinese cabbage (*Brassica rapa* var. *pekinensis*), kohlrabi (*Brassica oleracea* var. *gongylodes*), Pak-choi (*Brassica rapa* var. *chinensis*), spinach mustard (*Brassica pervirdis*), turnip (*Brassica rapa* var. *rapa*)

			S_{tot} (mg g^{-1})
—	7.5	6.5	Median
—	4	—	25% quartile

Continued

TABLE 7.6 (*Continued*)

S Nutritional Status			
Deficient	**Adequate**	**High**	**Parameter**
—	12.8	—	75% quartile
—	2.5–19.2	—	Range
—	30	1	(*n*)
			N:S ratio
—	—	—	Median
—	—	—	25% quartile
—	—	—	75% quartile
—	—	—	Range
—	—	—	(*n*)
			Sulfate (mg kg^{-1})
—	—	—	Median
—	—	—	25% quartile
—	—	—	75% quartile
—	—	—	Range
—	—	—	(*n*)

Nonbrassica vegetables: Asparagus (*Asparagus officinalis*), Arugula salad (*Eruca sativa*), cantaloupe, honeydew (*Cucumis melo*), celery (*Apium graveolens*), cucumber (*Cucumis sativus*), endive (*Cichorium endiva*), fenugreek (*Trigonella foenum-graecum*), garden sorrel (*Rumex acetosa*), lettuce (*Lactuca sativa* spp.), onion (*Allium cepa*), spinach (*Spinacia oleracea*), tomato (*Lycopersicon esculentum*), wild radish (*Raphanus raphanastrum*), zucchini (*Cucurbita pepo*)

			S_{tot} (mg g^{-1})
2.9	4.0	10	Median
1	3.0	7	25% quartile
3.9	7.0	10	75% quartile
0.6–4.9	1.6–14.0	7–10	Range
13	47	5	(*n*)
			N:S ratio
—	—	—	Median
—	—	—	25% quartile
—	—	—	75% quartile
—	—	—	Range
—	—	—	(*n*)
			Sulfate (mg kg^{-1})
1100	11750	—	Median
—	—	—	25% quartile
—	—	—	75% quartile
—	—	—	Range
1	2	—	(*n*)

Source: Compiled from references given in Schnug (103), Bergmann (143), Eaton (144), Reuter and Robinson (294), and Mills and Jones (296).

of the data in Table 7.6 indicates that the sampled plant part during the main vegetative development seems to be of minor relevance for generally addressing the sulfur nutritional status. However, for following up, for instance, nutritional or pathogen-related changes in sulfur metabolism, it might even be necessary to do so in defined parts of a plant organ or on a leaf cell level.

The results in Table 7.6 reveal that Poaceae and fodder crops have been studied intensely in relation to sulfur nutritional supply. For all crops, the total sulfur concentration was used most often to characterize the sulfur nutritional status. The range of variation was distinctly lower for total sulfur

than for sulfate concentrations, independent of the crop type. It is also remarkable that the ranges in the three classes overlap regularly for all groups of crops and sulfur fractions. With the exception of the fodder crops, however, the 25 and 75% quartiles separate samples from the three nutritional levels efficiently if total sulfur concentrations were determined. For sulfate, such partition was feasible too, except in Poaceae. Generally, an insufficient sulfur supply is indicated by total sulfur concentrations of <1.7 mg S g^{-1}. In the case of Poaceae and nonbrassica vegetables, this value may be lower at 0.94 mg S g^{-1} or higher at 2.9 mg S g^{-1} (Table 7.6; Section 7.3.1). Sulfate concentrations of <150 mg SO_4-S kg^{-1} indicate an insufficient sulfur supply. An adequate sulfur supply is reflected by total sulfur concentrations of 1.7 to 4 mg S g^{-1}; brassica crops show a higher optimum range with values of 4.8 (oil crops) to 7.5 (vegetables) mg S g^{-1} (Table 7.6). Values of 16 to 20 for N/S ratio, and 150 to 1600 for sulfate-S concentrations reflect a sufficient sulfur supply. In comparison, values of >2800 mg SO_4-S kg^{-1} denote an excessive sulfur supply (Table 7.6). Sulfate is usually not determined in brassica oil crops and vegetables as the degradation of glucosinolates might falsify the result (see Section 7.5). For fodder crops, total sulfur concentrations of even 3.2 mg S g^{-1} may be disproportionate, whereas the corresponding value for nonbrassica vegetables would equal 10 mg S g^{-1}.

