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THE INFLUENCE OF RAPE GLUCOSINOLATES ON THE GROWTH OF TWO PATHOGENIC FUNGI SPECIES: *FUSARIUM ROSEUM* AND *RHIZOCTONIA SOLANI*

DANUTA WALIGÓRA, MAREK KORBAS, DOROTA REMLEIN-STAROSTA
INSTITUTE OF PLANT PROTECTION, MICZURINA 20, 60-318 POZNAŃ, POLAND
e-mail: M.Korbas@ior.poznan.pl

Abstract: The influence of oilseed rape glucosinolates on the grown in vitro pathogenic fungi was studied. Two pathogenic to oilseed rape fungi species: *Fusarium roseum* and *Rhizoctonia solani* were taken into consideration. It was observed that glucosinolates added to the medium limited the growth of both tested fungi to some extent, especially when higher concentrations of glucosinolates was supplied.

Key words: oilseed rape glucosinolates, pathogenic fungi

I. INTRODUCTION

Glucosinolates are sulphur-containing glycosides which are found within the *Brassicaceae* and related families. They occur in all tissues of cruciferous plants, but the amount and the composition of these compounds differs significantly among species and varieties (Classais-Besnard and Larher 1991; Krzymańska et al. 1997; Rosa et al. 1996). Results of many studies have suggested that glucosinolates and their hydrolitic products mediate the interaction between cruciferous plants and their pests and diseases (Birck et al. 1992; Doughty et al. 1991; Koristas et al. 1991; Mithen and Magrath 1992; Oleszek 1995; Waligóra and Krzymańska 1995). The introduction of "00" (low erucic, low glucosinolate) oilseed rape cultivars containing significantly less glucosinolates in seeds as compared to previously grown "0" (low erucic, high glucosinolate) cultivars led to the concern that oilseed rape might become more susceptible to pests and diseases due to the presumed defensive role of these compounds (Giamoustaris and Mithen 1995; Oleszek 1995). Every year the pathogenic fungi cause considerable losses in oilseed rape crops. Fungi of the genera *Fusarium* and *Rhizoctonia* are of a great threat to oilseed rape plants as they are casual agents of pre-emergence damping-off and seedling dumping-off. It is of great importance to state whether the level of glucosinolates content does influence the development of these two pathogens in "in vitro" conditions.

II. MATERIALS AND METHODS

The influence of oilseed rape glucosinolates on the development of 2 species of pathogenic fungi: *Fusarium roseum* and *Rhizoctonia solani* – in cultures in vitro – was studied. The research was performed according to EPPO Bulletin (1991), designed for

studying the sensitivity of fungi to various chemical compounds. Glucosinolates used in experiments were extracted from oilseed rape seeds according to the method described by Jerzmanowska (1967). The isolates of fungi were derived from oilseed rape plants collected on experimental plots. Inoculum was prepared using 21-day old cultures grown on PDA (Potato Dextrose Agar) at temperatures 18-20°C. Tests were performed on PDA medium enriched with glucosinolates dissolved in water and applied at concentrations that ranged from 400 to 1000 ppm. Petri dishes containing PDA and 1 ml of water served as control.

The experiments were performed in 5 replications. Small discs (diam. 5 mm) of 3-week old cultures of tested pathogen, grown on inverted face-down plates, were used to inoculate agar plates (diam. 9 cm). Mycelial discs were placed face-down in the centre of prepared agar plates and incubated afterwards at 20°C in the dark, in face-down position. The size of colonies was estimated after 3 and 6 days of incubation by measuring two perpendicular diameters of surface area of mycelium along lines previously marked at the bottom of the plate.

III. RESULTS AND DISCUSSION

The results of conducted experiments concerning the influence of glucosinolates on the grown in vitro pathogenic fungi species are presented in Figures 1 and 2.

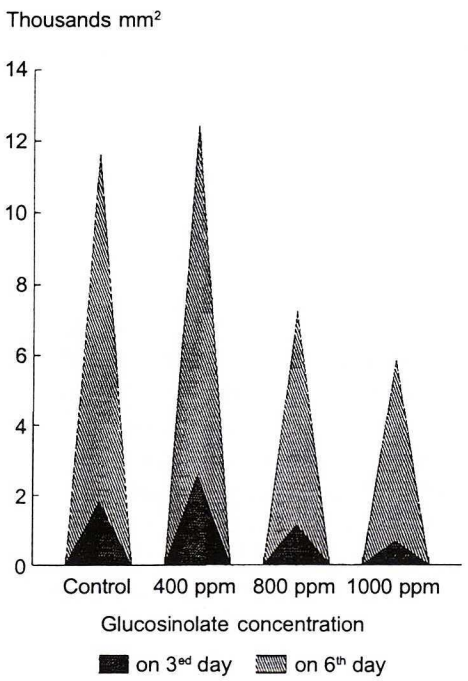

Fig. 1. Mean area of *Fusarium roseum* colony on medium amended with glucosinolates.

