SHORT COMMUNICATION

The role of glucosinolates of *Brassica* genus in the crop system

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ABSTRACT

Glucosinolates with *Brassica* genus as secondary metabolites have a lot of functions and effects. Glucosinolates form less than 2% of the overall sulphur content at the beginning of vegetation in different parts of the plants and during growth their content is decreasing and forms less than 0.1%. This low representation doubts their storage function. With its chemical composition, they are ranked among natural pesticides with active and passive resistance against diseases and pests. They show repellent effects and properties of natural biofumigators in soil after ploughing in their biomass as green fertilizing, or after ploughing in after harvest the leftovers of rape. The principle of these effects is decomposition products of glucosinolates – bioactive isothiocyanates. Very important from this point of view are turnip rape Rex and *Brassica juncea*, whose content of these compounds is the highest one and they are resistant against the attack of *Ceutorrhynchus pleurostigma*. The same effect showed also when attacked by *Phoma lingam*. With other winter *Brassicas* either hybrid or linea and summer rape is this defensive system suppressed because of their lowered content due to breeding interferences, leading to limitation of their anti-nutritional negative effects. It is possible to state the final result after finding out the production of the above matter, roots, and after evaluation of the sorbal characteristics of the soil and evaluation of the state of health of the following crop or vegetable. After this overall analysis, it will be possible to evaluate the biofumigation properties of accessible varieties of the *Brassica* genus.

Keywords: glucosinolates; sulphur; biocides; biofumigants; *Brassica juncea*; turnip rape; winter rape; summer rape; *Ceutorrhynchus pleurostigma*; *Phoma lingam*

Glucosinolates do not have any primary physiologic role. As secondary metabolites, they have or they are given many functions:

– Storage of sulfur, mainly in seeds.
– Natural protection of the plant. Next to the prepared passive defensive system (Giamoustaris and Mithen 1995). It has also an active one (Takasugi et al. 1986).
– Biofumigation effect of *Brassicacae* of following crops (Kirkegaard et al. 1999).

The aim of this work is the evaluation of glucosinolates with the *Brassica* genus during growth leading to clarification of their role in the plant, evaluation of their natural protection and biofumigation potential leading to purification of the soil without the use of synthetic pesticides.

MATERIAL AND METHODS

The experiment was carried out at the experimental ground of the Research Station of the Faculty of Agronomy of the CUA in Červený Újezd, Prague in 2001 year. Soil – deep Cambi soil, storage of P, K, Mg good, colloid complex fully saturated, 405 m above sea level. Meteorological records are given in Table 1.

Content of total sulphur, sulphates and glucosinolates in different parts of the plant during growth were observed in variety Lirajet.

The content of glucosinolates during growth was monitored with: varieties of winter rape Lirajet (DSV Lippstadt, D), hybrid variety Pronto (NPZ H.G. Lembke KG, Holtsee, D), super early variety of winter rape Prestol (Monsanto SAS, F), summer rape Golda (Semundo Rellingen, D), turnip rape Rex (NPZ H.G. Lembke KG, Holtsee, D) and *Brassica juncea* (Brown mustard), (VNIIMK Krasnodar R a Agrada, CZ, hybrid of summer *Brassica juncea* and winter rape). The experiment was carried out by standard breeding technology (Vašák et al. 2000) in form of incidentally chosen plots in four repeated forms with an area of 10 m^2 each.

The creation of biomass was followed in ca 14 day's intervals by takings of 10 plants and drying them. The average sample of fresh mass was lyofilized and later analysed for the content of different glucosinolates.

Determination of different glucosinolates by the method of gas chromatography

The content of different glucosinolates was determined by gas chromatography after conversion to desulfoglu-

Table 1. Meteorological records from Červený Újezd, July 2000–July 2001

Month	Precipitations (mm)		Temperature (°C)			
	2000/2001	normal	maximum	minimum	average	normal
July 2000	56.2	69.0	25.5	7.5	15.9	17.4
August 2000	42.6	64.0	34.1	7.5	18.8	16.6
September 2000	22.5	42.0	25.0	4.0	13.8	13.1
October 2000	56.9	35.0	23.0	–1.0	10.7	7.7
November 2000	31.6	29.0	14.0	–3.3	2.5	2.5
December 2000	11.0	26.0	12.4	–12.5	0.5	–0.9
January 2001	14.2	26.5	10.0	–14.5	–2.1	–2.1
February 2001	3.5	22.3	12.3	–14.0	1.4	–1.0
March 2001	53.9	30.4	17.0	–8.5	3.6	3.0
April 2001	53.0	31.9	21.5	–2.4	7.1	7.4
May 2001	52.9	58.1	29.1	4.7	14.5	12.6
June 2001	58.5	63.9	28.2	4.5	14.4	15.6
July 2001	93.5	71.3	29.5	7.7	18.2	17.4

cosinolates and a following silylation of N-methyl-N-trimethylsilylheptafluorobutyramide to silylderivates of glucosinolates according to our own modification of the Heaney method (1986). Silylderivates were then recorded on the gas chromatograph Hewlett Packard HP 5890 by the method of inner standard. Sinigrin was used as an inner standard, and for *Brassica juncea* glucotropaeolin was used.

