

## QUANTITY OF GLUCOSINOLATES IN 10 CABBAGE GENOTYPES AND THEIR IMPACT ON THE FEEDING OF *MAMESTRA BRASSICAE* CATERPILLARS

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**Abstract** - In 2011, we studied the glucosinolate content in 5 cultivars and 5 cabbage hybrids grown outdoors in order to study their influence on the feeding of cabbage moth caterpillars (*Mamestra brassicae*). The selected genotypes were categorized into three groups, early (the growth period from 55 to 70 days), mid-early (80-90 days) and mid-late (110-140 days), while the samples of cabbage for glucosinolate analysis were taken at five intervals, during which we also assessed genotypes for the extent of damage caused by caterpillars. We found that the feeding of caterpillars affected primarily the mid-early and mid-late genotypes of cabbage, and that the glucosinolate content among the different cabbage genotypes varies. The highest content of the analyzed glucosinolates was established in mid-late genotypes. Glucobrassicin was the only glucosinolate found in all cabbage genotypes, yet its antixenotic effect ( $r=0.20$ ) was very low. We found that sinalbin negatively affects the feeding of cabbage moth caterpillars in mid-early cabbage genotypes ( $r=-0.34$ ), while the same effect of sinigrin on the extent of damage can be observed in mid-late genotypes ( $r=-0.27$ ). We have established a strong or moderate correlation between the gluconapin ( $r=0.87$ ) and progoitrin ( $r=0.66$ ) contents in mid-late genotypes and the extent of damage caused by caterpillars. Our research proves that different cabbage genotypes are responsible for different susceptibilities to damage by the cabbage moth, and that one of the factors of natural resistance of cabbage are also glucosinolates. Despite this, due to their variability in cabbage we cannot precisely determine the set of genotypes that would ensure a higher cabbage yield as a result of less damage caused by the cabbage moth. Thus, we need to identify in more detail the reasons for the time and quantum variability of glucosinolates in Brassicaceae.

**Key words:** Cabbage, genotype, glucosinolates, feeding preference, *Mamestra brassicae*

### INTRODUCTION

Cabbage, which is in Europe among the most important vegetables, can defend itself against attacks by harmful pests in different ways. The research into the natural resistance of cabbage against attacks of selected harmful pests has so far confirmed a negative correlation between the content of epicuticular wax on cabbage leaves and the extent of damage done by the cabbage moth (*Phyllotreta* spp.), the cabbage stink bug (*Eurydema* spp.) and the onion thrips

(*Thrips tabaci* Lindeman) (Trdan *et al.*, 2009); it was also found that the diamondback moth (*Plutella xylostella* L.) averagely leaves more eggs on the green genotypes of cabbage than on the red, though the harmful pest thrives better on the latter (Colares *et al.*, 2013). Considerable, yet not sufficiently explained, is also the influence of glucosinolates on the appearance of the cabbage moth, the cabbage stink bug (Bohinc *et al.*, 2012; Bohinc *et al.*, 2013ab) and the diamondback moth in cabbage (da Silva Carvalho *et al.*, 2010), although some research into the

oviposition of harmful pests (e.g. in the species *Delia floralis*) shows a greater significance of non-glucosinolates (Hopkins et al., 1997). Although the connection between the glucosinolate content in cabbage and its natural resistance against the cabbage moth has been already studied (Cartea et al., 2010), the significance of these substances is still not sufficiently explained, despite the fact that this research problem has been addressed for quite a long time (Cole et al., 1994; Gutbrodt et al., 2012).

Glucosinolates are characteristic secondary metabolites for the order Capparales (Schreiner, 2005). They can be found in 13 different botanical families; so far, they have been characteristic mostly for Brassicaceae (Bohinc et al., 2012). Their variability among plant species, among different organs of the same plant species, and their effect on some harmful pests have been dealt with by certain authors (Gouinguene and Stadler, 2005; Bohinc et al., 2013ab). Glucosinolates can differently influence the feeding of monophagous and polyphagous insects (Renwick, 2002; Bohinc et al., 2012). Among typical polyphagous insects is also the cabbage moth (*Mamestra brassicae* L.), which can successfully feed and develop on more than 70 host plants (Devetak et al., 2010).

Due to the known negative effects of synthetic insecticides – harmful pests can be resistant to them (Springate and Colvin, 2012) – their number on the market has been lately significantly reduced (Finch and Collier, 2000); as a result, there is a greater need to develop, optimize and implement environmentally acceptable ways of suppressing harmful pests in systems of food production. Here the knowledge about the natural resistance of cultivated plants against harmful pests is of the utmost importance. This also includes information about the preferences of polyphagous harmful pests for different species of hosts (Xue et al., 2010; Metspalu et al., 2013) or for different genotypes of the same plant species (Trdan et al., 2004, 2008).