The major criticism of critical values for the interpretation of tissue analysis is the small experimental basis, which often consists of not more than a single experiment (297). Besides the lack of data, the method of interpretation may also yield erroneous results. Methods based on regression analysis, like the 'broken stick method' by Hudson (298) and Spencer and Freney (241), or the 'vector analysis' by Timmer and Armstrong (299) investigate mathematical, but not necessarily causal, interactions between the nutrient content and yield, because the dictate of minimizing the sum of squared distances aims only to find a function that fits best across the data set. Like the method of Cate and Nelson (300,301), these methods have been designed primarily for the investigation of small data sets and plants grown under *ceteris paribus* conditions, where only the response to variations in the nutrient supply varied. Another quite significant disadvantage of critical values and critical ranges* (143,296,302), or 'no-effect values (NEV)'† (284) is that they ignore the nonlinearity of the Mitscherlich function describing the relationship between growth factors and yield (303). The ideal basis for critical values for the interpretation of tissue analysis are large sets of yield data and nutrient concentrations in defined plant organs that cover a wide range of growth factor combinations. The data may include samples from field surveys or field or pot experiments if the reference yield of 100% was obtained in all cases under optimum growth conditions. In Figure 7.15 and Figure 7.16, corresponding examples are given for the total sulfur concentration in shoots of cereals at stem extension and the N/S ratio in younger, fully developed leaves of oilseed rape at stem extension.

The data in Figure 7.15 reveal a characteristic bow-shaped bulk, which covers sulfur concentrations from 0.5 to 5.5 mg S g^{-1}. Sulfur deficiency can be expected at sulfur concentrations below 0.94 mg g^{-1} (Table 7.6). A symptomatological threshold for the expression of macroscopic symptoms of 1.2 mg S g^{-1} was determined for cereals by Schnug and Haneklaus (114). Total sulfur concentrations of 1.7 mg g^{-1} are considered as being adequate to satisfy the sulfur demand of cereal crops, whereas the data in Figure 7.15 show a further yield increase with higher sulfur concentrations. The reason is simply that the 100% yield margin corresponds to a grain yield of 10 t ha^{-1} (180), so that accordingly a total sulfur concentration of 1.7 mg S g^{-1} would be sufficient for 8.2 t ha^{-1}. A productivity level of 10 t ha^{-1} is extraordinarily high and restricted to areas of high fertility or inputs, whereas a level of 8 t ha^{-1} represents a high-yielding crop in many areas in the world. Thus, a total sulfur concentration of 4.7 mg g^{-1}, which is rated as reflecting a high sulfur supply, is marginal on high productivity sites.

Basic shortcomings of using, for instance, the N/S ratio for the evaluation of the sulfur nutritional status were discussed (Section 7.5) and are reflected in the data in Figure 7.16. Hence, there are no relationships between N/S ratio and yield in a way as was shown for total sulfur and cereals (Figure 7.15). Crop productivity seems to be fairly independent of variations in the N/S ratio within a range of 5:1 to 12:1 (Figure 7.16).

*Tissue concentration for 95% of maximum yield.

†Tissue concentration for maximum yield or the concentration above which no yield response occurs.

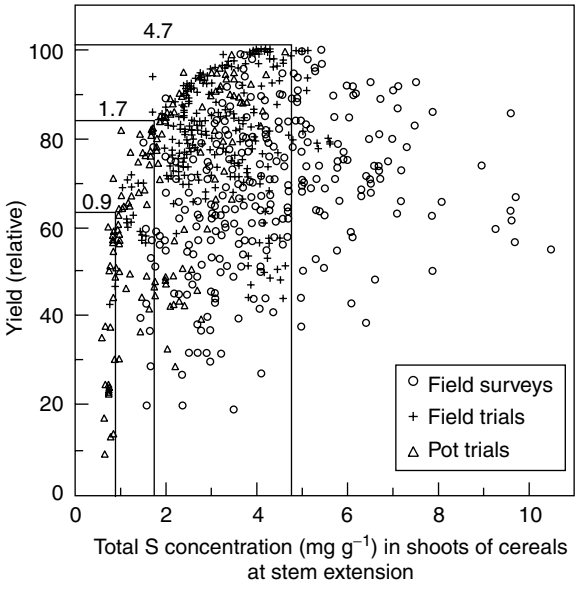

FIGURE 7.15 Scattergram of total sulfur in shoots and yield data for cereals in relation to experimental conditions (From Schnug, E. and Haneklaus, S., in *Sulphur in Agroecosystems*. Vol. 2, Part of the series 'Nutrients in Ecosystems', Kluwer Academic Publishers, Dordrecht, 1998, pp. 1–38.) and merged values thresholds for sulfur supply (see Table 7.7).

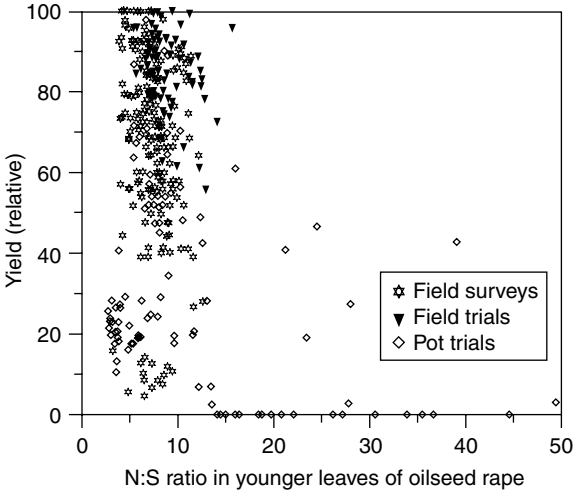

FIGURE 7.16 Relationship between N:S ratio in young leaves of oilseed rape at stem extension and relative seed yield. (From Schnug, E., Quantitative und Qualitative Aspekte der Diagnose und Therapie der Schwefelversorgung von Raps (*Brassica napus* L.) unter besonderer Berücksichtigung glucosinolatarmer Sorten. Habilitationsschrift, D.Sc. thesis, Kiel University, 1988.)