Determination of total sulphur: Total sulphur was determined on the apparatus ICP-AES Trace SCAN.

Determination of sulphates: Sulphates were determined on the analysator SAN Plus, System-Skalar after the extraction of dry matter of the plants into water.

For statistical evaluation, the analysis of dispersion of the programme system Statgraphics was used.

RESULTS AND DISCUSSION

Glucosinolates – the reserve substance of sulphur

During the study of the total sulphur, sulphates and glucosinolates distribution in the individual parts of plants variety Lirajet during their growth, it become obvious that glucosinolates (Table 2) represent less than 2% of the total sulphur at the beginning of vegetation. Their content decreases in the growth course to 0.1% of their total content. This fact is in accordance with the findings of Fielsend and Milford (1994), and Zhao et al. (1993). These minority contents of glucosinolates infirm the sulphur reserve function (Zukalová and Vašák 2002). The sulphates represent the greatest part of the sulphur

Table 2. Content of total sulphur, sulphates and glucosinolates in different parts of the plant during growth

	Phase	Total sulphur (µmol/g dry matter)	Sulphate (% of total sulphur)	Glucosinolates (µmol/g dry matter)
Rosette	leaf	291.56	39	1.38
	roots	99.06	17	0.96
Budding	leaf	199.38	73	1.22
	stem	108.44	56	1.41
	roots	118.44	23	0.41
Full flower	leaf	135.63	68	0.88
	stem	42.19	48	0.24
	inflorescence	111.25	32	0.61
	roots	55.00	34	0.25
Ripening	stem	35.94	60	0.04
	inflorescence	69.69	83	1.32
	pods	61.25	47	1.20
	roots	43.13	36	0.06
Harvest	stem	17.19	62	0.01
	inflorescence	35.63	37	0.00
	pods	44.69	77	0.45
	seeds	107.81		4.86

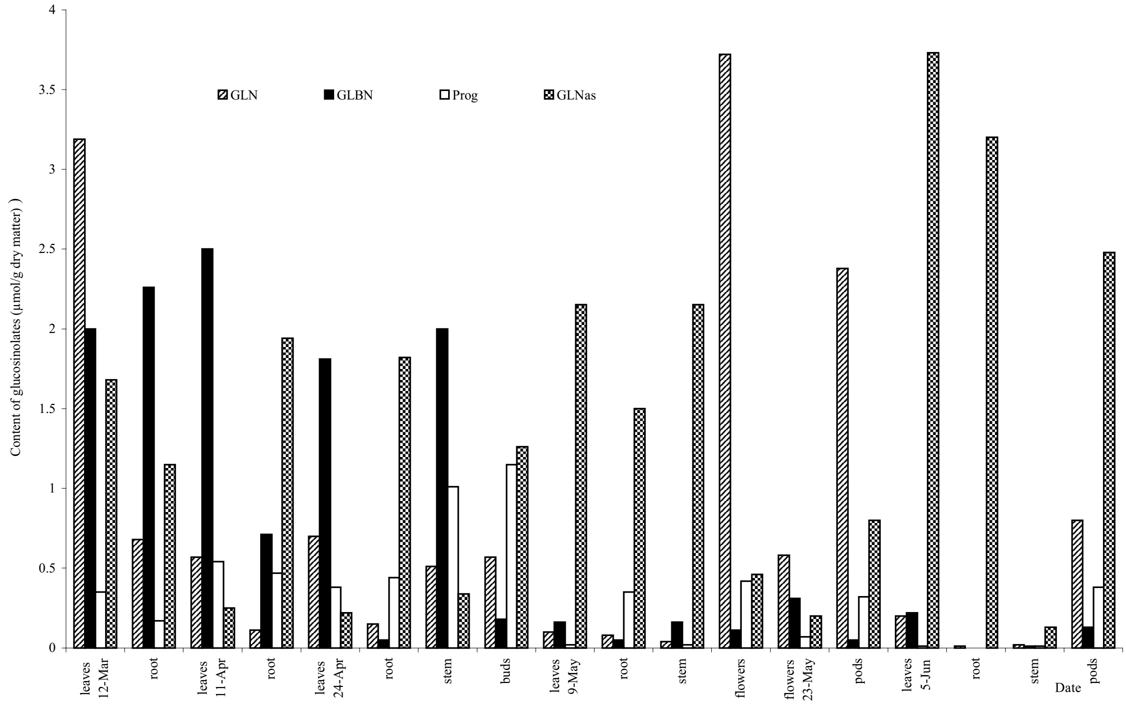

Figure 1. Content of glucosinolates during growth with the turnip rape Rex

total content (Table 2). These sulphates stay in the vegetative parts of plants and, therefore, they do not participate in the SO_4^{2-} transformation into organic substances. These findings lead to our considerations related to the sulphur non-effective economy in rape plant or to another function, so far unknown to us (Zukalová and Vašák 2002).