The purpose of our research was to study the glucosinolate content in different genotypes of cabbage in order to identify their influence on the extent of

feeding by cabbage moth caterpillars in a research area in Slovenia. We wish to prove that different genotypes of cabbage are differently susceptible to attacks by the cabbage moth, and that by selecting a genotype we can successfully control the extent of damage. The purpose of our research was based in the fact that glucosinolate content in the same plant species and even in the same genotype considerably depends also on environmental factors (Bohinc and Trdan, 2012); the results connected with the research of other genotypes of cabbage (Cartea et al., 2010) should therefore not be uncritically transferred into other environments.

## MATERIALS AND METHODS

### *Study site and plant material*

A field experiment was carried out in 2011 at the Laboratory Field of the Biotechnical Faculty, University of Ljubljana (46°04'N latitude, 14°31'E longitude, 300 m above sea level), Slovenia. The cabbage plants were grown in the Department of Agronomy's (Biotechnical Faculty) glasshouse according to the protocol described in Trdan et al. (2007). The survey consisted of 10 different cabbage genotypes (5 hybrids and 5 cultivars), which were classified into three groups of genotypes according the length of growing period: early ('Candisa F1', 'Pandion F1', 'Rdeče erfurtsko rano' [= 'Rdeče']) (the length of growing period between 55 and 70 days), mid-early ('Cheers F1', 'Grandslam F1', 'Futoško') (80-90 days), and mid-late ('Hinova F1', 'Holandsko pozno' [= 'Holandsko'], 'Kranjsko okroglo' [= 'Kranjsko'], 'Varaždinsko') (110-140 days).

### *Field evaluation*

The cabbage seedlings were planted on May 4, 2011 in 4 blocks. The plants were not sprayed with insecticides, and each genotype represented a separate treatment (arranged randomly) within the block. The seedlings were planted in a grid of 0.40 x 0.30 m; each block consisted of one bed (breadth 1 m, length 25 m). The beds were covered by black polyethylene mulch. Drip irrigation tape was installed under the

polyethylene mulch in the bed at a distance of 10-15 cm from the plant row. The injuries to the cabbage caused by *Mamestra brassicae* caterpillars were assessed 5 times (18 June, 9 July, 30 July, 5 August, 10 August) by the 5-grade visual scale. The plants were evaluated on the scale from 1 (no damage) to 5 (more than 25% leaf area eaten), as follows: 2) up to 2 % leaf area eaten, 3) between 3 and 10 % leaf area eaten and 4) 11-25 % leaf area eaten (OEPP/EPPO, 2002).

#### *Determination of glucosinolates*

Plant material (cabbage leaves) for the analysis of glucosinolates was sampled at five different intervals (the same days as the injuries of the caterpillars were assessed). The leaves were cut down with scissors. One sample represented a representative sample of the plants from one plot. The material was then freeze-dried (type: LIO-10P, producer: Kambič Laboratorijska oprema, Slovenia) and homogenized before extraction of glucosinolates. The lyophilized samples were stored in 50 ml bottles in a freezer (type: U3286S, producer: Sanyo) at -80°C. The glucosinolate extraction and analysis were performed according to ISO 9167:1-1992. The method was previously described by Bohinc et al. (2013a). In the samples, we determined the content of gluconapin, glucobrassicin, progoitrin, sinalbin, glucoiberin and sinigrin.

#### *Data analysis*

The differences in the glucosinolate content on the leaves of cabbage cultivars were analyzed using a general one-way ANOVA. Prior to analysis, each variable was tested for homogeneity of the variance (Bartlett's test) and the data found to be non-homogenous were transformed to log (Y) prior to ANOVA. Kruskal-Wallis (KW) tests were also applied to analyze the impact of different factors on the glucosinolate level. The differences in glucosinolate content ( $P < 0.05$ ) between the different cabbage cultivars were identified using Duncan's multiple range test. We calculated correlations between the concentration of an individual glucosinolate and the level of injury caused by the caterpillars on cabbage leaves. All the statistical

analyses were performed using Statgraphics Centurion XVI (2009).