Comprehensive data sets like those presented in Figure 7.15 allow for the accurate calculations of so-called upper boundary line functions, which describe the highest yields observed over the range of nutrient values measured. Data points below this line relate to samples where some other factor limited the crop response to the nutrient. An overview of the scientific background and development of upper boundary lines is given by Schnug et al. (304).

The Boundary Line Development System (BOLIDES) was elaborated to determine the upper boundary line functions and to evaluate optimum nutrient values and ranges. The BOLIDES is based on a five-step algorithm (Figure 7.17) (304). For the identification of outliers, cell sizes are defined for nutrient and yield values together with an optional number of data points per cell (Figure 7.17a). The cell size can be chosen variably with proposed values for X (nutrient content) corresponding to the standard deviations and for Y (yield) with the coefficient of variation. If another variable, often a stable soil feature such as organic matter or clay content, has a significant effect on the response to the nutrient, its presence is indicated by two or more distinct concentrations of points, each with its own boundary line response to the nutrient (Figure 7.17b). The data can be classified on the basis of this third variable, and the boundary line can be determined separately for each class. Next, a boundary step function is calculated for each class, starting from the minimum nutrient content up to the point of maximum yield, as well as from the maximum nutrient content up to the maximum yield (Figure 7.17c). Then the boundary line, usually a first-order polynomial function, is fitted according to the least-squares method (Figure 7.17d). The first derivative of the fitted polynomial gives predicted yield response to fertilization in relation to the nutrient content (Figure 7.17). The last step is the classification of the nutrient supply to determine optimum nutrient levels or optimum nutrient ranges. The optimum nutrient value corresponds with the zero of the first derivative of the upper boundary line and the sign of the second derivative at this point. For the determination of the optimum ranges, that is, the range of nutrient concentration that gives 95% of the maximum yield, standard, numerical root-finding procedures are used for real polynomials of degree 4 with constant coefficients (Figure 7.17).

Thus boundary lines describe the 'pure effect of a nutrient' on crop yield under *ceteris paribus* conditions (246,247,305,306). The comparison of the boundary lines for total sulfur and yield

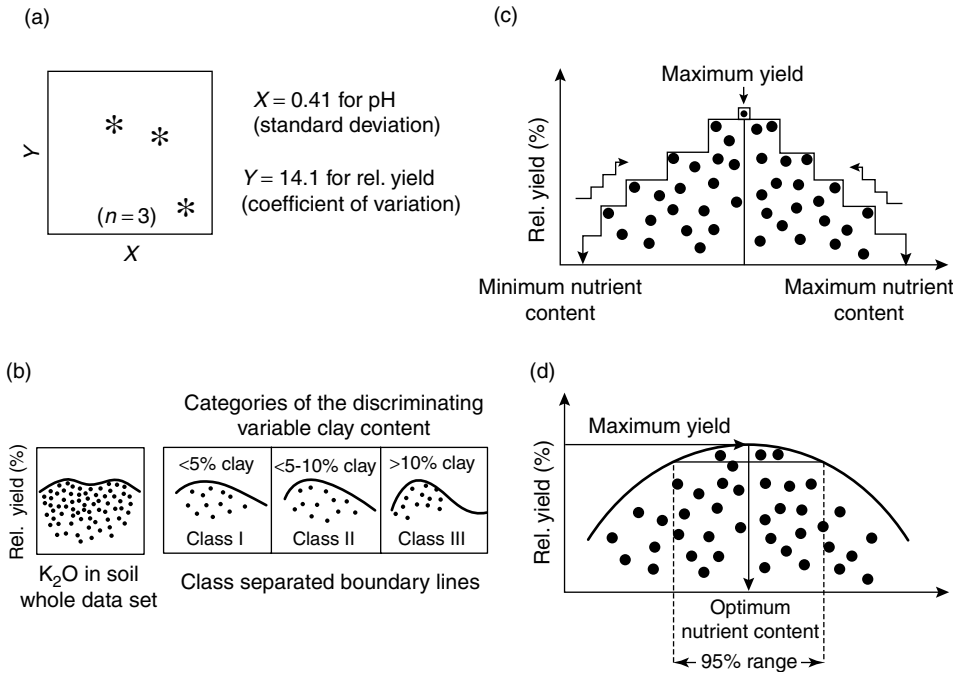

FIGURE 7.17 Structure of Boundary Line Development System (BOLIDES) for the determination of upper boundary line functions and optimum nutrient values and ranges in plants and soils: (a) identification of outliers; (b) discrimination against a third variable; (c) calculation of step functions; and (d) determination of the upper boundary line and calculation of optimum nutrient value and ranges. (From Haneklaus, S. and Schnug, E., *Aspects Appl. Biol*., 52, 87–94, 1998.)