Natural protection of the plant

In *Brassicas* – the glucosinolates belong amongst the natural biocides (Zukalová and Vašák 2002), which fulfil the function of a passive even active protective mechanism. For the evaluation of the natural protection of the *Brassica* plants, even their fumigation effects for the

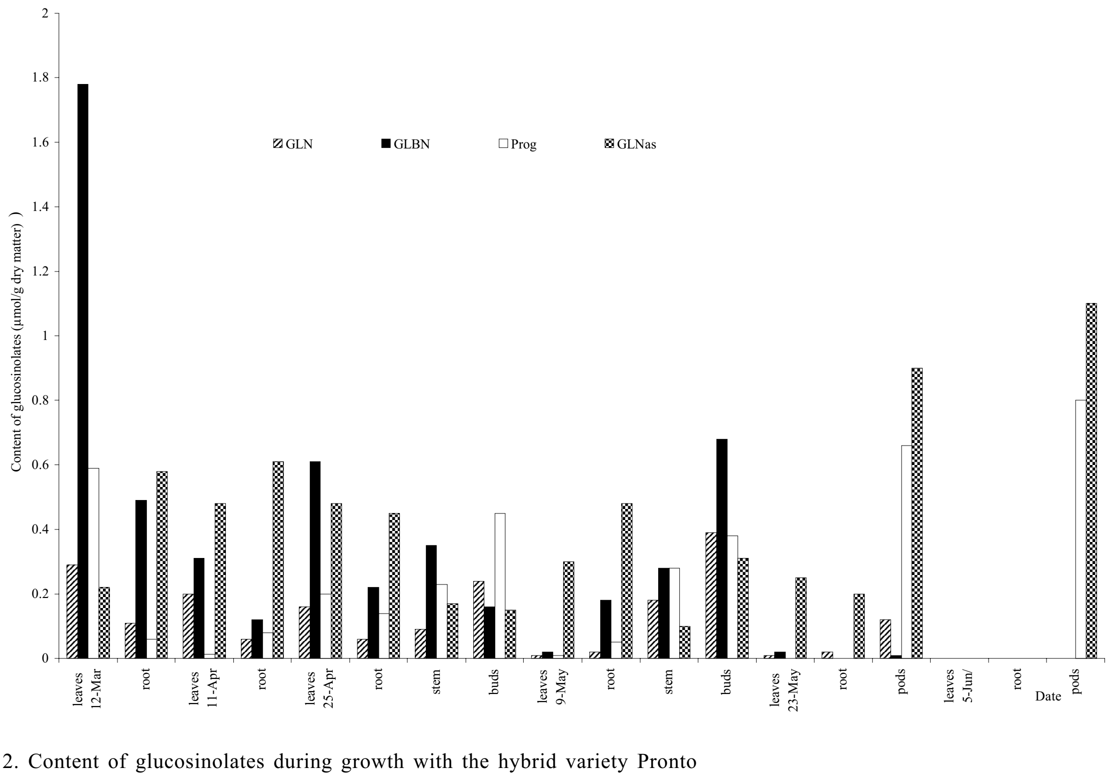

Figure 2. Content of glucosinolates during growth with the hybrid variety Pronto

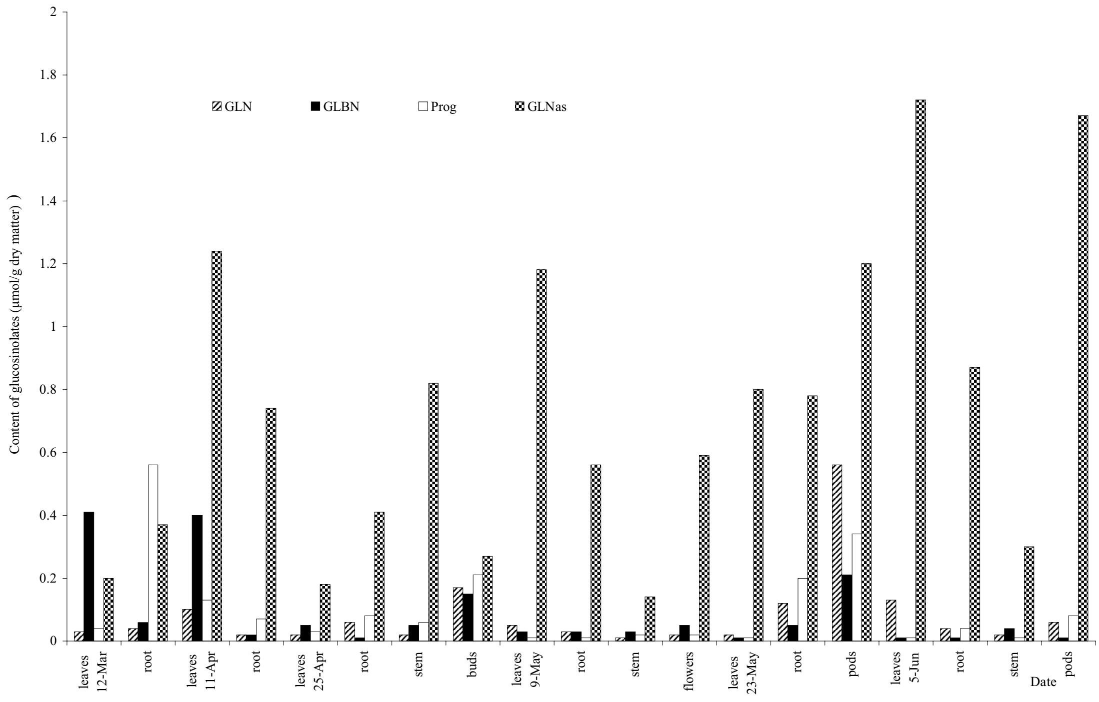

Figure 3. Content of glucosinolates during growth with summer rape Golda

subsequent crops, we studied the content and composition of glucosinolates and their decomposition products during growth.

There are important differences between turnip rape (Figure 1) and brown mustard *Brassica juncea* (Figure 5) and winter variety of *Brassica* – Prestol (Figure 4), hybrid variety Pronto (Figure 2) and spring variety of rapeseed Golda (Figure 3). Contrary to *Brassicas,* in the initial phases of growth turnip rape has a high content of gluconapin and glucobrassicanapin that is decreasing in the vegetative organs. In their further development glucosinolates, preponderantly gluconapin, go over into generative organs, in which they again decrease and pass into seeds. The content of gluconasturtiin is also relatively high in comparison to other species and types of *Brassica*. With winter *Brassicas*, hybrid and spring varieties on

