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Influence of sulfur fertilization on sulfur metabolites, disease incidence and severity of fungal pathogens in oilseed rape in Scotland

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Abstract

A harmonic mineral nutrition of plants that fully satisfies their nutrient demand is an important component of natural resistance mechanisms against biotic and abiotic stress. In Scotland, where atmospheric sulfur (S) depositions are extremely low, the infection pressure of various fungal pathogens proved to be higher than in regions with a higher S input. Soil applied S fertilization as sulfate significantly increased the natural resistance against fungal pathogens, but the trigger mechanisms are so far unknown and consequently, S induced resistance (SIR) can not be prompted regularly by S amendments.

Field experiments were conducted in Aberdeen and Inverness from 2000 to 2003 in order to evaluate the relationship between S applications and oilseed rape resistance to the three main fungal pathogens in this area (i.e. *Pyrenopeziza brassicae*, *Leptosphaeria maculans*, *Peronospora parasitica*). Generally, significant differences existed between sites and seasons with view to the infection of oilseed rape plants with fungal pathogens. In all three years and on both experimental sites, disease progression, expressed as disease incidence and disease severity, was not consistently reduced by S applications. However, a positive effect of S fertilization in spring on infections with *P. parasitica* was found on both sites in 2002. S fertilization increased the cysteine (0.70 µmol g^{-1} vs. 1.07 µmol g^{-1}), glutathione (15.2 µmol g^{-1} vs. 18.5 µmol g^{-1}) and glucosinolate content (3.81 µmol g^{-1} vs. 4.76 µmol g^{-1}) in young fully developed leaves of oilseed rape. Several lines of evidence show that these S compounds play an important role in the plant defence reactions. Factors possibly related to the initiation of SIR under natural conditions are discussed.

Key words: cysteine, glutathione, Leptosphaeria maculans, oilseed rape, Peronospora parasitica, Pyrenopeziza brassicae, Sulfur Induced Resistance (SIR)

Zusammenfassung

Einfluss der Schwefeldüngung zu Raps auf schwefelhaltige Metabolite und den Befall mit pilzlichen Erregern in Schottland

Eine harmonische Mineralstoffernährung ist ein wichtiger Bestandteil der natürlichen Resistenz von Pflanzen gegenüber biotischem und abiotischem Stress. In Schottland sind die natürlichen Schwefeleinträge extrem niedrig und der Infektionsdruck pilzlicher Erreger höher als in Regionen mit höheren Schwefelimmissionen. Bodenapplizierte Schwefeldüngung als Sulfat erhöhte die natürliche Resistenz gegenüber pilzlichen Schaderregern, wobei die induzierenden stoffwechselphysiologischen Mechanismen bislang unbekannt sind, so daß es derzeit nicht möglich ist Schwefel-Induzierte Resistenz (SIR) regelmäßig durch Düngung auszulösen.

In Aberdeen und Inverness wurden von 2000 bis 2003 Feldversuche durchgeführt, um den Einfluss der Schwefelzufuhr auf die Resistenz gegenüber den drei häufigsten pilzlichen Krankheiten (*Pyrenopeziza brassicae*, *Leptosphaeria maculans*, *Peronospora parasitica*) zu bestimmen. Die Rapspflanzen zeigten signifikante Unterschiede im Befall mit Pilzkrankheiten in Abhängigkeit vom Standort und Versuchsjahr. In allen drei Versuchsjahren führte die Schwefeldüngung auf beiden Standorten zu keiner konsistenten Verbesserung des Krankheitsverlaufs, ausgedrückt als Abnahme in der Befallsrate und –intensität. Im zweiten Versuchsjahr konnte jedoch ein positiver Einfluss der Schwefeldüngung auf den Befall mit *Peronospora parasitica* im Frühjahr festgestellt werden. Die Schwefeldüngung führte zu einem signifikanten Anstieg der Cystein- (0.70 µmol g^{-1} vs. 1.07 µmol g^{-1}), Glutathion- (15.2 µmol g^{-1} vs. 18.5 µmol g^{-1}) und Glucosinolatgehalte (3.81 µmol g^{-1} vs. 4.76 µmol g^{-1}) in jüngeren voll ausdifferenzierten Blättern. Bisherige Untersuchungen haben gezeigt, daß diese Metabolite eine wichtige Rolle bei der Abwehr der Pflanze von Schaderregern spielen. Faktoren, die möglicherweise SIR unter Feldbedingungen initiieren, werden diskutiert.

