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GLUCOSINOLATE CONTENT AND PATHOGENIC FUNGI OCCURRENCE IN SEEDS OF SPRING OILSEED RAPE FERTILISED WITH SULPHUR…*

ABSTRACT

Susceptibility of winter oilseed rape to freezing temperatures, increased an interest in cultivation of spring oilseed rape. The relationship between fertilisation with sulphur, glucosinolate content and pathogenic fungi occurrence on the seeds of spring oilseed rape 'Star' was estimated. Sulphur applied in ionic or elementary form at three different doses: 0, 20, 60 kg × ha^{-1} modified glucosinolate level. Total alkenyl glucosinolate content depended mainly on the way of sulphur application and the kind of its form, while the dose of sulphur applied was a main factor influencing indolyl glucosinolate content on investigated seeds. Sulphur fertilisation partially reduced intensity of *Alternaria brassicae,* the only pathogenic fungus frequently occurring on oilseed rape seeds.

Key words: alkenyl glucosinolate, *Alternaria brassicae*, fertilization, glucosinolate, sulphur, winter oilseed rape,

INTRODUCTION

Oilseed rape is a plant of high requirement for sulphur, which for double improved varieties amounts 88 kg S for the yield of 35 dt × ha^{-1} (Wielebski and Wójtowicz 1993). Sulphur fertilisation, especially in conditions of low S content, increased seed yield and the content of sulphuric amino acids in protein, however it may reduce the content of oleic acid in lipids and increase glucosinolate content in seeds (Janzen and Bettany 1984, Budzyński and Ojczyk 1995, Rotkiewicz et al. 1996, Szulc *et al.* 2000).

Toxicity of glucosinolate hydrolytic products to fungi and bacteria has been the subject of several studies. Research on the activity of these compounds and their influence on pathogens occurring on oilseed rape showed that isothiocyanates are toxic to *Peronospora parasitica* (Greenhalgh and Mitchel 1976), *Mycosphaerella brassicae* (Harthill and Shutton 1980), *Leptosphaeria maculans* (Mithen *et al.* 1986) and

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Alternaria spp. (Milford *et al.* 1989). The last one may appear at all growth stages causing black leaf and pod spot and it has been recognised as a very destructive fungal oilseed rape pathogen (Sadowski et al. 2000), which can be transmitted with sowing material is considered a primary source of infection.

Taking into consideration an important role of sulphur in oilseed rape metabolism, its influence on yield and level of the compounds, which determine quality of seeds, a relationship between sulphur fertilization of spring rape variety, glucosinolate level in seeds and occurrence of *Alternaria brassicae* was investigated.

MATERIAL AND METHODS

Seeds of oilseed rape (*Brassica napus* L. cv. Star) were from a field experiment on good wheat soil. Effects of increasing doses of sulphur (0, 20, 60 kg × ha^{-1}) on the relation between glucosinolate content in seeds and occurrence of pathogenic fungi was tested in 1997–1998.

Elemental (Siarkol extra – 80% S) and ionic sulphur (Na_2S0_4) were applied in the doses 20 and 60 kg × ha^{-1} into soil or on the leaves. The level of fertilisation with NPK was constant (N = 120 kg × ha^{-1}, P_2O_5 = 60 kg × ha^{-1}, K_2O =120 kg × ha^{-1}). Sulphur either elemental or ionic was applied in spring before the sowing. Foliar fertilisation, 6.7% sulphur water solution was applied over three growth phases: first dose (20 kg × ha^{-1}) at the rosette phase and the next doses after forming of stem (20 kg·ha^{-1}) and at the beginning of oilseed rape flowering (20 kg × ha^{-1}). The seeds were analysed for glucosinolate content by High Performance Liquid Chromatography (HPLC) according to EN ISO 9167–l: 1992.

In order to determine occurrence of pathogenic fungi on seeds a blotting paper method was applied. Oilseed rape seeds were put on plastic Petri dishes on blotting paper (Whatman 1) soaked with 2,4–D and incubated under 12h UV light/12h dark cycles (Capelli *et al.*, 1998). There were 4 replications for each variant combination (100 seeds per replication). Fungal colonies were characterised with the use of mycological keys.

RESULTS AND DISCUSSION

Response of winter oilseed rape to sulphur fertilisation resulted in yield increase and glucosinolate accumulation (Budzyński and Ojczyk 1995, Wielebski and Wójtowicz 1993, Wielebski *et al.* 1999). Our preliminary data revealed that both a form of S applied and the way of its application seemed to influence the total glucosinolate content of spring oilseed rape (Szulc *et al.* (2000).

Our present studies showed differences of alkenyl and indolyl glucosinolate content between growing seasons. The highest level of alkenyl glucosinolates was found in 1997 in the seeds from plant which were foliar fertilised with ionic sulphur, and in 1998 in the seeds from plants grown on soil ferilised with elemental form of sulphur. Over two years of the investigation there was no significant difference in the level of these compounds after application of 20 and 60 kg × ha^{-1} sulphur (Table 1).