## RESULTS

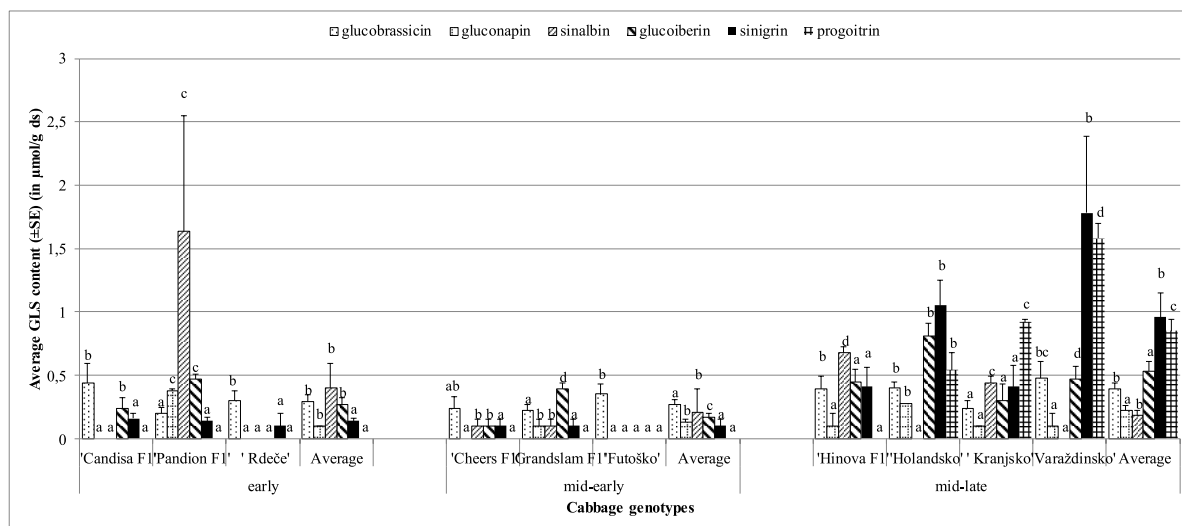
We found that the content of glucobrassicin in the cabbage was significantly influenced by the date of sampling (ANOVA:  $F=3.24$ ,  $Df=4$ ,  $P=0.0149$ ; KW test:  $H=22.18$ ,  $Df=4$ ,  $P=0.0002$ ) and the genotype (ANOVA:  $F=1.79$ ,  $Df=9$ ,  $P=0.0078$ ; KW test:  $H=18.95$ ,  $Df=9$ ,  $P=0.0256$ ), while the content of gluconapin was influenced by the date of sampling (ANOVA:  $F=7.13$ ,  $Df=3$ ,  $P=0.0445$ ; KW test:  $H=4.55$ ,  $Df=3$ ,  $P=0.0501$ ). However, we found out that the content of gluconapin (ANOVA:  $F=4.05$ ,  $Df=1$ ,  $P=0.0455$ ; KW test:  $H=14.04$ ,  $Df=1$ ,  $P=0.0442$ ) and sinalbin (ANOVA:  $F=18.58$ ,  $Df=3$ ,  $P=0.0082$ ; KW test:  $H=16.0$ ,  $Df=3$ ,  $P=0.0088$ ) differed between genotypes, and that the content of sinalbin was not conditioned by the date of sampling (ANOVA:  $F=1.48$ ,  $Df=2$ ,  $P=0.3130$ ; KW test:  $H=4.5$ ,  $Df=2$ ,  $P > 0.05$ ).

The content of sinigrin was also significantly influenced by the genotype of cabbage (ANOVA:  $F=16.55$ ,  $Df=5$ ,  $P=0.0083$ ; KW test:  $H=17.22$ ,  $Df=5$ ,  $P=0.0005$ ) and the date of sampling (ANOVA:  $F=12.03$ ,  $Df=3$ ,  $P=0.0063$ ; KW test:  $H=13.27$ ,  $Df=3$ ,  $P=0.0018$ ); we also found that the content of progoitrin in cabbage was conditioned by the genotype (ANOVA:  $F=3.84$ ,  $Df=2$ ,  $P=0.0080$ ; KW test:  $H=4.48$ ,  $Df=2$ ,  $P=0.0082$ ) and the date of assessment (ANOVA:  $F=4.05$ ,  $Df=2$ ,  $P=0.0039$ ; KW test:  $H=3.07$ ,  $Df=2$ ,  $P=0.0050$ ).

In view of our data, we can confirm that the content of glucoiberin in cabbage is influenced by the date of sampling (ANOVA:  $F=9.16$ ,  $Df=3$ ,  $P=0.0002$ ; KW test:  $H=14.52$ ,  $Df=3$ ,  $P=0.0022$ ) and the genotype (ANOVA:  $F=25.14$ ,  $Df=9$ ,  $P=0.0099$ ; KW test:  $H=23.93$ ,  $Df=9$ ,  $P=0.0044$ ).

#### *The influence of the length of growth period in the genotypes of cabbage on the content of the analyzed secondary metabolites (general analysis)*

The content of glucobrassicin does not differ between early, mid-early and mid-late genotypes of



**Fig 1.** Average glucosinolate content ( $\pm$ SE) ( $\mu\text{mol/g ds}$ ) in 10 cabbage cultivars from 3 groups according to the length of growing period. Lowercase letters represent differences between glucosinolate content in different cultivars belonging to the same group. Glucosinolates present in traces ( $<0.1 \mu\text{mol/g ds}$ ) are evaluated as  $0.1 \mu\text{mol/g ds}$ .

