Milestones in plant sulfur research on sulfur-induced-resistance (SIR) in Europe

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Until the 1970’s of the last century sulfur (S) was mainly regarded as a pollutant being the main contributor of acid rain, causing forest dieback in central Europe. When Clean Air Acts came into force at the start of the 1980’s SO2 contaminations in the air were consequently reduced within the next years. S changed from an unwanted pollutant into a lacking plant nutrient in agriculture since agricultural fields were no longer “fertilized” indirectly by industrial pollution. S deficiency was first noticed in Brassica crops that display an especially high S demand because of its content of S-containing secondary metabolites, the glucosinolates. In Scotland, where S depositions decreased even faster than in continental Europe, an increasing disease incidence with Pyrenopeziza brassicae was observed in oilseed rape in the beginning 1990’s and the concept of sulfur-induced-resistance (SIR) was developed after a relationship between the S status and the disease incidence was uncovered. Since then a lot of research was carried out to unravel the background of SIR in the metabolism of agricultural crops and to identify metabolites, enzymes and reactions, which are potentially activated by the S metabolism to combat fungal pathogens. The S status of the crop is affecting many different plant features such as color and scent of flowers, pigments in leaves, metabolite concentrations and the release of gaseous S compounds which are directly influencing the desirability of a crop for a variety of different organisms from microorganisms, over insects and slugs to the point of grazing animals. The present paper is an attempt to sum up the knowledge about the effect of the S nutritional status of agricultural crops on parameters that are directly related to their health status and by this to SIR. Milestones in SIR research are compiled, open questions are addressed and future projections were developed.

Keywords: nutrient induced resistance, S fertilization, plant S metabolism, fungal diseases, biotrophic and necrotrophic pathogens

NUTRIENT INDUCED RESISTANCE

Already Justus von Liebig identified in 1873 the nutritional status of a crop as crucial for its susceptibility against diseases. Interactions between mineral elements and plant diseases are established for several macro- and microelements. An overview of current knowledge on the effect of mineral nutrition on plant diseases was compiled by Datnoff et al. (2007).

A sufficient nutrient supply is the first agricultural measure against infection and determines the course of pathogenesis. In general, the greatest benefit can be expected when all essential nutrients are applied in sufficient amounts; however, the response to a particular nutrient may be different when going from deficiency to sufficiency than from sufficiency to excess (Huber and Haneklaus, 2007). For nitrogen it was shown that fertilizer application above recommended rates can lead to significantly greater disease incidences (Walters and Bingham, 2007). Strengthening the natural plant resistance is an important aspect of fertilization practice and modern fertilizers deliver the possibility to individually treat each kind of nutrient deficiency by tailored-made products. All essential plant nutrients have a direct impact on plants, pathogens, and microbial growth so that all of them as well as their proportions are important in disease control and will affect disease incidence or severity (Huber and Haneklaus, 2007). This illustrates an important problem in investigating the metabolic background of sulfur induced resistance (SIR): Plant pathogen response is determined by several interacting factors—different nutrients and their interactions, soil parameters, climatic conditions, pathogens, water supply and much more. Therefore, it is nearly impossible to investigate the response to a certain pathogen in relation to S under natural conditions without having interacting parameters.

PROGRESS IN RESEARCH ON SULFUR INDUCED RESISTANCE (SIR)

The fungicidal effect of foliar-applied elemental S ($S^0$) was already discovered by William Forsyth in 1802 and $S^0$ was used as the most important fungicide until the development of organic fungicides. The effects of foliar-applied elemental S have to be clearly distinguished from the health promoting effects of soil applied S on which SIR is based. The term SIR which denotes the reinforcement of the natural resistance of plants against fungal pathogens through triggering the stimulation of metabolic processes.
processes involving S by targeted soil-applied fertilizer strategies was first introduced by Schnug et al. (1995). In subsequent studies the term sulfur enhanced defense (SED) was used as synonym to prevent misinterpretation of the term resistance in a physiological context (Rausch and Wachter, 2005; Kruse et al., 2007).

Different research areas are of major relevance when investigating the background of SIR. The most important milestones in plant S research with respect to SIR are summarized in Table 1. Here important discoveries such as the detection of the Foyer-Halliwell-Asada pathway or the mustard oil bomb are listed as well as important technical developments.