Increasing sulphur doses significantly influence indolyl glucosinolate content. Two–year investigation proved also the effect of the kind of sulphur fertilisation. The mean content of indolyl glucosinolate in seeds was the highest when sulphur was applied to soil in elemental form (Table 2). There are not many papers referring to the effect of sulphur nutrition on indolyl glucosinolate level. In the pot experiments Wielebski *et al.* (1999) demonstrated that sulphur fertilisation decreased indolyl glucosinolate

Table 1

The influence of sulphur fertilisation on alkenyl glucosinolate content in seeds

Year	Control S_0	Dose and form of S applied [kg × ha^{-1}]							
		20				60			
		Soil fertilisation		Foliar fertilisation		Soil fertilisation		Foliar fertilisation	
		Elementary	Ionic	Elementary	Ionic	Elementary	Ionic	Elementary	Ionic
Glucosinolate content [μM·× g^{-1} d.w.]									
1997	8.45	7.24	7.11	8.22	8.09	7.04	7.37	7.34	8.99
LSD^*_{II} = 0,594									
1998	7.29	9.59	7.23	7.23	5.46	8.85	7.55	7.45	7.16
LSD_{II} = 0.543, $LSD_{I\cdot II}$ = 0.940, LSD_{III} = 0.543, $LSD_{I\cdot III}$ = 1.095									
$\bar{x}$	9.02	8.41	7.17	7.72	6.77	7.94	7.46	7.39	8.07
LSD_{III} = 0.316, $LSD_{I\cdot III}$ = 0.602									

*/LSD_I – for sulphur dose
LSD_{II} – for the way of application
LSD_{III} – for the form of sulphur

Table 2

The influence of sulphur fertilisation on indole glucosinolate content in seeds

Year	Control S_0	Dose and form of S applied [kg ×·ha^{-1}]							
		20				60			
		Soil fertilisation		Foliar fertilisation		Soil fertilisation		Foliar fertilisation	
		Elementary	Ionic	Elementary	Ionic	Elementary	Ionic	Elementary	Ionic
Glucosinolate content [μM ×·g^{-1} d.w]									
1997	5.44	4.84	4.67	4.12	4.95	4.63	4.48	4.01	4.39
LSD_I^* = 0,831									
1998	3.70	5.08	5.61	5.19	5.15	5.82	4.63	3.93	4.66
LSD_I = 0.801									
$\bar{x}$	4.57	4.96	5.14	4.65	5.05	5.22	4.55	3.97	4.52
LSD_I = 0.424, LSD_{II} = 0.315									

*/LSD_I – for sulphur dose
LSD_{II} – for the way of application

content. Similar relationship was noted by us but only in the seeds harvested in 1997.

The results of mycological analysis indicated that *Alternaria brassicae* was the only pathogenic fungus frequently occurring on seeds. The products of alkenyl glucosinolate hydrolysis showed different fungistatic effects. Among them only glucobrassicanapin and gluconapoleiferin proved to be fungistatic. The first tended to limit pathogen occurrence only in 1997 while a negligible action of the latter was expressed as a small negative correlation coefficient (Fig. 1). The relationship between total alkenyl glucosinolate content and *Alternaria brassicae* occurrence on seeds was not statistically significant.

Relationship between the content of indolyl glucosinolates, 4–OH–glucobrassicin and glucobrassicin, and occurrence of *A. brassicae* was statistically proven. Which of them limited *A. brassicae* occurrence on seeds more efficiently could not be determined because of small differences between correlation coefficients (Fig. 2). This group of compounds are important precursors of phytohormones. Fungi can take part in converting indolyl glucosinolates into IAA (Wallsgrove *et al.*, 1999).