cabbage (ANOVA:  $F=1.57$ ,  $DF=2$ ,  $P>0.05$ ; KW test:  $H=3.93$ ,  $Df=2$ ,  $P>0.05$ ), yet we can confirm that the content of gluconapin is conditioned by the length of growth period of the genotypes (ANOVA:  $F=4.14$ ,  $Df=2$ ,  $P=0.0039$ ; KW test:  $H=3.23$ ,  $Df=2$ ,  $P=0.0050$ ). Mid-late genotypes on average contained  $0.39\pm 0.05 \mu\text{mol/g ds}$  of glucobrassicin, in mid-early genotypes we found  $0.26\pm 0.04 \mu\text{mol/g ds}$ , and in early genotypes  $0.29\pm 0.05 \mu\text{mol/g ds}$ . The content of gluconapin was the highest in mid-late genotypes. The content of sinalbin in general did not differ between the groups of genotypes (ANOVA:  $F=0.57$ ,  $Df=2$ ,  $P>0.05$ , KW test:  $H=0.38$ ,  $Df=2$ ,  $P>0.05$ ). The content of sinigrin was highest in mid-late genotypes ( $0.96\pm 0.19 \mu\text{mol/g ds}$ ), which means that the content differs in the individual groups of genotypes (ANOVA:  $F=7.82$ ,  $Df=2$ ,  $P=0.0007$ ; KW test:  $H=22.95$ ,  $Df=2$ ,  $P=0.0007$ ). The content of glucoiberin differed in individual groups of genotypes (ANOVA:  $F=10.15$ ,  $Df=2$ ,  $P=0.0074$ ; KW test:  $H=7.43$ ,  $Df=2$ ,  $P=0.0243$ ), and was on average the highest in mid-late genotypes ( $0.54\pm 0.08 \mu\text{mol/g ds}$ ). Progoitrin was identified only in mid-late genotypes (ANOVA:  $F=10.14$ ,  $Df=2$ ,  $P=0.0497$ ; KW test:  $H=7.98$ ,  $Df=2$ ,  $P=0.0354$ ).

The content of sinigrin was on average higher in mid-late genotypes ( $1.99\pm 0.31 \mu\text{mol/g ds}$ ), while in

early genotypes it was on average  $0.38\pm 0.01 \mu\text{mol/g ds}$ . The content of glucobrassicin ( $0.44\pm 0.05 \mu\text{mol/g ds}$ ) and gluconapin ( $0.57\pm 0.05 \mu\text{mol/g ds}$ ) was also higher in mid-late genotypes. Sinalbin was in our research present in a larger quantity in early genotypes ( $3.18\pm 0.58 \mu\text{mol/g ds}$ ), while the content of sinigrin was higher in mid-late genotypes ( $1.99\pm 0.32 \mu\text{mol/g ds}$ ).

The content of sinigrin was the highest in the samples of the cultivars 'Varaždinsko' ( $1.78\pm 0.75 \mu\text{mol/g ds}$ ) and 'Kranjsko' ( $2.31\pm 0.04 \mu\text{mol/g ds}$ ), the content of glucobrassicin was higher in the samples of the hybrid 'Hinova F1' ( $0.39\pm 0.14 \mu\text{mol/g ds}$ ) and the cultivars 'Varaždinsko' ( $0.48\pm 0.13 \mu\text{mol/g ds}$ ) and 'Holandsko' ( $0.45\pm 0.08 \mu\text{mol/g ds}$ ), while the average content in the samples of the cultivar 'Rdeče' was  $0.29\pm 0.08 \mu\text{mol/g ds}$ , and in the hybrid 'Pandion F1' it was  $0.18\pm 0.04 \mu\text{mol/g ds}$ . The content of gluconapin was significantly the highest in the samples of the cultivar 'Holandsko' ( $0.27\pm 0.06 \mu\text{mol/g ds}$ ) and the hybrid 'Pandion F1' ( $0.24\pm 0.08 \mu\text{mol/g ds}$ ) (Fig. 1).

Gluconapin was present in traces ( $< 0.1 \mu\text{mol/g ds}$ ) in the hybrids 'Candisa F1' at the first assessment (18 June) and 'Grandslam F1' at the second assess-

**Table 1.** Average values of five glucosinolates (except glucobrassicin) occurring in different cabbage genotypes ( $\mu\text{mol/g ds}$ ) during the growth period

Date of sampling	Cabbage genotype	Glucosinolate				
		glucoiberin	progotrin	sinigrin	sinalbin	gluconapin
18 <sup>th</sup> June	Pandion F1'	in traces	x	0.10±0.00	x	in traces
	'Rdeče'	in traces	x	0.10±0.00	in traces	x
	'Cheers F1'	in traces	x	0.10±0.035	x	x
	'Holandsko'	0.50±0.03	0.50±0.01	0.10±0.10	x	x
	'Candisa F1'	0.40±0.03	x	0.36±0.10	in traces	in traces
	'Grandslam F1'	0.52±0.08	x	0.10±0.00	x	x
	'Varaždinsko'	x	x	1.00±0.12	x	x
	'Hinova F1'	x	x	0.10±0.05	in traces	x
	'Kranjsko'	x	x	x	x	x
	'Futoško'	x	x	0.10±0.10	x	x
9 <sup>th</sup> July	Pandion F1'	x	x	3.18±0.10		
	'Rdeče'	x	x	x	in traces	in traces
	'Cheers F1'	x	x	x	0.67±0.15	in traces
	'Holandsko'	x	x	0.10±0.00	in traces	in traces
	'Candisa F1'	x	x	x	in traces	
	'Grandslam F1'	x	x	x	in traces	in traces
	'Varaždinsko'	x	x	0.10±0.00	x	x
	'Hinova F1'	x	x	0.10±0.00	x	in traces
	'Kranjsko'	x	x	x	x	in traces
	'Futoško'	x	x	0.10±0.00	x	x
30 <sup>th</sup> July	Pandion F1'	0.35±0.08	x	0.10±0.10	x	0.36±0.10
	'Rdeče'	x	x	x	x	in traces
	'Cheers F1'	in traces	x	in traces	x	x
	'Holandsko'	in traces	x	0.27±0.05	x	x
	'Candisa F1'	x	x	in traces	in traces	in traces
	'Grandslam F1'	x	x	in traces	x	in traces
	'Varaždinsko'	x	x	in traces	x	x
	'Hinova F1'	0.32±0.05	x	in traces	x	x
	'Kranjsko'	x	x	in traces	x	x
	'Futoško'	x	x	in traces	x	x