The achievements made in gene transfer, by which the possibility to work with genetically modified plants was established, as well as the elucidation of the Arabidopsis genome promoted the progress in plant S research tremendously (Chilton et al., 1977). Experimentation with knock-out mutants delivered deep insight into plant metabolism and cross-talk between different pathways (Thomma et al., 1998; Kopriva, 2006; Parisy et al., 2006).

A lot of efforts were undertaken to understand the S assimilation pathway in plants, the transport of S into plants, and the storage and regulation of the S metabolism (Table 1). Since the completion of the Arabidopsis genome research has made considerable progress.

For example a range of S transporters carrying S containing metabolites within and between cells and over long-distance have been characterized, some of them just recently (Gigolashvili and Kopriva, 2014). In glucosinolate research the biosynthesis as well as its regulation was nearly explained in the last years (Halkier and Gershenzon, 2006).

Technical progress such as the development of macroarray hybridization can be seen as a further important milestone. Jost et al. (2005) recorded the reaction of more than 2000 selected genes of Arabidopsis thaliana to methyl jasmonate (JA) elicitation, a signaling compound in host-pathogen interactions. The authors could show that S-related genes were even more up-regulated due to methyl JA treatment than stress-related genes and that more than one pathway is involved in plant stress response. Gene expression of the ascorbate and glutathione metabolic pathways increased in response to JA as well as the synthesis of indole glucosinolates (Sasaki-Sekimoto et al., 2005). Moreover it was shown that imbalances in cytosolic cysteine alter the expression of groups of genes involved in pathogen response (Alvarez et al., 2012). Therefore, macroarray analysis delivers the opportunity to investigate and understand the network and cross-talk of metabolic pathways.

But despite of these great advances in scientific discoveries and technologies delivering several pieces of the puzzle of SIR, many questions remain open. It is still under discussion which reactions or compounds are responsible for the higher resistance of plants in relation to the S supply and how it is possible to induce a higher resistance and use this by advanced fertilizer application.

**PHYSIOLOGICAL BACKGROUND OF SIR**

Plants have developed several defense mechanisms in response to stress and react to a certain pathogen attack through a combination of constitutive and inducible defense with S-containing compounds being involved compiled by Bloem et al. (2005). In principle plants have three major strategies to combat pathogens: cell wall strengthening, apoplastic defense for inhibition of microbial enzymes and poisoning of the pathogen by toxic compounds like phytoalexins (Huckelhoven, 2007).

Initial pathogen recognition causes responses such as oxidative burst with the production of reactive oxygen species (ROS) and cell wall lignification (Swarupa et al., 2014). ROS serve as major signaling molecules in plant defense and are closely linked to the S metabolism via the Foyer-Halliwell-Asada pathway where glutathione is involved in the detoxification of ROS (Foyer and Halliwell, 1976). Via this link to ROS the S metabolism is linked to pathogen recognition and activation of the defense network.

The complexity of plant stress responses became obvious in several infection trials. S metabolites such as cysteine, glutathione, gaseous S emissions, phytoalexins, glucosinolates, and elemental S depositions have been investigated for their role in plant defense and how targeted S applications may prompt and enhance crop resistance to fungal pathogens (Bloem et al., 2007; Haneklaus et al., 2007, 2009). For most S containing metabolites a direct antifungal mode of action was proven (Table 2). Cysteine is the main precursor for all S containing compounds and is directly linked to stress response via its function related to systemic acquired resistance (Luckner, 1990). Cysteine displays a regulatory function in pathogen defense. It was shown that a specific cytosolic cysteine content is mandatory for the initiation of the plant immune response to pathogens and a link to the hypersensitive response (HR) was proven (Alvarez et al., 2012).