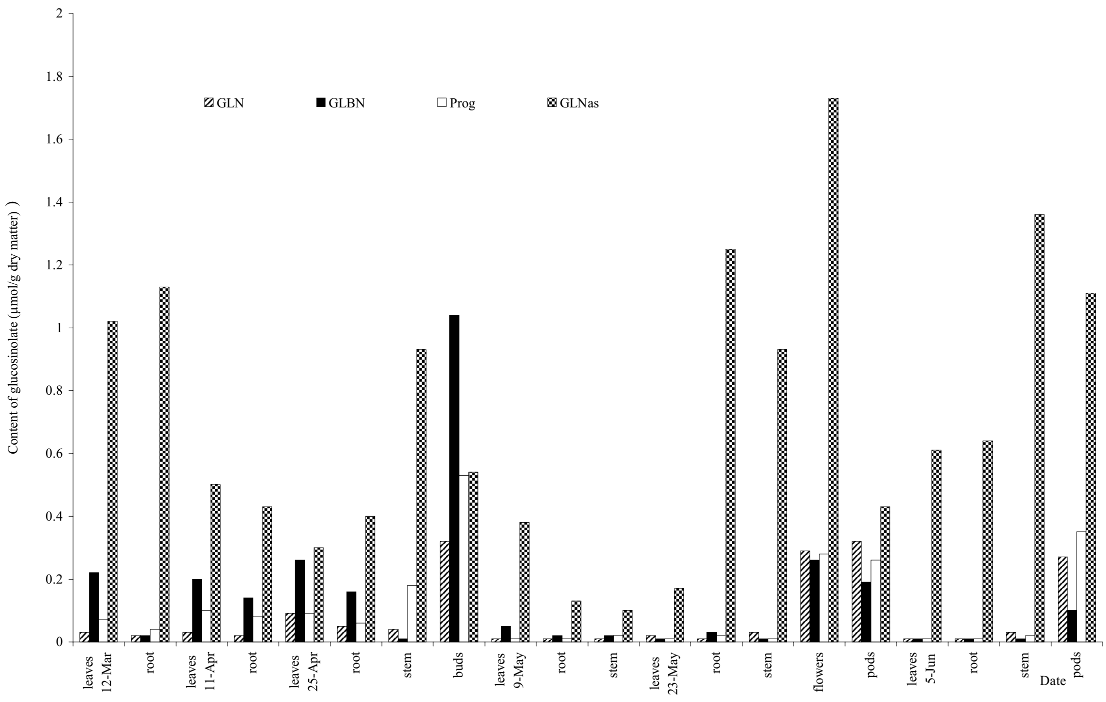

Figure 4. Content of glucosinolates during growth with winter rape Prestol

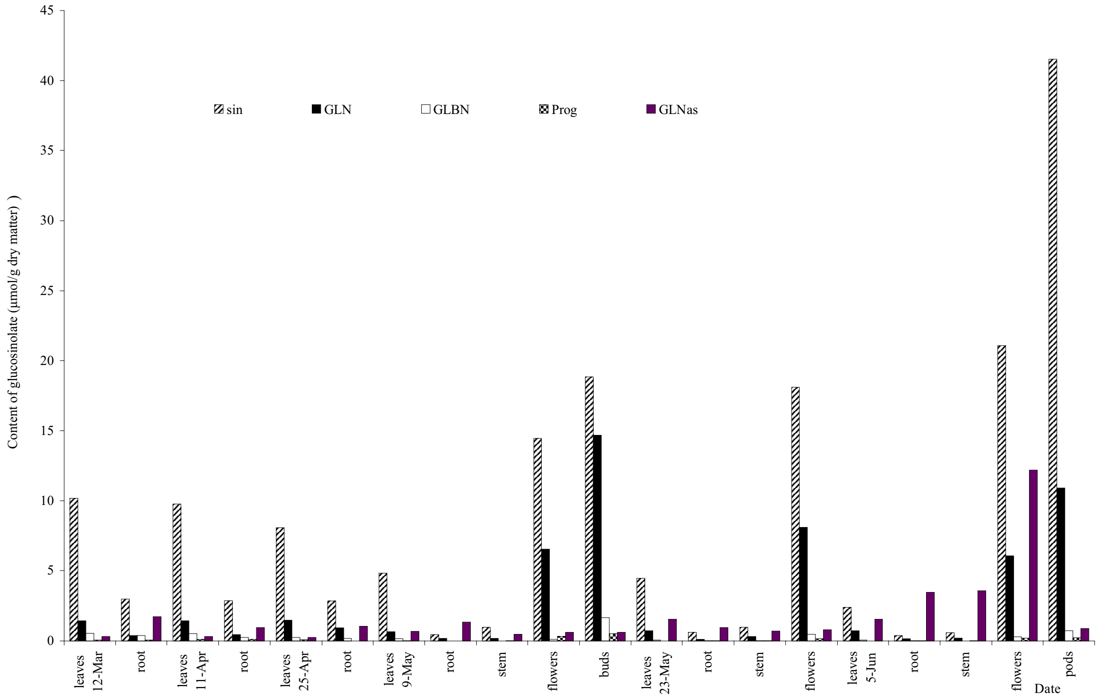

Figure 5. Content of glucosinolates during growth with *Brassica juncea*

the contrary to turnip rape in the early phases of development prevails glucobrassicin, which is dominant in the hybrid variety Pronto. Trends in the development of glucosinolates as in vegetative and generative organs are similar and they fully confirm the theory, that the pod walls are the place from where glucosinolates are transported into the seeds with the same structure as the seeds. Winter upper early rape Prestol with its content of glucosinolates is the closest in resembling summer rape. Very different is *Brassica* juncea, in which alkenyl glucosinolate sinigrin prevails, though enzymatic hydrolysis originates allyisothiocyanate (Table 5). This major glucosinolate is decreasing during growth in the vegetative parts of the plants, in generative it is increasing. The highest concentration is in pods, where it with its high content unambiguously and as model proves, that from the pod glucosinolates are transported into seeds.

These volatile compounds – isothiocyanates (Table 3) have a wide spectrum of anti-microbial effects and they act as repellent against some insects (Giamoustaris and Mithen 1995).

After two years, following of attack of pests in relation to the content of natural defenders of glucosinolates it is clear, that this natural defensive functions according their main occurrence, when the content of volatile isothiocyanates is the highest (Figures 1–5).