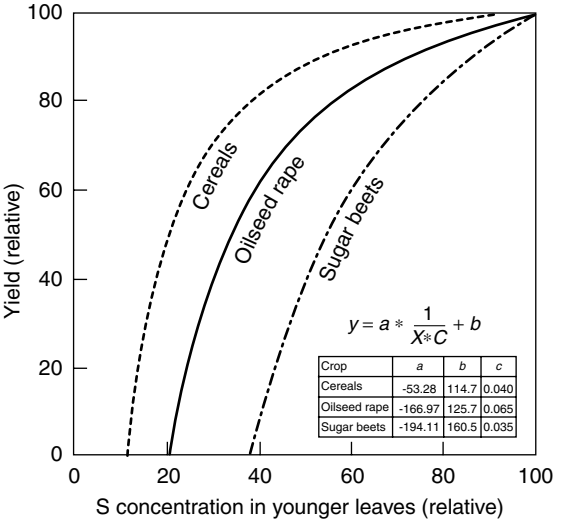

FIGURE 7.18 Comparison of boundary line functions for yield and total sulfur concentration in tissue of cereals, oilseed rape, and sugar beet. (From Schnug, E. and Haneklaus, S., in *Sulphur in Agroecosystems*, Vol. 2, Part of the series 'Nutrients in Ecosystems', Kluwer Academic Publishers, Dordrecht, 1998, pp. 1–38.)

(both relative) for oilseed rape, cereals, and sugar beet (Figure 7.18) reveals the physiological differences between these crops. The boundary lines for cereals and oilseed rape are for seed yields, and that for sugar beet for root yields. The optimum sulfur ranges proved to be the same for sugar beet root yield and sugar yield.

For all crops, the boundary lines show a steep increase at the beginning, which reflects the response of the photosynthetic system to sulfur deficiency. In cereals, the boundary line continues over a long range and asymptotically toward the value above which no further yield increase (NEV) is to be expected from increasing sulfur concentrations. This part of the boundary line most likely reflects the proportion of sulfur that is bound to the proteins of the cereal grain. In sugar beet, the boundary line reaches the NEV much faster after a steep increase, which is in line with the fact that sugar beet roots take up only small amounts of sulfur (205). Oilseed rape, with its internal storage system for S, which is based on the enzymatic recycling of glucosinolates (90,289), shows a steadier ascent of its boundary line. Therefore, within oilseed rape varieties, those with genetically low glucosinolate contents ('double low' or '00' varieties) show a steeper increase of their boundary lines than those with genetically high glucosinolate concentrations (103,116).

The nonlinearity of the boundary lines reveals once more the limited value of critical values. Above total sulfur concentrations of 6.5, 4.0, and 3.5 mg g^{-1} in foliar tissue of oilseed rape, cereals, and sugar beet, respectively, no further yield increases are to be expected by increasing tissue sulfur concentrations (NEVs). This result corresponds to the usually assigned 'critical values,' which are valid for 95% of the maximum yield, of 5.5, 3.2, and 3.0 mg S g^{-1} for rape, corn, and sugar beets, respectively. However, in this range of the response curve, there is still no linearity between tissue sulfur levels and yield.

The relationship between sulfur concentration in plant tissue and yield, which reflects the physiological patterns in the internal nutrient utilization, is specific for each plant species, and can be best established by boundary lines (Figure 7.17). In comparison, the relationship between fertilizer dose and sulfur concentration in plant tissues is much less dependent on physiological factors but is strongly influenced by factors affecting the physical mobility and losses of sulfur from soils. Therefore, this transfer function bears the largest part of insecurity for the effectiveness of sulfur fertilization. Thus, for the derivation of fertilizer recommendations, the common relationship between fertilizer dose and yield is best split into two partial relationships: (a) fertilizer dose versus nutrient

uptake and (b) nutrient uptake versus yield (307). If tissue analysis is to be used for fertilizer recommendations, concentrations need to be calibrated against sulfur doses. This strategy was proved for nitrogen (308), and the setting up of sulfur response curves is recommended for sulfur too.

Professional Interpretation Program for Plant Analysis (PIPPA) software not only evaluates the status of individual plant nutrients but also appraises results from multiple elemental analyses (309). In PIPPA, boundary line and transfer functions are integrated for each element so that the yield-limiting effect is calculated for each specified nutrient, and finally fertilizer recommendations are given (309).

7.5.3 Sulfur Status and Plant Health

Although the significance of individual nutrients for maintaining or promoting plant health saw some interest in the 1960s and 1970s (143), research in the field of nutrient-induced resistance mechanisms has been scarce because of its complexity, and because of its limited practical significance due to the availability of effective pesticides.