Glutathione displays a central function in plant defense as well: it is an important redox buffer in cells as it exists in a reduced form (GSH) which can react with another molecule of reduced glutathione (GSH) to form the oxidized disulfide form (GSSG) and which is restored by the enzyme glutathione reductase (Leustek et al., 2000). The ratio of reduced to oxidized glutathione delivers already an important information as it decreases under stress conditions that consume reducing equivalents. Moreover, glutathione is supposed to be involved in stress signaling, the detoxification of xenobiotics, it is the precursor of phytochelatines, which are important for heavy metal detoxification, acts as transport and storage form of reduced S and has a regulatory function in S assimilation (Leustek et al., 2000). These manifold functions illustrate the major importance of glutathione in plant S metabolism and stress response.

A direct antifungal mode of action was determined for S-rich proteins, phytoalexins such as camalexin, elemental S and the degradation products build from glucosinolates (Mithen, 1992; Kuć, 1994; Cooper et al., 1996; Wallsgrove et al., 1999; Hughes et al., 2000; Williams et al., 2002; Stet et al., 2004; Glawischnig, 2007). The toxicity of S-rich proteins such as thionins is explained by their ability to generate ion channels in cell membranes of pathogens and by the disturbing ion concentration gradients and cellular homeostasis (Shai, 1999; Hughes et al., 2000).

The antifungal mode of action of S0 can be explained by its lipophilic character. S0 may enter directly through the fungal cell wall disturbing redox reactions in the metabolism of the pathogen (Beffa, 1993). Beffa (1993) suggested that the fungicidal action of S0 is mainly related to the oxidation of important sulfhydryl...
Table 1 | Discoveries and progress in plant sulfur (S) research with respect to sulfur induced resistance (SIR) during the twentieth century.

| Year | Scientific discoveries | References |
|------|------------------------|------------|
| 1802 | William Forsyth discovered the fungicidal effect of elemental S | Forsyth, 1802 |
| 1860 | S was recognized as an essential plant nutrient, required for growth | Woodard, 1922 |
| 1872 | Robert Angus Smith coined the term “acid rain” | Seinfeld and Pandis, 1998 |
| 1956 | The common structure of glucosinolates was discovered | Ettlinger and Lundeen, 1996 |
| 1973 | Elucidation of the major steps in glucosinolate biosynthesis | Underhill et al., 1973 |
| 1976 | First description of the Foyer-Halliwell-Asada-cycle | Foyer and Halliwell, 1976 |
| 1977 | Agrobacterium tumefaciens-mediated gene transfer | Chilton et al., 1977 |
| 1979 | SO₂ exposure increase the glutathione content in sensitive trees | Grill et al., 1979 |
| 1982 | Description of the glutathione metabolism in higher plants and its function in transport, storage and detoxification of xenobiotics | Rennenberg, 1982 |
| 1984 | Detection of hydrogen sulfide (H₂S) emissions from leaf tissue in response to L-cysteine feeding | Sekiya et al., 1982 |
| 1986 | Demonstration that leaf glucosinolates of Brassica napus can control fungal infection by Leprosphora maculans | Mithen et al., 1986 |
| 1989 | Plants can take up and use atmospheric H₂S as S source | De Kok et al., 1989 |
| 1990 | Localization of the γ-glutamylcysteine synthetase in higher plants | Hell and Bergmann, 1990 |
| 1994 | The term “sulfur induced resistance” (SIR) was introduced after field trials unraveled a relationship between S nutrition and plants susceptibility toward fungal diseases | Schnug et al., 1995 |
| 1995 | Significance of glutathione in plants under stress was demonstrated | Rennenberg and Brunold, 1994 |
| 1996 | Concept of “biofumigation” was developed | Angus et al., 1994 |
| 1999 | Overexpression of serineacetyltransferase (SAT) caused increased cysteine and glutathione contents accompanied by an increased resistance to oxidative stress | Blaszczyk et al., 1999 |
| 2000 | Interaction of sulfate reduction with N nutrition and major role of O-acetylserine in this regulation was shown at the transcriptional level | Koprivova et al., 2000 |
| 2001 | Identification and biochemical characterization of Arabidopsis thaliana sulfite oxidase | Eilers et al., 2001 |
| 2003 | Application of DNA macroarray technique to investigate the gene-to-metabolite networks regulating the S metabolism of Arabidopsis | Hirai et al., 2003 |
| 2004 | The regulatory function of the O-acetylserine(thiol)lyase (OAS-TL) in the S assimilation pathway was shown | Wirtz et al., 2004 |
| 2005 | Introduction of the term “sulfur enhanced defense” (SED) | Rausch and Wachter, 2005 |
| 2006 | Higher susceptibility of S deficient oilseed rape for different pathogens | Dubuis et al., 2005 |
| 2009 | Identification of PAD2 as a γ-glutamylcysteine synthetase and the importance of glutathione in pathogen defense | Parisy et al., 2006 |
| 2012 | Indole glucosinolate biosynthesis and hydrolysis is required for callose accumulation in response to microbial pathogens | Clay et al., 2009 |
| 2012 | A shift from plant COS uptake to COS release with fungal infection | Clay et al., 2009 |
| 2012 | Regulatory role of cytosolic cysteine/cytosolic OAS-TL in plant immune response | Alvarez et al., 2012; Tahir et al., 2013 |