Schlüsselworte: Cystein, Glutathion, Leptosphaeria maculans, Peronospora parasitica, Pyrenopeziza brassicae, Raps, Schwefel-Induzierte Resistenz (SIR)

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1 Introduction

There is a limited number of well-described host-pathogen interactions for which the role of individual plant nutrients such as N, P and K is well established (Huber 1991). Information about the interactions of S and diseases is limited, and results in relation to the S metabolites putatively involved in Sulphur Induced Resistance (SIR) are often inconsistent and in some cases apparently controversial (Salac et al. 2005). Sulphate assimilation primarily results in the amino acid cysteine, itself showing fungicidal effects (Vidhyasekaran 2000) and being the basic compound for other S metabolites, such as glutathione, glucosinolates, phytoalexins, pathogenesis related proteins and H_2S, possibly linked to resistance mechanisms of plants (Haneklaus et al. 2005).

The atmospheric S depositions are extremely low in Scotland (Dore et al. 2003) and the infection pressure from fungal pathogens is high. Light leaf spot (*Pyrenopeziza brassicae*), stem canker (*Leptosphaeria maculans*) and downy mildew (*Peronospora parasitica*) are the main fungal diseases in oilseed rape, which cause considerable productivity losses each year (Fig. 1; Fitt et al. 1997).

The aim of the present experiments was to establish the effect of S fertilization on S-containing metabolites, to determine the influence of the S supply on disease incidence and severity of relevant fungal diseases of oilseed rape in Scotland, and last but not least to evaluate the results obtained with a view to prompting and controlling SIR under field conditions.

2 Materials and Methods

A quadri-factorial field experiment was carried out in Aberdeen and Inverness from 2000 to 2003. The experimental design and analytical methods are described comprehensively by Salac (2005). The progression of fungal diseases was recorded regularly during the whole growth period. Plants were visually rated for disease incidence (%-age of plants infected) and disease severity (%-age of leaf area infected). Younger, fully developed leaves of winter oilseed rape were randomly taken from each plot at

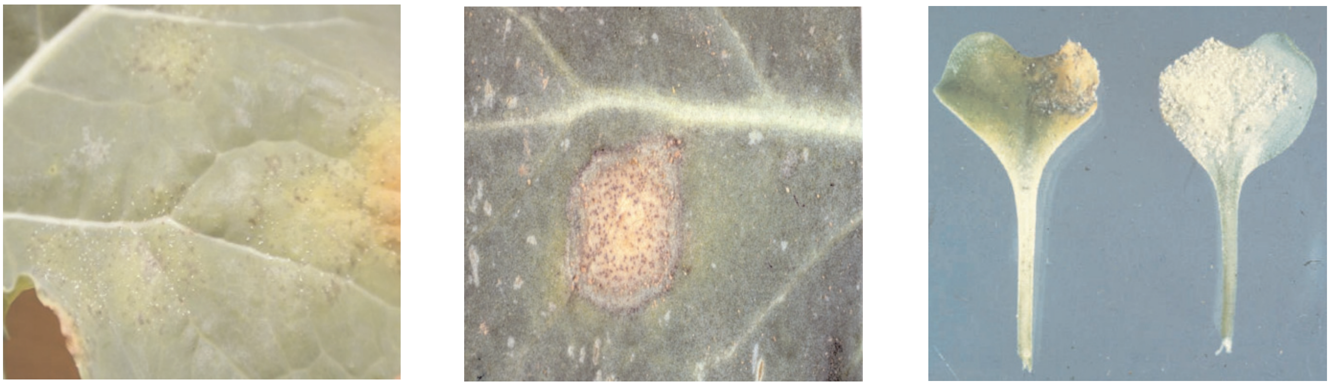

Fig. 1:
Macroscopic symptoms of infections of oilseed rape leaves by *P. brassicae*, *L. maculans* and *P. parasitica* (from left to right).

Table 1:
Influence of S fertilization on the cysteine, GSH and total GSL content in younger, fully developed leaves (d.w.) of winter oilseed rape at the start of stem elongation (GS 3.3 - 3.5) during three vegetation periods (2000 - 2003) in Aberdeen and Inverness.