Since the beginning of the 1980s, atmospheric sulfur depositions have been declining drastically after Clean Air Acts came into force, and severe sulfur deficiency advanced to a major nutritional disorder in Western Europe (114,310,311). Increased infections of agricultural crops with fungal pathogens were observed, and diseases spread throughout the regions that were never infected before (312). Sulfur fertilization, applied to the soil as sulfate, proved to have a significant effect on the infection rate and infection severity of different crops by fungal diseases (148). Sulfur fertilization increased the resistance against various fungal diseases in different crops under greenhouse (313,314) and field conditions (315–317). Based on these findings, the concept of sulfur-induced resistance (SIR) was developed; research in this field has strengthened since then, and the advances made are discussed comprehensively by Bloem et al. (318) and Haneklaus et al. (148).

The term SIR stands for the reinforcement of the natural resistance of plants against fungal pathogens through triggering of the stimulation of metabolic processes involving sulfur by targeted fertilizer application strategies (148). A sufficient sulfur supply and an adequate availability of plant-available sulfate are presumably a prerequisite for inducing S-dependent resistance mechanisms in the plant so that the required sulfur rates and sulfur status may be higher than the physiological demand.

The mechanisms possibly involved in SIR may be related to processes of induced resistance (319), for example, via the formation of phytoalexins and glutathione, or the requirement of cysteine for the synthesis of salicylic acid by β-oxidation and the cysteine pool itself. Another option is the release of reduced sulfur gases, such as H_2S, which is described in the literature as being fungitoxic. The H_2S may be produced prior to or after cysteine formation (see Section 7.2 and (320)). Two enzymes that could be responsible for the H_2S release are L-cysteine desulfhydrase (LCD) and *O*-acetyl-L-serine(thiol)lyase (*O*AS-TL). The LCD catalyzes the decomposition of cysteine to pyruvate, ammonia, and H_2S. The *O*AS-TL is responsible for the incorporation of inorganic sulfur into the amino acid cysteine, which can be subsequently converted into other sulfur-containing compounds such as methionine or glutathione. The H_2S is evolved in a side reaction because of the nature of the pyridoxal 5′-phosphate cofactor and the specific reaction mechanism of the *O*AS-TL protein (321). There is wide variation with regard to specifications about the release of H_2S, ranging from 0.04 ng g^{-1} s^{-1} in whole soybean plants on a dry matter basis (322) to 100 pmol min^{-1} cm^{-1} in leaf discs of cucumber (323). Thus, H_2S emissions of cut plant parts may be 500 times higher than in intact plants (Table 7.7).

The release of H_2S by plants is supposedly regulated by interactions in the N and sulfur metabolic pathways. Lakkineni et al. (327) demonstrated a distinct increase in H_2S emissions when leaf discs of mustard, wheat, and groundnut (*Arachis hypogaea* L.) were fed with sulfate or cysteine (Table 7.8). Supply of additional nitrogen with the sulfate did not cause H_2S emissions to increase (Table 7.8). Lakkineni et al. (330) suggested a preferable synthesis of nitrogen- or sulfur-containing products at the level of substrate availability.

TABLE 7.7
Survey of Different Investigations of the Release of Hydrogen Sulfide from Terrestrial Plants

Measured H_2S Evolution	Plant/ Plant Part	Reference	Estimated H_2S Emission (nmol g^{-1} d.w. h^{-1})
0.04–0.08 ng g^{-1} d.w. s^{-1}	Soybeans (whole plant)	322	2.1–8.5
5.58–6.21 pmol kg^{-1} s^{-1}	Conifers (whole plant)	324	0.02
2.22 µg kg^{-1} h^{-1}	Spruce seedlings (*Picea abies* L. Karsten)	325	0.07
0.04–0.46 nmol min^{-1} $leaves^{-1}$	Attached leaves of different plants	326	8–92[a]
0.49–0.94 nmol g^{-1} f.w. h^{-1}	Leaf extract of *Brassica. napus*	327	3.3–6.3[b]
0.80–1.11 nmol g^{-1} f.w. h^{-1}	Leaf discs of mustard	327	5.3–7.4[b]
1.7–3.9 nmol min^{-1} $leaves^{-1}$	Detached leaves of different plants	326	340–780[a]
8 nmol g^{-1} f.w. min^{-1}	Maximum emission of detached leaves	326	3200[b]
2.4–3.9 nmol g^{-1} f.w. min^{-1}	Leaves of spinach and cucumber	65	960–1560[b]
40 pmol min^{-1} cm^{-2}	Leaf discs of different plants	323	800[c]
50–100 pmol min^{-1} cm^{-2}	Leaf discs of cucumber	328	1000–2000[c]
Total S emission from higher plants			Total S Emission (nmol S^{-1} d.w. h)
12–1062 ng S kg^{-1} d.w. min^{-1}	42 types of terrestrial plants	329	0.02–1.99

[a]Assuming a medium leaf weight of 2 g fresh weight and a leaf water content of 85%.
[b]Assuming a medium leaf water content of 85%.
[c]Assuming a dry weight of 3 mg cm^{-2}.
Source: From Bloem, E. et al., *J. Plant Nutr.*, 28, 763–784, 2005.