Native glucosinolates display no fungal toxicity in contrast to their hydrolysis products, the isothiocyanates (ITC), which display a strong antimicrobial activity (Manici et al., 1997). Fungal inhibition is caused by irreversible reactions of ITC’s with functional groups of proteins resulting in enzyme inactivation (Brown and Morra, 1997). In accordance with this not only the biosynthesis of indole glucosinolates was up-regulated by ethylene signaling after pathogen recognition in Arabidopsis but also the expression of myrosinase enzymes which catalyze their hydrolysis (Clay et al., 2009). Additionally the biosynthesis of indole glucosinolates was shown to be required for callose depositions in response to microbial pathogens (Clay et al., 2009). Therefore,
glucosinolate biosynthesis seems to be involved in pathogen defense in more than one way in glucosinolate containing plants.

The concentrations of all S containing metabolites, the amino acids cysteine and methionine as well as primary and secondary S compounds were reduced with S deficiency or can be increased by S fertilization (Salac et al., 2005; Bloem et al., 2007). It was observed that the gas exchange of H\textsubscript{2}S and carbonyl sulfide (COS) between plants and atmosphere changed in relation to S supply and fungal infection. As long as enough S is available plants release H\textsubscript{2}S into the atmosphere. This happens most likely to build glutathione under these conditions. H\textsubscript{2}S exchange shifted from uptake, indicated by the negative value in S deficient plants, to H\textsubscript{2}S release in the fertilized ones. COS was taken up in S fertilized as well as in non-fertilized plants.

Amongst others flavonoids and phenolics are shown to be major biochemical marker against fungal infections (Shanmugam et al., 2010; Datta and Lal, 2012). Cell wall strengthening is another important resistance response against fungi as it helps to inhibit pathogen entry. Accumulation of cell wall-bound phenolics, the monomers of lignin, is part of this process (Swarupa et al., 2010; Datta and Lal, 2012). Cell wall strengthening is another biochemical marker against fungal infections (Huckelhoven, 2007). Moreover several studies show a close link between the S metabolism, mineral deficiency or increased internal demand and hormonal signaling by methyl jasmonate and possibly other hormones (Hirai et al., 2003; Saito, 2004; Jost et al., 2005) (Figure 1).

Table 3 gives an example which changes occur in the S metabolism in response to S fertilization and fungal infection (Bloem et al., 2012). Brassica napus was artificially infected by Sclerotinia sclerotiorum and the plants displayed strong symptoms of S deficiency without S application.

With increasing S supply total S and \textsubscript{SO}_{4}-S increased in leaves as well as the cysteine and glutathione content (Table 3). Only \textsubscript{γ}-glutamylcysteine, which is an intermediate in the biosynthesis of glutathione, was lower with S fertilization indicating to a fast turn-over to build glutathione under these conditions. H\textsubscript{2}S exchange shifted from uptake, indicated by the negative value in S deficient plants, to H\textsubscript{2}S release in the fertilized ones. COS was taken up in S fertilized as well as in non-fertilized plants.