ment (9 July). The significantly highest quantity of sinalbin was present in samples of the hybrid 'Pandion F1' ( $1.64\pm 0.92 \mu\text{mol/g ds}$ ), while it was also detected in samples of the hybrids 'Cheers F1' ( $0.26\pm 0.10 \mu\text{mol/g ds}$ ) and 'Hinova F1' ( $0.24\pm 0.09 \mu\text{mol/g ds}$ ) and the cultivars of 'Kranjsko' ( $0.27\pm 0.10 \mu\text{mol/g ds}$ ). Progoitrin was confirmed in the samples of the mid-

late cultivars 'Holandsko' ( $0.45\pm 0.05 \mu\text{mol/g ds}$ ) and 'Varaždinsko' ( $0.35\pm 0.05 \mu\text{mol/g ds}$ ), glucoiberin was among the studied glucosinolates found in the significantly highest quantity in the samples of the cultivar 'Varaždinsko' ( $0.47\pm 0.24 \mu\text{mol/g ds}$ ), while its content was lowest in early hybrids 'Candisa F1' ( $0.39\pm 0.01 \mu\text{mol/g ds}$ ) and 'Pandion F1' ( $0.47\pm 0.07$

Table 1. Continued

5 <sup>th</sup> Aug	'Pandion F1'	0.58±0.02	x	0.41±0.04	in traces	in traces
	'Rdeče'	x	x	in traces	x	in traces
	'Cheers F1'	in traces	x	in traces	x	in traces
	'Holandsko'	0.62±0.10	0.23±0.02	in traces	x	in traces
	'Candisa F1'	in traces	x	in traces	x	in traces
	'Grandslam F1'	0.28±0.04	x	x	x	in traces
	'Varaždinsko'	in traces	0.47±0.02	1.82±0.21	x	x
	'Hinova F1'	1.54±0.10	x	in traces	x	in traces
	'Kranjsko'	in traces	x	in traces	x	x
'Futoško'	in traces	x	in traces	x	x	
10 <sup>th</sup> Aug	'Varaždinsko'	in traces	x	1.12±0.10	in traces	x
	'Hinova F1'	0.30±0.08	x	1.21±0.19	x	x
	'Kranjsko'	0.50±0.08	x	2.41±0.15	0.44±0.05	x
	'Holandsko'	1.23±0.16	0.62±0.05	2.71±0.10	in traces	0.81±0.10