The protection against *Ceutorrhynchus pleurostigma* is from this point of view very important; its main occurrence is during IX/X month (Table 4), when the contents of glucosinolates are the highest. During both experimental years the attack by this pest was the lowest with *Bras-*

Table 3. Systematic and trivial names of the main glucosinolates forming bioactive isothiocyanates with *Brassica* species by hydrolytic decomposition

Systematic name	Trivial name	Abbreviation	*R*	*R*–isothiocyanate
Aliphatic				
2-propenyl (allyl)-GSL	sinigrin	Sin	CH_2=CH–CH_2	allyl-ITC
3-butenyl-GSL	glukonapin	GLN	CH_2=CH–$(CH_2)_2$	butenyl-ITC
4-pentenyl-GSL	glukobrassicanapin	GLBN	CH_2=CH–$(CH_2)_3$	pentenyl-ITC
Aromatic				
2- fenylethyl-GSL	glukonasturtiin	GLNas	$C_6H_5(CH_2)_2$	fenylethyl-ITC

For abbreviation see Figures 1–4
Pro = progoitrin – a glucosinolate creating a very unstable isothiocyanate, which immerdiately cycles under the origin of vinylthiooxazolidinethion
ITC = isothiocyanate
GSL = glucosinolate

Table 4. Important pests and diseases of *Brassica* and their main occurrence

Pest (disease)	Main occurrence
Pollen beetle (*Meligethes aeneus* F.)	IV.–V.
Cabbage seed weevil (*Ceutorrhynchus assimilis* Payk)	V.
Brassica pod midge (*Dasineura brassicae* Winn)	V.
Cabbage gall. weevil (*Ceutorrhynchus pleurostigma* Marsh)	IX.–X.
Phoma (*Phoma lingam*)	
Cabbage weevil and Cabbage stem weevil (*Ceutorrhynchus napi* Gyll and *pallidactilus* Marsh)	III.–IV.