### Table 3: Possible mode of action of S-containing plant compounds in stress resistance and in response to fungal infection.

| Compound          | Mode of action in stress resistance and after fungal infection                                                                 | References                  |
|-------------------|-------------------------------------------------------------------------------------------------------------------------------|-----------------------------|
| Cysteine          | –Precursor for all relevant S containing metabolites<br>–Cytosolic cysteine has a regulatory function in the establishment and signaling of the plant response to pathogens<br>–Increase with fungal infection<br>–Link to salicylic acid and by this to systemic acquired resistance via CoASH and essential for the initiation of the hypersensitive response (HR) | Luckner, 1990; Bloem et al., 2007; Alvarez et al., 2012 |
| Glutathione       | –Participation in antioxidative defense<br>–Detoxification of xenobiotics by targeting them into the vacuole<br>–Involved in phytochelatine biosynthesis/ detoxification of heavy metals<br>–Messenger in the hypersensitive response (HR)<br>–Fast accumulation after fungal attack | Edwards et al., 1991; Rea et al., 1998; Leustek and Saito, 1999; Cobbett, 2000; Foyer and Rennenberg, 2000; Vanacker et al., 2000 |
| S-containing volatiles | –H\textsubscript{2}S causes disturbances in redox reactions<br>–Release of H\textsubscript{2}S and COS increased with fungal infections | Bloem et al., 2007, 2012 |
| S-rich proteins   | –Pathogen-induced or constitutive expression (defensins)<br>–Thionins are enhanced locally and systemically after infection<br>–Toxic mode of thionins: disruption of the cell wall structure; generation of ion channels | Hughes et al., 2000; Stec et al., 2004; Kruse et al., 2007 |
| Phytalexins       | –De-novo synthesis after pathogen attack | Kuc\textsuperscript{a}, 1994 |
| S\textsuperscript{0} | –S\textsuperscript{0} accumulates after fungal infection in vascular tissue<br>–Disturbances of the respiratory chain<br>–Oxidation of sulfhydryl groups | Beffa, 1993; Cooper et al., 1996; Williams et al., 2002 |
| Glucosinolates    | –Their degradation products (isothiocyanates) exhibit a toxic and repellent effect → reason for its use in biofumigation | Mithen, 1992; Wallsgrove et al., 1999 |
Infection with *S. sclerotiorum* caused significant changes in the S metabolism. The total S content decreased as well as cysteine and γ-glutamylcysteine while glutathione significantly increased. Additionally plants were analyzed for their potential to take up or release H$_2$S and COS in the first days after infection (Bloem et al., 2012). In Table 3 the data from 2 days after infection are shown when the strongest plant response was observed. H$_2$S release was significantly increased by infection. The change in COS was even more striking as COS was changed from uptake to release (Bloem et al., 2012). The data clearly revealed that plants responded to the infection by several changes in their S metabolism. Nevertheless, the visual scoring revealed that the infection rate was not reduced by the higher S supply at this stage of infection (see Bloem et al., 2012).

Likewise Raj and Srivastava (1977) showed that the total S content of infected tissue of *Brassica juncea* was inversely correlated with the pathogenicity of different isolates of *Macrophomina phaseolina* and suggested that the pathogens are able to...
A direct relationship between fungal infection and S metabolism as shown exemplary in Figure 1 was also found for other host-pathogen interactions. Infection of oilseed rape with *Pyrenopeziza brassicae* increased the cysteine and glutathione content in leaves as well as the activity of the L-cysteine-desulphydrase, an enzyme that releases H2S during cysteine degradation (Bloem et al., 2004). A higher release of H2S after fungal infection was determined in grapes (*Vitis vinifera* L.) infected with *Uncinula necator* (Bloem et al., 2007). Gaseous S compounds seem to be involved in stress response but to date their function is not fully understood. A possible role could be in stress signaling or as regulatory compounds comparable to the effect in mammalian cells where H2S is involved in the regulation of the intracellular redox-homeostasis and glutathione generation (Ju et al., 2013).

Also Kruse et al. (2007) determined a steep and fast increase not only for H2S, but also for cysteine, glutathione and phytoalexins during the initial phase of pathogenesis. The important role of cysteine in pathogenesis was proven by Alvarez et al. (2012) and Tahir et al. (2013). Alvarez et al. (2012) could show that mutants with increased cytosolic cysteine content are resistant to biotrophic as well as necrotrophic pathogens, while mutants with decreased cytosolic cysteine contents are more susceptible. Also Tahir et al. (2013) found that decreased cytosolic cysteine contents resulted in enhanced disease susceptibility against infection with virulent and non-virulent *Pseudomonas syringae* strains.