x-not able to detect, in traces is evaluated as <0.1 µmol/ g ds

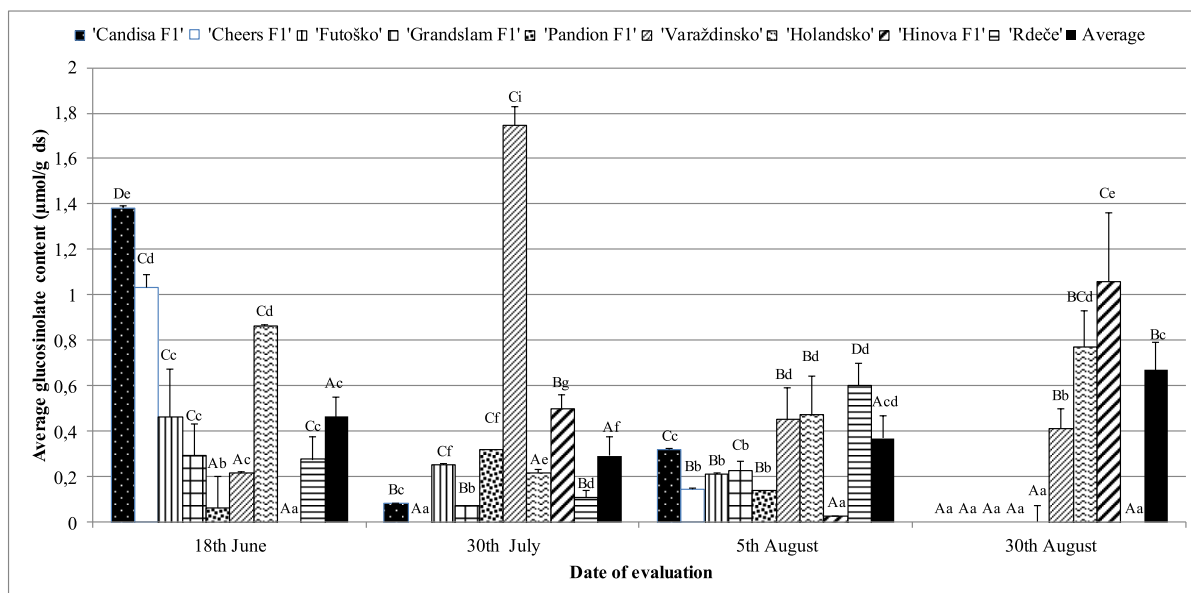


Fig 2. Average glucobrassicin content ( $\pm$ SE) (in  $\mu\text{mol/g ds}$ ) in different cultivars (lowercase letters present the differences between cabbage cultivars on the same date of assessment; uppercase letters represent the differences between different dates of assessment concerning the same glucosinolate). To simplify Fig 2, 9<sup>th</sup> July is not presented.

$\mu\text{mol/g ds}$ ). In the samples of the cultivar 'Rdeče', the said glucosinolate was present in traces (Table 1).

#### *Content of glucobrassicin at different intervals in the growth period*

Glucobrassicin was the only glucosinolate found

in all genotypes of cabbage. Fig. 2 shows the content of this substance at four from the five intervals of sampling. The content of glucobrassicin was at the first date of assessment highest in the genotype 'Candisa F1' ( $1.38\pm 0.01 \mu\text{mol/g ds}$ ), while in 'Hinova F1' we did not establish any content of this glucosinolate. On the third date of assessment, we

**Table 2.** Correlation between the mean level of injury caused by *Mamestra brassicae* caterpillars and glucosinolate concentration ( $P < 0.05$ , Duncan's multiple range test) on mid-early and mid-late cabbage genotypes.

Cabbage group	Glucosinolate	r	a	b	p
mid-early genotypes	glucoiberin	-0.25	1.3091	-0.6948	0.0456*
	sinalbin	-0.34	1.50726	-0.6682	0.0454*
	sinigrin	-0.04	1.27132	0.7751	0.9600
mid-late genotypes	glucobrassicin	0.20	1.0580	0.1643	0.0498*
	gluconapin	0.87	-0.81	3.74	0.0241*
	sinalbin	-0.02	1.2078	-0.0666	0.9600
	sinigrin	-0.27	1.1087	0.0290	0.0426*
	progoitrin	0.66	0.2895	2.0231	0.0358*
	glucoiberin	0.27	0.9798	0.2210	0.1793

confirmed in the genotype 'Varaždinsko' the highest content ( $1.74 \pm 0.085$ ), while at the fourth date assessment we found the highest content in the genotypes 'Varaždinsko' ( $0.45 \pm 0.14 \mu\text{mol/g ds}$ ) and 'Holandsko' ( $0.47 \pm 0.17 \mu\text{mol/g ds}$ ). At the last date of assessment, the content of glucobrassicin was among the highest in the samples of the genotype 'Hinova F1' ( $1.06 \pm 0.03 \mu\text{mol/g ds}$ ).

*The influence of glucosinolate content on the extent of damage in mid-early and mid-late genotypes of cabbage*

Among the studied correlations between glucosinolate content and the extent of damage done by the caterpillars of *Mamestra brassicae* on cabbage leaves, we can point out the activity of glucoiberin ( $r = -0.25$ ,  $P < 0.05$ ) and sinalbin ( $r = -0.34$ ,  $P < 0.05$ ) (Table 2) in mid-early genotypes. A significant influence of the remaining glucosinolates was not established.

In mid-late genotypes of cabbage, we can talk about the significant influence of the four selected glucosinolates. We thus noted a weak correlation between the content of glucobrassicin and the extent of damage ( $r = 0.20$ ,  $P < 0.05$ ), and between sinigrin and the extent of damage ( $r = -0.27$ ,  $P < 0.05$ ), a strong correlation between the content of gluconapin and the extent of damage ( $r = 0.87$ ,  $P < 0.05$ ), and a moderate

correlation between the content of progoitrin and the extent of damage ( $r = 0.66$ ,  $P < 0.05$ ). We found that there was a significant negative correlation between the content of sinigrin and the extent of damage ( $r = -0.27$ ,  $P < 0.05$ ).

In the remaining glucosinolates, we did not detect any significant correlation; the remaining values are presented in the Table 2.

## DISCUSSION

The results of our research confirm the findings of some past studies (Moyes et al., 2000; Bohinc et al., 2013ab), namely that glucosinolate content in plants depends on different factors (the significance of many is still not precisely explained) and that their content in plants varies through the growth period. It has already established that differences in the content of these secondary metabolites also occur between genotypes of the same plant species (Kim et al., 2010). Our research defines these correlations in more detail. The results of our research indicate a higher content, and consequently the significance of glucosinolates for mid-early and mid-late genotypes of cabbage. For this reason, we studied in detail the correlation between the extent of damage done by the polyphagous cabbage moth caterpillars (*Mamestra brassicae*) and the content of glucosinolates in the selected groups of cabbage.