Table 5. Occurrence of pests during vegetation with different *Brassicas* (taking from 50 plants) and % of attacked plants

	Date	Occurrence of pests on 50 terminals of plants					% of attack of plant				
		A	B	C	D	E	F		G		H
							2001	2000	2001	2000	
Rex (1)	11. 4. 2001										
	17. 4. 2001		2								
	2. 5. 2001										
	7. 5. 2001										
	15. 5. 2001	17			2	4					
	21. 5. 2001	3			1						
	23. 5. 2001						20	0			
	12. 6. 2001								66	48	84
Pronto (2)	11. 4. 2001										
	17. 4. 2001										
	2. 5. 2001	2	9								
	7. 5. 2001	1									
	15. 5. 2001	26			11						
	21. 5. 2001	6									
	23. 5. 2001						98	–			
	12. 6. 2001								96		100
Golda (3)	11. 4. 2001		2								
	17. 4. 2001		2								
	2. 5. 2001		1		1						
	7. 5. 2001	1			2						
	15. 5. 2001	11			3	2					
	21. 5. 2001	5									
	23. 5. 2001						100	27			
	12. 6. 2001								100	87	78
Prestol (4)	11. 4. 2001		2								
	17. 4. 2001										
	2. 5. 2001		1								
	7. 5. 2001				1						
	15. 5. 2001	12									
	21. 5. 2001	2			1						
	23. 5. 2001						99	30			
	12. 6. 2001								78	17	76
B. juncea (5)	11. 4. 2001		3								
	17. 4. 2001		1								
	2. 5. 2001		4	6	18						
	7. 5. 2001		1								
	15. 5. 2001	75				4					
	21. 5. 2001	258			2						
	23. 5. 2001						13	0			
	12. 6. 2001								74	20	94

A – *Meligethes aeneus*, B – *Ceutorrhynchus napi*, C – *Ceutorrhynchus quadridens*, D – *Ceutorrhynchus assimilis*, E – *Dasineura brassicae*, F – *Ceutorrhynchus pleurostigma*, G – *Phoma lingam*, H – *Ceutorrhynchus napi* + *Ceutorrhynchus pallidactilus*

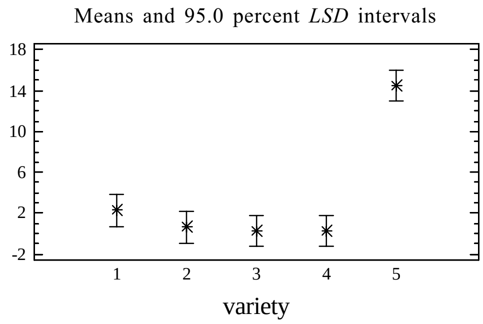

Figure 6. Analysis of variance for above ground – aliphatic isothiocyanate
Multifactor ANOVA – above ground aliphatic

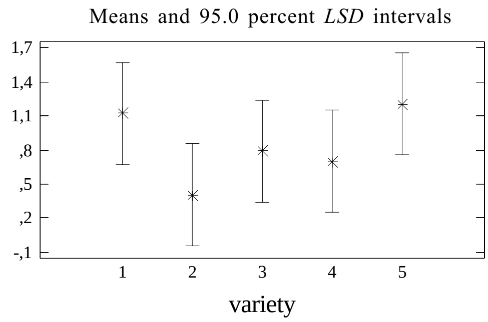

Figure 7. Analysis of variance for above ground – aromatic isothiocyanates
Multifactor ANOVA – above ground aromatic

Variety 1–5 see Tables 5 and 6

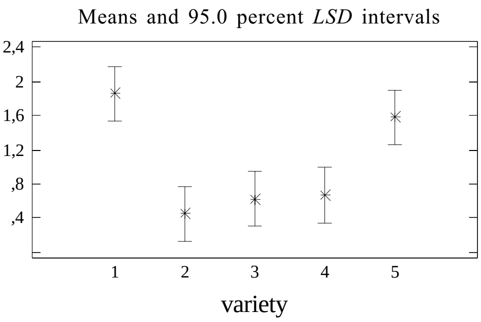

Figure 8. Analysis of variance for roots – aromatic isothiocyanates
Multifactor ANOVA – root aromatic

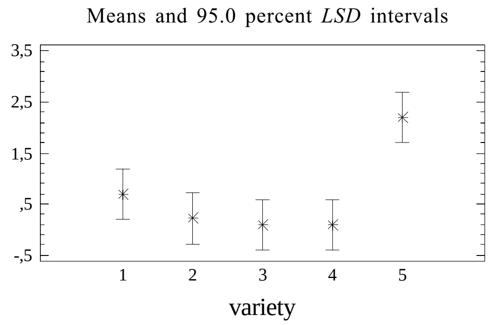

Figure 9. Analysis of variance for roots – aliphatic isothiocyanates
Multifactor ANOVA – root aliphatic

sica Rex with a high content of gluconapin producing butenyl isothiocyanate and *Brassica juncea* with a high content of sinigrin, by which hydrolysis originates a very volatile allylisothiocyanate (Tables 3 and 5). Similarly was the attack by phoma (*Phoma lingam*), which was at its lowest with variety Rex and *Brassica juncea*. The occurrence of other pests moves into the period with a low content of these resistant substances and that is why there are not so evident differences.