Though the sequence, magnitude and efficacy of all individual S metabolites involved in the activation and strengthening of plant defenses by S fertilization are not yet fully known, these could be released in a chain reaction triggered by the pathogen and mediated by the S status of the plant (Haneklaus et al., 2006). It seems possible that infection triggers the activation of all effective resistance mechanisms of the host.

Trials where the effect of S nutrition on fungal infection was studied are compiled in Table 4. Plant pathogens are often divided into biotrophs and necrotrophs despite of the fact that there are several transitions. Biotrophs feed on living host tissue while necrotrophs cause die-off and feed on the remains (Glazebrook, 2005). SIR was proven for biotrophic and necrotrophic pathogens (Table 4). Different mechanisms in pathogen response are important for biotrophic and necrotrophic pathogens and a schematic model is summarized in Figure 2.

Generally defense reactions that cause the die-off of cells such as oxidative burst and hypersensitive response (HR) are only beneficial when repelling the attack of a biotrophic pathogen. In contrast, it is not predicted that the cell death of a host plant will limit the growth of necrotrophic pathogens. It is the opposite way round; necrotrophic fungi can elicit a defense response such as oxidative burst in a susceptible host plant causing necrosis (Winterberg et al., 2014). SA dependent defense is more frequently observed against biotrophs and JA/ET dependent defense against necrotrophs but there are exceptions. The fact that pathogenesis-related proteins are not expressed and JA dependent signaling is not activated against a special biotrophic pathogen does not mean that they are not active in case they are triggered (Glazebrook, 2005). S containing compounds are involved in both defense lines (see also Figure 1). Glutathione is involved in detoxification of ROS, many pathogenesis-related proteins contain S (phytoalexins, thionins, defensins) and SA needs coenzyme A (CoASH) as a precursor. Therefore, it is hardly possible to predict the efficacy of S against special fungi based on the lifestyle of the pathogen.

It is hard to explain why in some trials a clear relationship between the S supply and the extent of fungal infection was found whilst in others with the same pathosystem no such response was observed. Probably it is the timing and extent of plants defense response which decides over resistance or susceptibility while the nutritional status of the crop determines the extent of defense. Moreover, the type of pathogen and its pathogenicity, infection severity and other environmental factors are important as well.

**PRACTICAL RELEVANCE OF SIR**

Optimizing the S nutritional status of a plant is equivalent of enhancing the capability of a plant to cope with stress. The identification of the mechanisms causing SIR is an important...
Table 4 | Influence of soil S application on pathogen development of different host pathogen interactions.