Variable/Treatment		Aberdeen			Inverness		
		2000/2001	2001/2002	2002/2003	2000/2001	2001/2002	2002/2003
Cysteine	***S0***	1.04	0.62	0.71	0.81	0.50	0.54
	S100	1.19	0.91	0.83	1.46	1.25	0.74
	$LSD_{5\%}$	0.24	0.09	0.05	0.11	0.16	0.12
GSH	***S0***	19.5	21.1	11.3	11.9	12.9	14.2
	S100	19.2	27.5	11.0	11.9	30.5	10.6
	$LSD_{5\%}$	3.89	1.39	1.69	1.71	5.03	1.87
GSL	***S0***	3.78	3.83	4.26	5.88	2.59	2.49
	S100	4.07	4.35	4.97	7.90	4.20	3.11
	$LSD_{5\%}$	0.60	0.95	0.49	0.77	0.73	0.65

note: S0 = 0 kg ha^{-1} S; S100 = 100 kg ha^{-1}

the start of stem elongation (GS 3.3 - 3.5). After shock-freezing in liquid N and freeze-drying, the leaf material was fine-ground in a coffee mill prior to the analysis of the free cysteine, glutathione (GSH) and glucosinolate (GSL) content. For statistical analysis the SPSS software package version 10 was employed (SPSS, 1999). Statistically significant differences between means were determined by employing the t-test ($LSD_{5\%}$).

3 Results and Discussion

In the present field experiments S fertilization significantly increased the cysteine and GSL content in young leaves of oilseed rape (Table 1). The effect of S applications on the GSH content was not consistent, and varied annually (Table 1). Only in the second year of experimentation did S fertilization significantly increase the GSH

Table 2:
Correlation coefficients (r) for the relationship between disease incidence and disease severity of *P. brassicae*, *L. maculans* and *P. parasitica* at the start of stem elongation (GS 3.3 - 3.5) during three vegetation periods (2000 - 2003) in Aberdeen and Inverness.

Incidence (%)	Aberdeen			Inverness		
	2000/2001	***2001/2002***	***2002/2003***	***2000/2001***	***2001/2002***	***2002/2003***
	Severity (%)					
P. brassicae	***P. brassicae***					
	0.518***	0.501***	0.560***	0.941***	0.262*	0.853***
L. maculans	***L. maculans***					
	0.961***	0.819***	n.s.	n.s.	n.s.	n.s.
P. parasitica	***P. parasitica***					
	n.s.	0.838***	0.707***	0.851***	0.860***	0.960***

note: n.s. – not significant

Table 3:
Effect of S fertilization (S0 – S100) on disease incidence and severity of infections with fungal pathogens before winter (GS 1.1 – 1.12) and at the start of stem elongation (GS 3.3 – 3.5) during three vegetation periods in Aberdeen and Inverness (non-fungicide treated plots).

		2000/2001		***2001/2002***		***2002/2003***	
		1.1 - 1.12	***3.3 - 3.5***	***1.1 - 1.12***	***3.3 - 3.5***	***1.1 – 1.12***	***3.3 - 3.5***
		Aberdeen					
P. brassicae	***I***	n.i.	-5.1	-5.0	-6.9	-20	-5.0
	S	n.i.	-4.0	-0.24	-0.2	-3.69	1.65
L. maculans	***I***	n.i.	-0.63	5.0	0.63	n.i	n.i.
	S	n.i.	-0.01	0.07	0	n.i.	n.i.
P. parasitica	***I***	n.i.	n.i.	5.0	5.0	n.i.	n.i.
	S	n.i.	n.i.	0.03	0.06	n.i.	n.i.
		Inverness					
P. brassicae	***I***	n.i.	-4.4	-5.0	0	25	-3.1
	S	n.i.	-0.17	-0.45	2.5	1.9	0.17
L. maculans	***I***	n.i.	0	n.i.	n.i.	n.i.	n.i.
	S	n.i.	0.03	n.i.	n.i.	n.i.	n.i.
P. parasitica	***I***	n.i.	n.i.	5.0	3.1	35	n.i.
	S	n.i.	n.i.	0.09	0.04	0.15	n.i.

note: I – incidence; S – severity; n.i. – no infection; negative values indicate a higher disease incidence and severity in S fertilized plots; positive values indicate a reduction of infection levels by S fertilization; S0 = 0 kg ha^{-1} S; S100 = 100 kg ha^{-1}

content on both sites. Changes in the GSH content are known to be related to various environmental factors (Tausz et al. 2003) and investigations of Salac et al. (2004) showed this to be the case for the infection severity of *L. maculans*.