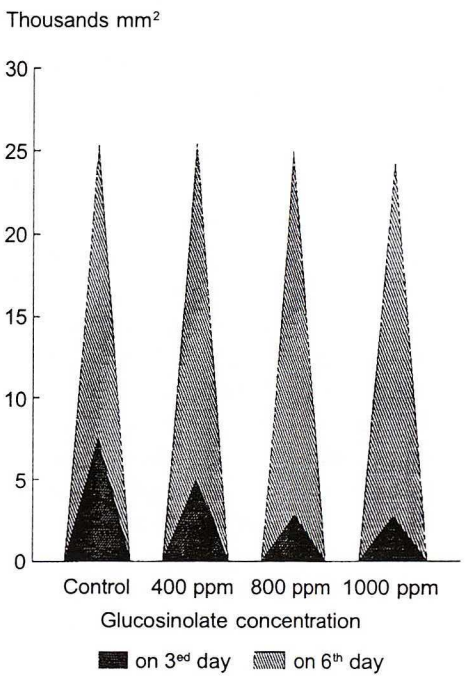

Fig. 2. Mean area of *Rhizoctonia solani* colony on medium amended with glucosinolates.

Observations performed on 3 days old colonies of *Fusarium roseum* (Fig.1) revealed the inhibition of growth in these experimental combinations where glucosinolates were applied at higher concentrations: 800 and 1000 ppm. At the concentration of 400 ppm the growth of mycelium was even better than in the control. This effect was observed both after 3 and 6 days of rearing. Mean area of *Fusarium roseum* colony on the medium amended with 1000 ppm of glucosinolates was, after 6 days, almost twice smaller as the size of colony reared on the control medium.

Three days after inoculation of *Rhizoctonia solani* its growth rate was lower in each combination with glucosinolates comparing to the control (Fig. 2). However, after 6 days of rearing the differences in the size of colonies among experimental combinations were not very differentiated. The size of mycelium was little smaler in combinations where glucosinolates were applied at the concentrations of 800 ppm and 1000 ppm. Then again the difference among experimental combinations were not so significant as in case of *Fusarium roseum.*

It can be concluded that in all experiments some effect of glucosinolates on mycelial growth of two tested fungi species was observed and the effect of inhibition was stronger in the case of *Fusarium roseum.*

The level of glucosinolates' content in artificial medium (PDA) used in all experiments and in rape plant was comparable, because counting over these amounts with reference to the standard (sinigrin) shows that 100 ppm of glucosinolates in the diet corresponds to about 0.24 μM/g of these compounds in rape leaves, instead 1000 ppm corresponds to about 2.41 μM/g and approximate range of concentrations was used in in vitro experiments.

There are many reports confirming the thesis that, glucosinolates and their breakdown products play a major role in pest and desease development on Crucifers. Changes in glucosinolates content of vegetative parts of oilseed rape arising from plant breeding programmes leading up to improve nutritional value of this plant, may influence the relationship between oilseed rape and its pests and deseases. Mithen (1993) stated that manipulation of leaf glucosinolates content had little effect on pathogen interactions, although there was a significant positive relationship between pod glucosinolates content and pod infection by *Alternaria brrasicae.*

Results of many investigation concerning the problem of antifungal activity of glucosinolates proved that glucosinolates indicate strong inhibiting action towards different strains of pathogenic fungi. Some scientists had tested such activity using the mixture of glucosinolates against different microorganisms (Makulec et al. 1995). However, in such experiments minimal inhibitory concentration of glucosinolates was relatively high, what might be caused by using the mixture of glucosinolates. Presence of other compounds in this mixture could dissemble the activity of glucosinolates by themselves.

We also used the extract containing mixture of glucosinolates in our experiments. As it was stated, it showed some inhibiting properties towards both of tested fungi species and the effectivenes of inhibiting increased as the glucosinolate concentration in the diet increased.

It was stated that after initial tissue damage breakdown products of glucosinolates are accumulated and it results in restricting of the further infection development, so the low and

double-low varieties of rape can be infected in nature more intensive than traditional varieties. It was in some cases observed in practice. Mithen et al. (1986) had stated that the ability of the pathogen to spread in tissue varied considerably among different lines of *Brassica.* The extent of colonization was related to the level of glucosinolates: plants showing localised lesions had high levels of glucosinolates and those with large lesions or systematic infection had low levels of these compounds.

So, it seems that the reduction of the level of glucosinolates in green parts of rape plants may not be profitable concerning its susceptibility to the infestation by some species of pathogenic fungi. Magrath at al. (1993) suggested that it would be possible within conventional breeding programme to modify the leaf and seed glucosinolate profile of oilseed rape with the use of synthetic *Brassica napus* lines. It would give the possibility to differentiate the glucosinolates content in seeds and green parts within a plant variety, what would be, from the practical point of view, the optimal solution.