| Host                  | Pathogen                  | Pathogen classification | Lifestyle | Trial | S-fertilization         | Change in pathogen development                                                                 | References |
|-----------------------|---------------------------|-------------------------|-----------|-------|-------------------------|-------------------------------------------------------------------------------------------------|------------|
| CONFIRMED SIR         |                           |                         |           |       |                          |                                                                                                |            |
| Brassica napus L.     | Sclerotinia sclerotiorum  | A                       | necrotrophic (heterotrophic) | PT     | 120 mg S kg\(^{-1}\) soil | Disease index (DI) was reduced by 5% in comparison to a control without S application             | Wang et al., 2003 |
| Zea mays              | Bipolaris maydis (Southern leaf blight) | A                       | necrotrophic | PT     | 120 mg S kg\(^{-1}\) soil | DI was reduced by 37% in comparison to a control without S application                             | Wang et al., 2003 |
| Brassica napus L.     | Botrytis cinerea (Gray mold) | A                       | necrotrophic | VWC    | 0.5 mM MgSO\(_4\)       | Lesions were 24-times larger in S-starved plants of cultivar Express and 3.7-fold larger in cultivar Bienvenue | Dubuis et al., 2005 |
| Brassica napus L.     | Leptosphaeria maculans    | A                       | facultative necrotrophic, initially biotrophic | VWC    | 0.5 mM MgSO\(_4\)       | Lesions were 1.9-times larger in S-starved plants (cultivar Bienvenue)                           | Dubuis et al., 2005 |
| Arabidopsis thaliana  | Alternaria brassicicola   | A                       | necrotrophic | WC     | 50 μM vs. 500 μM SO\(_4\) | DNA from A. brassicicola was 3-time more abundant on plants grown on 50μM SO\(_4\) in comparison to plants grown on 500μM                         | Kruse et al., 2012 |
| Brassica napus L.     | Pyrenopeziza brassicae    | A                       | hemi-biotrophic | FT     | Plots with and without S fertilization | A non-resistant and a resistant oilseed rape variety were compared with and without S application and fungicide treatment: the non-resistant variety showed a much stronger response to fungicide under S deficiency | Schnug et al., 1995 |
| Solanum lycopersicum L. | Verticillium dahliae     | A                       | hemi-biotrophic | WC     | 0.016 mM vs. 25 mM K\(_2\)SO\(_4\) | Supra-optimal S supply significantly reduced the number of infected cells and the amount of V. dahliae gDNA in vascular tissue of the hypocotyl | Bollig et al., 2013 |
| Vitis vinifera L.     | Uncinula necator          | A                       | obligate biotrophic | FT     | 250 or 500 kg S\(^{0}\) ha\(^{-1}\) (soil applied) | Proportion of infected leaves and berries decreased by more than 90% with soil S application | Haneklaus et al., 2007 |
| Solanum tuberosum L.  | Rhizoctonia solani       | B                       | necrotrophic | FT     | 50 kg S\(^{0}\) ha\(^{-1}\) (soil applied) | Soil applied S\(^{0}\) reduced infection rate by 41% in comparison to control without S application | Klikocka et al., 2005 |
| Triticum aestivum L.  | Rhizoctonia cerealis     | B                       | necrotrophic | PT     | 120 mg S kg\(^{-1}\) soil | DI was reduced by 44% in comparison to a control without S application                               | Wang et al., 2003 |
| Brassica napus L.     | Peronospora parasitica    | O                       | obligate biotrophic | FT     | 100 kg S ha\(^{-1}\) | Decrease in disease incidence and severity was found                                              | Salac et al., 2005 |
| Brassica napus L.     | Phytophthora brassicae    | O                       | hemi-biotrophic | VWC    | 0.5 mM MgSO\(_4\)       | Lesions were 3.3-times larger in S-starved plants of cultivar Express                             | Dubuis et al., 2005 |

(Continued)
CONFLICTING RESULTS OF S FERTILIZATION ON PATHOGEN DEVELOPMENT

| Host     | Pathogen                        | Lifestyle     | Trial | S-fertilization | Change in pathogen development | References |
|----------|---------------------------------|---------------|-------|-----------------|--------------------------------|------------|
| Gossypium L. | Fusarium oxysporum             | Neocortexic   | PT    | 160 mg S kg⁻¹  | Soil DI was reduced by 8% in comparison to a control, but with higher S application rates (40, 80, 120 mg S kg⁻¹ soil) DI increased again and was significantly higher than in the control, depending on season, year, and site. | Wang et al., 2003 |
|          |                                 |               | FT    | 100 kg S ha⁻¹  | Soil DI was reduced by 47% in comparison to a control, but with higher S application rates (40, 80, 120 mg S kg⁻¹ soil) DI increased again and was significantly higher than in the control, depending on season, year, and site. | Salac et al., 2005 |
|          |                                 | Hemibiotrophic| PT    | 40 mg S kg⁻¹  | Soil DI was significantly reduced when 160 mg S kg⁻¹ soil were applied. | Salac et al., 2005 |
|          |                                 | Facultative   | FT    | 100 kg S ha⁻¹  | Soil DI was significantly reduced when 160 mg S kg⁻¹ soil were applied. | Salac et al., 2005 |

Pathogen classification: A, Ascomycete; B, Basidiomycete; O, Oomycete.

Trial: PT, Pot trial; WC, Water culture; FT, Field trial; VWC, Vermiculite water culture.