During experimentation, *P. brassicae* caused the most severe symptoms. Ratings for infection rates and severity varied within each season and between sites, with generally higher values recorded in Aberdeen. On an average, the disease incidence and severity of *P. brassicae* at the start of stem elongation (GS 3.3 – 3.5) was of 87 % and 9.9 %, respectively in Aberdeen and of 39 % and 3.0 %, respectively in Inverness. Values of >25 % of plants infected by *P. brassicae* at stem extension indicate a severe infection (Steed and Fitt, 2000). The correlation analysis revealed significant positive relationships between disease incidence and disease severity for *P. brassicae* and *P. parasitica* (Table 2). In the case of *L. maculans*, no relationship was found on the experimental site in Inverness.

The relative effect of S fertilization on disease incidence and severity of *P. brassicae, L. maculans* and *P. parasitica*, which were assessed before winter (GS 1.1 - 1.12) and at the start of stem elongation (GS 3.3 - 3.5) is shown in Table 3.

The values reflect differences between score values determined for control plots and those plots which received S dressings. The results reveal that in all three years and on both experimental sites S fertilization in autumn and in spring did not prompt generally SIR against the three pathogens. In the case of *P. parasitica* a detrimental effect of S applications on disease incidence and severity was found on both sites in 2002 (Table 3).

A positive effect of S fertilization on the infection severity of *P. brassicae* was noted in 2003. The limited effect of S applications might be related to pathogen specific differences in infecting the plant and thereby in the initiation of response mechanisms. There is circumstantial evidence that plants activate specific defence compounds in response to each type of challenge (Stout and Bostock 2000). In addition, other important conditions for the efficiency of SIR might be the infection pressure of individual pathogens and the number of coincidental stressors. The timing, the form and doses of S applications might also be significant under monofactorial host-pathogen conditions. The results of Salac et al. (2005) revealed that the changes in the concentration of S metabolites in infected plant tissues were related to a certain disease severity. A detailed knowledge about the role and efficacy of S-containing metabolites in crop resistance to fungal pathogens and their triggers is vital for taking advantage of SIR by targeted fertilizer strategies.

References

Bloem EM, Riemenschneider A, Volker J, Papenbrock J, Schmidt A, Salac I, Haneklaus S, Schnug E (2004) Sulphur supply and infection with *Pyrenopeziza brassicae* influence L-cysteine desulphydrase activity in *Brassica napus* L. J Exp Bot 55(406):2305-2312

Dore A, Heywood E, Vieno M, Fournier N, Smith R, Weston K, Hall J, Sutton M (2003) Status Report to DEFRA

Fitt BDL, Gladders P, Turner JA, Sutherland KG, Welham SJ, Davies JML (1997) Asp Appl Biol 48:135-142

Haneklaus S, Bloem E, Schnug E (2005) In: Datnoff L et al (eds) (2005) Mineral elements and plant disease. Minneapolis : APS Press (in press)

HGCA (2003) HGCA Recommended List WOSR 2003[online]. Available at <http://www.oilseedrape.com/pages/trial/dis_res_tables.htm> [cited 2003]

Huber DM (1991) In: Pimentel D (ed) Handbook of pest management in agriculture. Boca Raton : CRC, pp 405-494

Salac I, Haneklaus S, Gassner A, Bloem E, Schnug E (2004) Phyton 44(2):219-233

Salac I (2005) Influence of the sulphur and nitrogen supply on S metabolites involved in Sulphur Induced Resistance (SIR) of *Brassica napus* L. Landbauforsch Völkenrode SH 277, 134 p

Salac I, Haneklaus S, Bloem E, Booth EJ, Sutherland KG, Walker KC, Schnug E (2005). Sulfur nutrition and its significance for crop resistance - a case study from Scotland. Landbauforsch Völkenrode SH 283:111-119

SPSS (1999) SPSS Base 10.0. Application guide. SPSS Inc, USA

Steed J, Fitt BDL (2000) HGCA project report, No OS41

Stout MJ, Bostock RM (2000) In: Agrawal AA et al (eds) Induced plant defences against pathogens and herbivores. Minnesota : APS Press

Tausz M, Gullner G, Kömives T, Grill D (2003) In: Abrol P, Ahmad A (eds) Sulphur in plants. Dordrecht : Kluwer, pp 221-224

Vidhyasekaran P (2000) Physiology of disease resistance in plants : vol II. Boca Raton : CRC