We thus established that glucobrassicin, which is one of the glucosinolates whose content in plants is also influenced by environmental factors (Kang et al., 2006; Bohinc and Trdan, 2012), was in our research present in all five cultivars and five hybrids of cabbage. In the research carried out by Bohinc et al. (2013ab), glucobrassicin was also the only glucosinolate present in all studied species of Brassicaceae. In view of the sensitiveness of this glucosinolate to environmental factors, our research established a weak correlation ( $r=0.20$ ) between its concentration and the extent of damage in mid-late genotypes; we cannot classify it as a key secondary metabolite that would condition antixenosis in cabbage. Our finding that the content of progoitrin is higher in mid-late genotypes of cabbage is also consistent with the findings of our earlier research (Bohinc et al., 2013b). However, the added value of our results is the confirmed moderately strong positive correlation ( $r=0.66$ ) between the content of progoitrin and the extent of damage done by cabbage moth caterpillars to leaves (a similar correlation was confirmed by Newton et al. (2010) for the louse *Brevicoryne brassicae* and cabbage), which was in the same group of cabbage genotypes confirmed also for gluconapin ( $r=0.87$ ), whose antixenotic effects could be according to the reports by Fritz et al. (2010) increased by foliar application of jasmonic acid to cabbage.

Despite the fact that in our research sinigrin was, in comparison with other types of glucosinolates, present in large amounts in all mid-late cultivars, its influence on the extent of damage done by cabbage moth caterpillars was relatively weak ( $r=-0.27$ ), so we cannot confirm the finding of Olsson and Jonasson (1994), who attribute to this substance a great antixenotic effects on leaf-eating caterpillars in cabbage. Sinigrin is known to have anticarcinogenic effects (Wang et al., 2012), and is consequently attributed a greater importance for healthy nutrition than in plant protection. The negative influence of sinigrin and sinalbin (which is a known stimulator of oviposition in cabbage root fly (*Delia* spp.)) on the feeding of *Mamestra configurata* caterpillars was mentioned also by Ulmer et al. (2001) (Gouinguene and Stadler,

2005), and we can to some extent confirm such correlation ( $r=-0.34$ ) in mid-early genotypes of cabbage.

In view of the results of our research, in which we established that the content of the analyzed glucosinolates was the highest in mid-late cultivars, we can say that glucosinolates can be an important factor deterring cabbage moth caterpillars from feeding on these genotypes; these caterpillars are usually more harmful at the end of the growth period of the cabbage genotypes from the said group (Brandsaeter et al., 1998; Zalokar, 2011).

We can thus summarize that the selection of a cabbage genotype can be one of the indirect (alternative) measures for reducing the harmfulness of cabbage moth caterpillars. Much has been written about the positive effects of glucosinolates in human nutrition (Bjorkman et al., 2011), while, due to their variability (confirmed also in our research), we cannot speak of their universal applicability in plant protection (Bohinc et al., 2012). As key substances influencing the susceptibility of the studied cabbage genotypes to attacks by cabbage moth caterpillars were identified the glucosinolates gluconapin and progoitrin in mid-late genotypes, and sinalbin (by increasing its quantity in cabbage we reduced its susceptibility to attacks by the harmful pest) in mid-early genotypes, yet the potential nature of their antixenotic effects in cabbage will still have to be studied in more detail.

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## REFERENCES

- Bjorkman, M., Klingen, I., Birch, A. N. E., Bones, A. M., Bruce, T. J. A., Johansen, T. J., Meadow, R., Molmann, J., Seljasen, R., Smart, L. E. and D. Stewart (2011). Phytochemicals of Brassicaceae in plant protection and human health - Influences of climate, environment and agronomic practice. *Phytochem.* **72**, 538-556.
- Bohinc, T. and S. Trdan (2012). Environmental factors affecting the glucosinolate content in Brassicaceae. *J. Food Agric. Environ.* **10**, 357-360.