TABLE 7.8
Influence of Sulfate, Cysteine, and Nitrate on the Emission of H_2S from Leaf Discs of Mustard, Groundnut, and Wheat

	H_2S Emission (nmol g^{-1} f.w. h^{-1})		
Treatment	**Mustard**	**Wheat**	**Groundnut**
Control (H_2O)	0.80	1.27	0.25
Sulfate (5 mM)	1.15	1.85	—
Cysteine (5 mM)	1.11	2.19	0.80
Sulfate + nitrate (5 mM)	0.81	1.29	—
Cysteine + nitrate (5 mM)	0.72	2.63	—

Source: From Lakkineni, K.C. et al., in *Sulphur Nutrition and Sulphur Assimilation in Higher Plants; Fundamental, Environmental and Agricultural Aspects*, SPB Academic Publishing, The Hague, 1990, pp. 213–216.

H_2S and DMS emissions by plants are, however, supposedly not involved in SIR against fungal pathogens belonging to the class Basidiomycetes, as fumigation experiments with fungal mycelium of *Rhizoctonia solani* revealed that the pathogen metabolized both gases efficiently (331).

The amino acids cysteine and methionine are the major end products of sulfate assimilation in plants and bind up to 90% of the total sulfur (320). Conditions of sulfur deficiency will result in a decrease of sulfur-containing amino acids in proteins (5). As the amino acid composition is genetically determined, this effect is limited, and thereafter the total protein content will be reduced (5). Amino acid type and concentration in plant tissues are related to the susceptibility of plants to

pathogens (332). Amino acids occur in the free state in plants, and the amino acids cysteine and methionine are enriched in resistant plant tissues. Soil-applied sulfur significantly increased the free cysteine content in the vegetative tissue from 0.5 to 1.2 μmol g^{-1} d.w. (63). Bosma et al. (333) reported a two- to five-fold increase in the content of water-soluble nonprotein sulfhydryl compounds in clover (*Trifolium* spp.) and spinach after fumigation with H_2S under field conditions, whereby the cysteine content increased 10-fold. De Kok (18) reported similar results for fumigation experiments with sulfur dioxide.

Glutathione is a major, free, low-molecular, nonprotein, thiol compound and is an important reservoir for nonprotein reduced sulfur in plants (66). A relationship between glutathione content and the extent of protection against fungal diseases exists (72). A low glutathione content in plants does not inevitably imply, however, a higher susceptibility of the plant, as a rapid accumulation of glutathione in response to pathogen attack was noted (334), and this observation proved to be decisive in pathogenesis (72). Sulfur-deficient plants have very low glutathione concentrations, and sulfur fertilization significantly increases the free thiol content (Table 7.3; Section 7.2.3). Basically, sulfur-deficient plants are expected to be more vulnerable to stress factors, which are usually compensated by the glutathione system so that sulfur fertilization should have a positive effect on resistance mechanisms.

Phytoalexins are important for plant defense (335). Phytoalexins are secondary plant metabolites which are synthesized de novo and accumulate in response to diverse forms of stress, including pathogenesis (336). The immunity is generally of short duration and is concentrated around the infected area. According to this definition, the formation of elemental sulfur, the stress-induced formation of pathogenesis-related (PR) proteins, and a novel class of LMW antibiotics, all come under the term phytoalexins. At the moment however, the influence of the sulfur nutritional status on phytoalexin synthesis can only be speculated from the dependency of their precursors on the sulfur supply. The influence of the sulfur nutritional status on the synthesis of PR-12, PR-13, and PR-14 proteins and elemental sulfur depositions in plant tissues remains obscure too (148).

7.6 SULFUR FERTILIZATION

The optimum timing, dose, and sulfur form used depends on the specific sulfur demand of a crop and application technique. Under humid conditions, the sulfur dose should be split in such a way that sulfur fertilization in autumn is applied to satisfy the sulfur demand on light, sandy soils before winter and to promote the natural resistance against diseases. At the start of the main vegetative growth, sulfur should be applied together with nitrogen. With farmyard manure, on an average 0.07 kg sulfur is applied with each kg of nitrogen. In mineral fertilizers and secondary raw materials, sulfur is available usually as sulfate, elemental sulfur, and sulfite. Sulfate is taken up directly by plant roots, whereas sulfite and elemental sulfur need prior oxidation to sulfate, whereby the speed of transformation depends on the particle size and dimension of the thiobacillus population in the soil (Figure 7.19) (337,338).