Decompositive products of hydrolysis of glucosinolates – isothiocyates were on the contrary studied as attractants for *Meligethes aeneus* F. (Ruther and Thiemann 1997) and *Ceutorrhynchus assimilis* Payk. These papers study the repercussion of the attack of pests according to the yellow colour in combination with bioactive isothiocyanates. According to literature, the efficiency of these attractants is not unambiguous, but our results during the two experimental years have clearly shown *Meligethes aeneus* a high attractivity of allylisothiocyanate originating through decomposition of sinigrin with *Brassica juncea* (Table 5) in comparison with other tested *Brassica* varieties.

The glucosinolates of the third group participate in the active protection (Zukalová and Vašák 2002), which include the glucosinolates with the indole group. These very unstable compounds require a special study.

Biofumigation effect of *Brassicacae* on following crops

We have studied the composition of the main glucosinolates (Figures 1–5) from the viewpoint of their biofumigation potential, leading to purification of the soil without the use of synthetic fumigants. Decomposive products of glucosinolates volatile isothiocyanates (Table 3) play an important role in the suppression of pathogens remaining in the soil. Isothiocyanates are an important substance of synthetic biofumigants and that is why these bioactive compounds would require special study. From previous information followed, that aromatic isothiocyanates (Sarwar et al. 1998) have a higher toxicity and that is why we have considered this fact when studying the different varieties of *Brassica*.

By statistical analysis, it is evident (Figures 6–9), that the content of alkenyl isothiocyanates in the above ground matter differed widely *Brassica juncea*, which is caused by a high content of sinigrin. With aromatic isothiocyanates, it is a fenylethylisothiocyanate and there are no statistically important differences between the different varieties of *Brassica* in the above ground matter. With roots there is the same trend for alkenyl isothiocyanates as with above ground matter, where there again differentiated *Brassica juncea* because of the

Table 6. Content of alkenyl and aromatic isothiocyanates (μmol/g of dry matter) and creation of dry matter of above ground matter and roots with different *Brassicas* during growth

Date/Variety	Above ground matter		Root		Above ground matter	Root
	alkenyl ITC	aromatic ITC	alkenyl ITC	aromatic ITC	dry matter of 10 plants (g)	dry matter of 10 plants (g)
Rex (1)						
12. 3.	5.19	1.68	2.94	1.15	14.93	37.04
11. 4.	3.07	0.25	0.82	1.94	45.07	14.5
25. 4.	1.92	0.61	0.2	1.82	99.86	23.8
9. 5.	1.43	1.59	0.13	1.5	203	26.54
23. 5.	1.66	0.5	0.1	1.56	317.32	24.26
5. 6.	0.46	2.11	0.01	3.2	466.4	20.43
Pronto (2)						
12. 3.	2.07	0.22	0.6	0.58		
11. 4.	0.51	0.48	0.18	0.61	100.64	24.47
25. 4.	0.54	0.27	0.28	0.45	197.54	37.26
9. 5.	0.52	0.24	0.2	0.48	358.86	50.8
23. 5.	0.08	0.57	0.02	0.2	385.8	44.12
5. 6.	0.07	0.65	0.02	0.35	771.86	31.9
Golda (3)						
12. 3.	0.44	0.2	0.1	0.37	13.55	26.93
11. 4.	0.5	1.24	0.04	0.74	34.37	10.33
25. 4.	0.15	0.42	0.07	0.41	79.8	19.6
9. 5.	0.06	0.64	0.06	0.56	213.94	21.46
23. 5.	0.4	1	0.17	0.78	337.14	28.34
5. 6.	0.09	1.23	0.05	0.87	483.26	19.6
Prestol (4)						
12. 3.	0.25	1.02	0.04	1.13	13.73	30.21
11. 4.	0.23	0.5	0.16	0.43	52.97	13.93
25. 4.	0.58	0.59	0.21	0.4	102.34	19.26
9. 5.	0.05	0.24	0.02	0.13	222.4	27.94
23. 5.	0.28	0.82	0.04	1.25	330.74	32.14
5. 6.	0.15	1.03	0.02	0.64	499.86	21.57
B. juncea (5)						
12. 3.	12.18	0.31	3.72	1.74	14.59	30.49
11. 4.	11.71	0.31	3.58	0.95	40.64	14.29
25. 4.	9.82	0.26	3.94	1.04	35.34	11.06
9. 5.	21.03	0.79	0.63	1.34	111.46	17.86
23. 5.	11.07	1.01	0.74	0.93	159.26	19.4
5. 6.	21.15	4.55	0.55	3.48	622.34	33.07

above stated reasons. In case of fenylethylisothiocyanates of the roots, there are statistically important differences among turnip rape Rex, *Brassica juncea* and *Brassicas*. Among spring, winter and hybrid rape there is no difference in the case of aromatic isothiocyanates. In the content of aromatic isothiocyanates turnip rape Rex and *Brassica juncea* are comparable with the highest contents. From this point of view, it is possible to judge, that the highest biofumigation effect has turnip rape Rex, *Brassicea juncea* as far as natural bioactive substance contained in them are concerned.