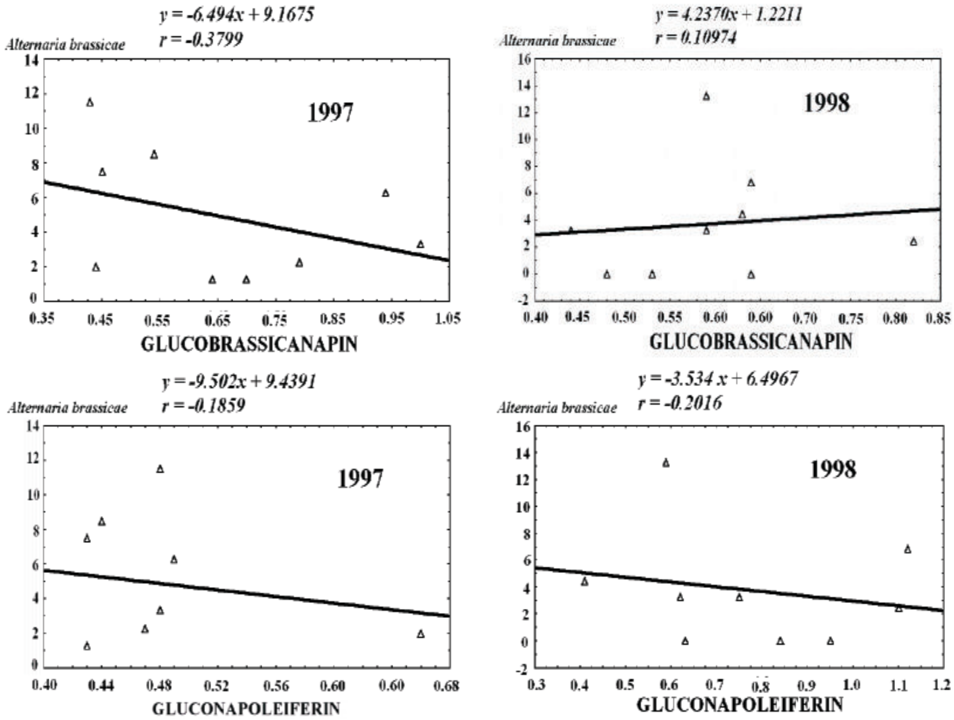

Fig. 1 Relationship between alkenyl glucosinolate content [mM × g^{-1} d. m.] in seeds and occurrence of *Alternaria brassicae* [%]

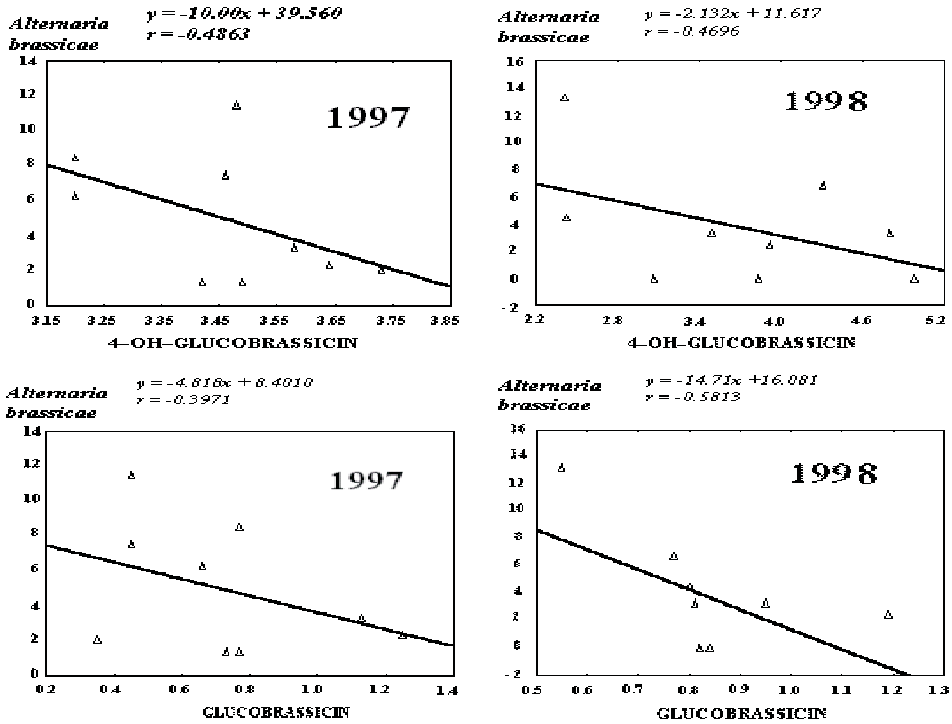

Fig. 2 Relationship between indolyl glucosinolate content [mM × g^{-1} d. m.] in seeds and occurrence of *Alternaria brassicae* [%]

Ambiguous results obtained in this research have confirmed the observation of Buchwaldt (1985) who did not observed any glucosinolate impact on development of fungal pathogens in leaves.

Some pathogenic fungi of *Brassicaceae* can overcome the plant glucosinolates–myrosinase defence system by degradation or biotransformation of glucosinolates into non–toxic or less toxic products. Xiao–Ming Wu and Johan Meijer (1999) have reported on such properties of *Phoma lingam*, *Verticillium dahliae* and *Sclerotinia sclerotiorum*.

Referring to our research, it should be emphasised that glucosinolate hydrolytic products, especially derived from indolyl glucosinolates can consist a part of plant defence mechanism against *A. brassicae*. A similar idea concerning *Sclerotinia sclerotiorum* was presented by Wallsgrove's *et al.* (1999). Limiting concentration of indolyl glucosinolate in seeds by breeding can negatively affect not only on plant growth (Krzymański 1995), but also increases probability of infection of oilseed rape seeds with pathogenic microorganisms (Giamoustaris and Mithen 1995).

Antibacterial activity of glucosinolate mixture extracted from oil meal has also been demonstrated. These compounds limited growth of some bacterial genera, such as: *Escherichia coli*, *Pseudomonas* sp., *Strepto–coccus aureus* (Makulec *et al.* 1995).

CONCLUSIONS

1. The effect of sulphur fertilisation on glucosinolate content was proved. The level of alkenyl glucosinolates depended on the method and form of sulphur application. A dose of sulphur influenced indolyl glucosinolate content.
2. *Alternaria brassicae* was the only pathogenic fungus frequently found on seeds.
3. A negative correlation between indolyl glucosinalate content and the number of *Alternaria brassicae* colonies occurring on seeds was found. It may indicate that this group of compounds take part in oilseed rape defence mechanism.

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