The main secondary-sulfur-containing raw materials from the flue gas desulfurization process are gypsum and spray dry absorption (SDA) products, which are a mixture of calcium sulfite and calcium sulfate in a mass ratio of about 8:1 (340). SDA products with fly ash contents $< 8\%$ may contain up to 68% calcium sulfite, whereas this percentage in products with fly ash contents between 20 and 85% will not exceed 47% (341). A phytotoxic effect of sulfite applied by SDA products was observed when it was used as a culture substrate and on soils with a $pH < 4$ (337). The time required for complete oxidation of sulfite is about 2 weeks (342). Sulfite oxidation proceeds faster with increasing oxygen content and soil pH, and decreasing soil moisture content (343,344). When sulfur was applied at rates of ≤ 80 kg ha^{-1} to exclusively satisfy the sulfur demand of agricultural crops, no negative impact on crop performance and subsequent crops in the rotation was detected (337,342,345,346).

In general, the efficiency of sulfur uptake by rape is highly dependent on the sulfur status of the shoots (Figure 7.20). There is a close relationship between the initial sulfur content and its increase

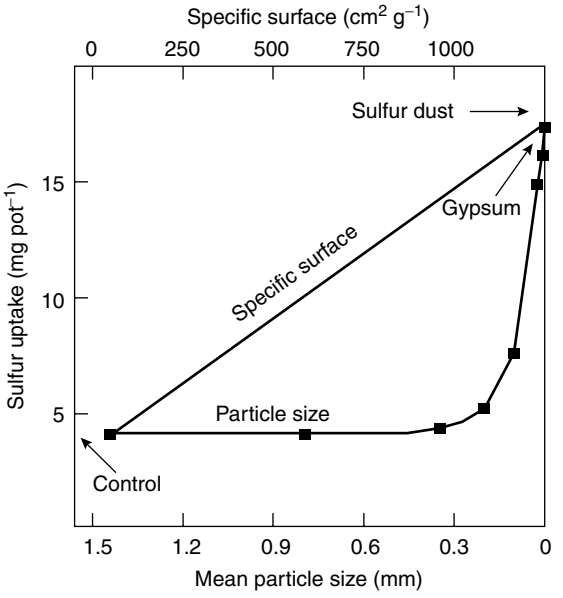

FIGURE 7.19 Sulfur uptake of maize plants 32 days after sowing, in relation to particle size and specific surface of elemental sulfur in a pot experiment. (From Fox, R.L. et al., *Soil. Sci. Soc. Am. Proc.*, 28, 406–408, 1964.)

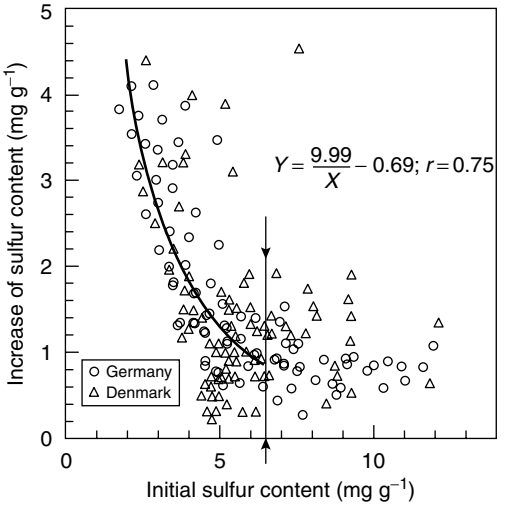

FIGURE 7.20 Influence of sulfur fertilization (20 kg S ha^{-1}) on the total sulfur concentration of oilseed rape leaves, in relation to the initial sulfur supply. (From Schnug, E. and Haneklaus, S., *Landbauforschung Völkenrode*, Sonderheft 144, 1994.)

by fertilization. Under sulfur-limiting growth conditions, root-expressed sulfur transporters are highly regulated and induced (see Section 7.2.1 and Section 7.2.2). Besides that, sulfur fertilization improved root growth and thus access to sulfate (53).

An insufficient sulfur supply will not only reduce crop productivity, diminish crop quality, and affect plant health, but it also will impair nitrogen-use efficiency (53,347). Under conditions of

TABLE 7.9
Influence of Sulfur Fertilization on the Nitrate Reductase Activity and N-Use Efficiency of Sugarcane

S Dose (kg ha^{-1})	Nitrate Reductase Activity (nmol NO_2^- g^{-1} (f.w.) h^{-1})	Nitrogen-Use Efficiency (g (d.m.) g^{-1} (N) m^{-2})
0	1652	2.17
40	1775	2.23
80	1989	3.02
120	2020	2.54
160	1805	2.67

Source: From Shanmugam, K.S., *Fert. News*, 40, 23–26, 1995.

sulfur deficiency, nitrate and non-S-containing amino acids accumulate—actions which may reduce the nitrate reductase activity (see Section 7.2.4; 348). Sulfur fertilization promotes nitrate reduction and thus restricts nitrate accumulation in vegetative tissues. In Table 7.9, the influence of an increasing sulfur supply on the nitrate reductase activity and nitrogen-use efficiency is shown.