This result is supported also by orientational annual results of production of above ground matter and roots with the different varieties of *Brassica*, which do not expressively differ (Table 6). It is possible to state the final result after finding out the production of the above matter, roots, after evaluation of the sorbal characteristics of the soil and evaluation of the state of health of the following crop or vegetable. After this overall analysis, it will be possible to evaluate the biofumigation properties of accessible varieties of the *Brassica* genus.

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REFERENCES

Fieldsend J., Milford G.F.J. (1994): Changes in glucosinolates during crop development in single and double-low genotypes of winter oilseed, rape (*Brassica napus*). I. Production and distribution in vegetative tissues and developing pods during development and potential role in the recycling of sulphur within crop. Ann. Appl. Biol., *124*: 531–542.

Giamoustaris A., Mithen R. (1995): The effect of modifying the glucosinolate content of leaves of oilseed rape on its interactions with specialist and generalist pests. Ann. Appl. Biol., *126*: 347–363.

Heaney R.K., Spinks E.A., Hanley A.B., Fenwich G.R. (1986): Analysis of glucosinolates in rapeseed. Techn. Bull. Agric. Food Res. Coun., Food Res. Inst., Norwich, Colney Lane, Norwich NR4 7UA.

Kirkegaard J.A., Matthiessen J.N., Wong P.T.W. et al. (1999): Exploiting the biofumigation potential of *Brassicas* in farming systems. Proc. 10th Int. Rapeseed Congr. Canberra, Australia.

Ruther J., Thiemann K. (1997): Response of the pollen beetle *Meligethes aeneus* to volatiles emitted by intact plants and conspecifies. Entomol. Exp. Appl., *84*: 183–188.

Sarwar M., Kirkegaard J.A., Wong P.T.W., Desmarchelier J.M. (1998): Biofumigation potential of *Brassicas*. III. *In vitro* toxicity of isothiocyanates to soil-borne fungal pathogens. Plant and Soil, *201*: 103–112.

Takasugi M., Katsui N., Shirata A. (1986): Isolation of three novel sulphur-containing phytoalexins from the Chinese cabbage *Brassica campestris* L. spp. *pekinensis*. J. Chem. Soc., Chem Commun.: 1077–1078.

Vašák J. (2000): Variantní pěstitelské technologie. In: Vašák J. et al. (2000): Řepka. Agrospoj, Praha: 288–294.

Zhao F.J., Evans E.J., Bilsborrow P.E., Syers J.K. (1993): Sulphur uptake and distribution in double and single low varieties of oilseed (*Brassica napus* L.). Plant and Soil, *150*: 69–76.

Zukalová H., Vašák J. (2002): The role and effects of glucosinolates of *Brassica* species – a review. Rostl. Výr., *48*: 175–180.

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ABSTRAKT

Úloha glukosinolátů rodu *Brassica* v pěstebním systému

Glukosinolátům rodu *Brassica* jako sekundárním metabolitům je přisuzována řada funkcí a účinků. Glukosinoláty tvoří méně než 2 % celkové síry na počátku vegetace v jednotlivých částech rostlin a v průběhu růstu jejich obsah klesá na méně než 0,1 %. Toto nízké zastoupení zpochybňuje jejich zásobní funkci. Svým chemickým složením jsou řazeny mezi přirozené pesticidy s aktivní i pasivní obranou proti chorobám a škůdcům. Vykazují odpudivé účinky a mají i vlastnosti přírodních biofumigantů v půdě po zaorání jejich biomasy při zeleném hnojení nebo po zaorání posklizňových zbytků řepky. Principem těchto účinků jsou rozkladné produkty glukosinolátů – bioaktivní isothiokyanáty. Velmi významné z tohoto hlediska jsou řepice Rex a hořčice sareptská, které mají nejvyšší obsah těchto sloučenin a jsou odolné proti napadení krytonoscem zelným (*Ceutorrhynchus pleurostigma*). Stejný efekt se projevil i u napadení phomou (*Phoma lingam*). U ostatních odrůd ozimé řepky, hybridních i liniových a jarních, je tento obranný systém potlačen vzhledem k jejich sníženému obsahu v důsledku šlechtitelských zásahů, vedoucích k omezení jejich antinutričních negativních účinků. Konečný rezultát bude možné vyslovit po zjištění produkce nadzemní hmoty a kořenů a po zhodnocení sorpčních vlastností půdy a zdravotního stavu následné plodiny nebo zeleniny. Po tomto celkovém rozboru bude možné vyhodnotit biofumigační vlastnosti dostupných odrůd rodu *Brassica*.

Klíčová slova: glukosinoláty; síra; biocidy; biofumiganty; hořčice sareptská; řepice; ozimá řepka; jarní řepka; krytonosec zelný (*Ceutorrhynchus pleurostigma*); phoma (*Phoma lingam*)

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