OPEN QUESTIONS AND FUTURE PROJECTIONS

It is generally difficult to assign a change in plant metabolism to a specific stress factor, as usually a variety of abiotic and biotic stress factors occur at the same time and can induce antagonistic responses resulting in accumulation, degradation, and consumption of primary and secondary metabolites. Therefore, standardized experimental conditions are important to improve comparability of results.

Moreover, more field studies and infection trials are necessary accompanied by molecular research to unravel the relationship between S supply and fungal infection and by this to enable researchers and farmers to adopt the results into new fertilizer concepts. There are many unknowns affecting plant response such as timing of application, kind of fungal pathogen, crop species, climatic conditions, and cross-talk with other macro- and micronutrients. Therefore, to date it is not possible to induce a stress response by S application that will certainly reduce or prevent a crop from fungal infection. It is necessary to further elucidate the cross-talk between different pathways to understand which other parameters need to be optimized in order to reach the full potential of plants own pathogen defense.

Much more work in the field of phytopathology is necessary to solve the questions why only some pathogens are affected by S nutrition and which is the exact mode of action by which the S supply is affecting fungal pathogens. Could be the lifestyle (biotrophic, necrotrophic, heterotrophic) of a pathogen important for plants defense in relation to S or is the timing of infection in relation to plant development most important for the course of infection? Which part of metabolite changes is caused by the host and which one by the pathogen? These are only some of the manifold topics and questions where further research is necessary.

Moreover, it is not possible to transfer all results obtained from research with model plants such as Arabidopsis to other species so that studies on major agricultural crops are important especially as most agricultural crops do not contain glucosinolates.

Nevertheless, the manifold results, which point to a relationship between S nutrition and crop resistance, indicate that in future the crucial factors will be identified. There are still some agricultural diseases where the efficiency of chemical fungicides is limited. For example currently, no fungicides are available milestone for a sustainable agricultural production as the input of fungicides can be minimized by crop specific S fertilization and a higher resistance due to S will not be rapidly broken by new pathotypes. It is possible to optimize the S nutrition without understanding all mechanisms underlying SIR. For winter crops a first S application in autumn was shown to be advantageous with respect to disease resistance followed by the regular S application in spring. An increasing S supply is associated with higher contents of cysteine, glutathione, H₂S, and glucosinolates in Brassica crops so that plants with a higher content of phytoanticipins might not only have a priori a better protection against pathogens, but also be able to activate resistance mechanisms more rapidly and intensely. In addition, an instantly high S supply satisfying the elevated S demand after a fungal attack may play a pivotal role in SIR, even when the nutrient demand of the crop is well exceeded by such an S application (Haneklaus et al., 2009).
FIGURE 2 | Model of the response of plants to biotrophic and necrotrophic plant pathogens (adapted from Glazebrook (2005); displayed are the interactions of *Arabidopsis* with the biotrophs *Peronospora parasitica* and *Erysiphe* ssp. and with the necrotrophs *Alternaria brassicicola* and *Botrytis cinerea*; SA, salicylic acid; JA, jasmonic acid; ET, ethylene; broken line arrows indicate to a possible interaction but which was not found in the chosen experiments while the solid line arrows indicate to the observed plant-pathogen-response). The defense reaction of *Arabidopsis* against biotrophic pathogens start with gene-for-gene recognition of the pathogen followed by rapid activation of defense and the production of reactive oxygen species (ROS), the so-called “oxidative burst,” which is by self a signal for defense activation. ROS production is connected with the hypersensitive response (HR), also called “programmed cell death,” which limits the access of biotrophs that feed on living tissue to water and nutrients. HR is associated with the activation of the salicylic acid (SA) dependent signaling pathway that is connected with systemic acquired resistance (SAR) and the expression of pathogenesis-related proteins. For necrotrophic pathogens a different defense line takes place as they feed on dead plant tissue and host cell death is not predicted to limit their growth. Defense against necrotrophic pathogens is mainly mediated by JA and ET controlled defense as well as production of phytoalexins such as camalexin. The broken line arrows indicate that also mixed defense lines are possible for other biotrophic or necrotrophic pathogens.

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to control *Verticillium* wilt. Therefore, fertilizer strategies which improve the plants potential and resistance against fungal diseases are still of high importance not only in organic farming but in conventional agriculture as well.
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