The highest nitrate reductase activity occurred at a sulfur dose of 120 kg S ha^{-1} and the highest N-use efficiency at 160 kg S ha^{-1} (Table 7.9) (349). This result corresponds to an increase of 18.2 and 18.7%, respectively, for the two doses. In comparison, the net nitrogen utilization of oilseed rape and cereals was significantly increased by sulfur fertilization by about 7 to 16%. A sulfur application rate of 100 kg S ha^{-1} yielded the best results for oilseed rape during three consecutive years of experimentation (347).

The sulfur demands of agricultural crops vary highly, as do the recommended sulfur doses (Table 7.10). Recommended sulfur rates vary between 30 and 100 kg S ha^{-1} for oilseed rape, and between 20 and 50 kg S ha^{-1} for cereals (103,337,348). For other crops such as sugar beet, grassland, rice, and soybean, the highest crop productivity occurred at sulfur rates of 25, 40, 45, and 60 kg S ha^{-1}, respectively (351–353).

Aulakh (364) gives a detailed overview of sulfur uptake and crop responses to sulfur fertilization in terms of yield and quality, with special attention being paid to crops grown in India. Sulfur fertilizer can be applied to the soil or given as foliar dressings. As the sulfur dose is limited when applied via the leaves, this form of fertilization can only be a complementary measure to correct severe sulfur deficiency. Usually, for foliar applications, either Epsom salts or elemental S are used. Calculated from changes in the sulfur uptake by seeds, only 0 to 3% of foliar-applied sulfate-S with Epsom salts was utilized, while 33 to 35% of sulfur applied as elemental sulfur product (Thiovit®) was utilized (338). Foliar-supplied sulfate moved into leaves much faster than elemental sulfur and was supposedly trapped in vacuoles so that it did not contribute to increased yield. The better results with elemental sulfur were explained by the fact that it needs to be oxidized before significant quantities can be absorbed by leaves. As oxidation is slow, sulfate supply from foliar-applied elemental sulfur fits better to the metabolic demand of the leaves and avoids excess sulfate concentrations in the cytosol and their deposition in vacuoles.

The problem of severe sulfur deficiency still exists on a large scale as the widespread regular appearance of macroscopic symptoms reveal, even more than 20 years after addressing this nutrient disorder (147). The reason is most likely the wide variation of official sulfur fertilizer recommendations in Europe (Table 7.11), recommendations, which only partly acknowledge site-specific features and productional peculiarities.

On-farm experimentation employing precision agriculture tools would be an ideal approach for setting up site-specific sulfur response curves (see Section 7.5.2 and (366)).

TABLE 7.10
Sulfur Demand (kg S t^{-1}) of Agricultural Crops

Crop	Based Plant Part	S Demand (kg S t^{-1})	Reference
Poaceae			
Barley	Grain	1.2–1.9	354, 205
(winter varieties)	Straw	1.6–2.1[a]	354, 205
Barley	Grain	1.2–1.4	205
(summer varieties)	Straw	0.7–1.5[a]	205
Oats	Grain	1.7	354
Rice	Total	3.2	355
Sugarcane	Total	0.3	355
Wheat	Grain	1.6–2.2	354, 205
(winter varieties)	Straw	1.1–2.8[a]	205
Wheat	Grain and straw	4.3	355
Oil crops			
Mustard	Total	16.0–17.3	355, 356, 357, 358
Oilseed rape	Total	16	103
Groundnut	Pods	3.3–5.9	355, 357, 358,
		(20.9)	359, 360, 361
Soybean	Seeds	4.3–8.8	357, 358, 362
Sunflower	Seeds	7.1–12.7	356, 357, 358
Legumes			
Chickpea	Total	8.7	355
Pigeon pea	Total	7.5	355
Root crops			
Potato	Tuber	1.2–1.6	205
Sugar beet	Beet root	0.3–0.4	205
	Leaves	0.7–1.9[a]	205
Fodder crops			
Grass	Herbage	1.7	354
Red clover	1st cut	2.2–4.3	363
	2nd cut	2.0–4.0	363
	3rd cut	2.0–3.8	363
Vegetables			
Swedes	Roots[b]	3.0	354
	Tops[b]	1.4[a]	354
Turnip	Roots[b]	2.5	354
	Tops[b]	1.1[a]	354
Marrowstem kale	Whole plant[b]	4.0	354

[a]Yield of harvested product.
[b]Dry matter yield.

TABLE 7.11
Official Sulfur Fertilizer Recommendations and Optimum Fertilizer Doses Based on Scientific Experimentation for Various Crops in Europe

Crop	Range of Officially Recommended S Fertilizer Dose (kg ha^{-1})
Cabbage	30–50
Cereals	10–30
Grassland, cut	30–40
Grassland, grazed	0–30
Grass, silage	0–30
Oilseed rape	20–60
Peas	10–30
Potatoes	0–20
Sugar beet	0–40
Vegetables	20–40

Source: From Aulakh, M.S., in *Sulphur in Plants*, Kluwer Academic Publishers, Dordrecht, 2003, pp. 341–358.

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