VI. REFERENCES

1. Birch A.N., Griffith D.W., Hopkins R.J., MacFarlane Smith W.H., McKinlay R.G. 1992. Glucosinolates responses to swedge, kale, forage and oilseed rape to root damage by turnip root fly (*Delia floralis*) larvae. J. Sci. Food Agric., 60: 1-9.
2. Classais-Besnard N., Larher F. 1991. Physiological role of glucosinolates in *Brassica napus.* Concentration and distribution pattern of glucosinolates among plant organs during a complete life cycle. J. Sci. Food Agric., 56: 25-38.
3. Doughty K.J., Porter A.J.R., Morton A.M., Kiddle G., Bock C.H., Wallsgrowe R. 1991. Variation in glucosinolates content in oilseed rape (*Brassica napus* L.) leaves. II. Response to infection by *Alternaria brassicae* (Berk.) Sacc. Ann. Appl. Biol., 118: 469-477.
4. EPPO Bulletin. 1991. No. 21: 291-354.
5. Giamoustaris A. Mithen R. 1995. The effect of modifying glucosinolate content of leaves of oilseed rape on its interaction with specialist and generalist pests. Ann. Appl. Biol., 126: 347-363.
6. Jerzmanowska Z. 1967. Substancje roślinne – metody wyodrębniania. Wydawnictwo PWN Warszawa, 298 pp.
7. Koristas V.M., Lewis J.A., Fenwick G.R. 1991. Glucosinolate responses of oilseed rape, mustard and kale to mechanical wounding and infestation by cabbage stem flea beetle (*Psylliodes chrysocephala).* Ann. Appl. Biol., 118: 209-221.
8. Krzymańska J., Lisiecka E., Nawrot D. 1997. The content and composition of glucosinolates in genetically different varieties of oilseed rape (*Brassica napus* L.). J. Plant Protection Res., 37 (1/2): 104-108.
9. Magrath R., Herron C., Giamoustaris A., Mithen R. 1993. The inheritance of aliphatic glucosinolates in *Brassica napus.* Plant Breeding 111: 55-72.
10. Makulec I., Marczak E., Lipkowski A., Baranowska B. 1995. Przeciwbakteryjne i przeciwgrzybowe działanie glukozynolanów wyekstrahowanych ze śruty rzepaku. Rośliny Oleiste, t. XVI: 255-258.
11. Mithen R.F., Lewis B.G., Fenwick G.R.. 1986. In vitro activity of glucosinolates and their products againsts *Leptosphaeria maculans.* Trans. Br. Mycol., 87: 433-440.
12. Mithen R.F., Magrath R. 1992. Glucosinolates and resistance to *Leptosphaeria maculans* in wild and cultivated *Brassica* species. Plant Breeding 108: 60-68.
13. Mithen R. 1993. Leaf glucosinolate profiles and their relationship to pest and disease resistance in oilseed rape. Euphytica 63: 71-83.
14. Oleszek W. 1995. Glucosinolates – occurence and ecological significance. Wiadomości Botaniczne 39 (1/2): 49 -58.

15. Rosa E.A.S., Heaney R.K., Portas C.A., Fenwick G.R. 1996. Changes in glucosinolate concentration in *Brassica* crops (*B. oleracea* and *B. napus*) throughout growing seasons. J. Sci. Food Agric., 71: 237-244.
16. Waligóra D., Krzymańska J. 1995. Biological activity of glucosinolates isolated from rape (*Brassica napus*) to some pests. Proceedings of the 9th International Rapeseed Congress, July 1995, Cambridge vol. 3: 1022-1024.

Danuta Waligóra, Marek Korbas, Dorota Remlein-Starosta

WPŁYW GLUKOZYNOLANÓW RZEPAKOWYCH NA WZROST DWÓCH GATUNKÓW GRZYBÓW PATOGENICZNYCH: *FUSARIUM ROSEUM* I *RHIZOCTONIA SOLANI*

STRESZCZENIE

Testowano wpływ glukozynolanów wyizolowanych z nasion rzepaku (*Brassica napus* L.) na wzrost – w warunkach in vitro – dwóch gatunków grzybów patogenicznych porażających tę roślinę: *Fusarium roseum* i *Rhizoctonia solani.* Prowadzono hodowle grzybów na pożywce agarowo-ziemniaczanej (AGZ), do której dodawano glukozynolany w stężeniach od 400 do 1000 ppm.

Stwierdzono, że glukozynolany ograniczały w pewnym stopniu wzrost pola powierzchni grzybni obu gatunków grzybów – zwłaszcza w wyższych stężeniach. Efekt ten był silniejszy w przypadku grzyba *Fusarium roseum.*