- Bohinc, T., Goreta Ban, S., Ban D. and S. Trdan (2012). Glucosinolates in plant protection strategies – a review. *Arch. Biol. Sci.* Belgrade. **64**, 821-828.
- Bohinc, T., Košir, I.J. and S. Trdan (2013a). Glucosinolates as arsenal for defending Brassicas against cabbage flea beetle (*Phyllotreta* spp.) attack. *Zemdirbyste-Agriculture*. **100**, 199-204.
- Bohinc, T., Hrastar, R., Košir, I. J. and S. Trdan (2013b). Association between glucosinolate concentration and injuries caused by cabbage stinkbugs *Eurydema* spp. (Heteroptera: Pentatomidae) on different Brassicas. *Acta Sci. Agron*. **35**, 1-8.
- Brandsaeter, L.O., Netland, J. and R. Meadow (1998). Yields, weeds, pests and soil nitrogen in a white cabbage living mulch system. *Biol. Agric. & Hort*. **16**, 291-309 .
- Cartea, M.E., Francisco, M., Lema, M., Soengas, P. and P. Velasco (2010). Resistance of cabbage (*Brassica oleracea capitata* Group) crops to *Mamestra brassicae*. *J. Econom. Entomol*. **103**, 1866-1874.
- Colares, F., Silva-Torres, C. S. A., Torres, J. B., Barros, E. and A. Pallini (2013). Influence of cabbage resistance and colour upon the diamondback moth and its parasitoid *Oomyzus sokolowskii*. *Entomol. Exp. Appl*. **148**, 84-93.
- Cole, R. A. (1994). Locating a resistance mechanism to the cabbage aphid in 2 wild Brassicas. *Entomol. Exp. Appl*. **71**, 23-31.
- Da Silva Carvalho, J., De Bortoli, S.A., Thuler, R.T., Goulart, R.M. and H. L. Linhares Volpe (2010). Efeito de sinigrina aplicada em folhas de brássicas sobre características biológicas de *Plutella xylostella* (L.) (Lepidoptera: Plutellidae). *Acta Sci. Agron*. **32**, 16-20.
- Devetak, M., Vidrih, M. and S. Trdan (2010). Cabbage moth (*Mamestra brassicae* [L.]) and bright-line brown-eyes moth (*Mamestra oleracea* [L.]) – presentation of the species, their monitoring and control measures. *Acta Agric. Slo*. **95**, 149-156.
- Finch, S. and R.H. Collier (2000). Integrated pest management in field vegetable crops in northern Europe – with focus on two key pests. *Crop Prot*. **19**, 817-824.
- Fritz, V.A., Justen, V.L., Bode, A.M., Schuster, T. and M. Wang (2010). Glucosinolate Enhancement in Cabbage Induced by Jasmonic Acid Application. *Hortsci*. **45**, 1188-1191.
- Gouinguene, S. P. D. and E. Stadler (2005). Comparison of the sensitivity of four *Delia* species to host and non-host plant compounds. *Physiol. Entomol*. **30**: 62-74.
- Gutbrodt, B., Dorn, S., Unsicker, S.B. and K. Mody (2012). Species-specific responses of herbivores to within-plant and environmentally mediated between-plant variability in plant chemistry. *Chemoecol*. **22**, 101-111.
- Hopkins, R.J., Birch, A.N.E., Griffiths, D.W., Baur, R., Stadler, E. and R.G. McKinlay (1997). Leaf surface compounds and oviposition preference of turnip root fly *Delia floralis*: the role of glucosinolate and nonglucosinolate compounds. *J. Chem. Ecol*. **23**, 629-643.
- Lucas-Barbosa, D., van Loon, J.J.A. and M. Dicke (2011). The effects of herbivore-induced plant volatiles on interactions between plants and flower-visiting insects. *Phytochem*. **72**, 1647-1654.
- Kang, J.Y., Ibrahim, K.E., Juvik, J.A., Kim, D.H. and W.J. Kang (2006). Genetic and environmental variation of glucosinolate content in Chinese cabbage. *Hortsci*. **41**, 1382-1385.
- Kim, J.K., Chu, S.M., Kim, S.J., Lee, D.J., Lee, S.Y., Lim, S.H., Ha, S.H., Kweon, S.J. and H.S. Cho (2010). Variation of glucosinolates in vegetable crops of *Brassica rapa* L. ssp *pekinensis*. *Food Chem*. **119**, 423-428.
- Moyes C.L., Collin H.A., Britton G. and A.E. Raybould (2000). Glucosinolates and differential herbivory in wild populations of *Brassica oleracea*. *J. Chem. Ecol*. **26**, 2625-2641.
- Metspalu, L., Kruus, E., Jõgar, K., Kuusik, A., Williams, I. H., Veromann, E., Luik, A., Ploomi, A., Hiisaar, K., Kivimägi, I. and M. Mänd (2013). Larval food plants can regulate the cabbage moth, *Mamestra brassicae* population. *Bull. Insect*. **66**, 93-101.
- Newton, E., Bullock, J.M. and D. Hodgson (2010). Temporal consistency in herbivore responses to glucosinolate polymorphism in populations of wild cabbage (*Brassica oleracea*). *Oecologia*. **164**, 689-699.
- OEPP/EPPO (2002). Guidelines for the efficacy evaluation of insecticides. *Phyllotreta* spp. on rape. *OEPP/EPPO Bull*. **32**, 361-365.
- Olsson, K. and T. Jonasson (1994). Leaf feeding by caterpillars on white cabbage cultivars with different 2-propenyl glucosinolate (sinigrin) content. *J. Appl. Entomol*. **118**, 197-202.
- Renwick, J.A.A. (2002). The chemical world of crucivores: lures, treats and traps. *Entomol. Exp. Appl*. **104**, 35-42.
- Screiner, M. (2005). Vegetable crop management strategies to increase the quantity of phytochemicals. *Eur J Nutr*. **44**, 85-94.
- Springate, S. and J. Colvin (2012). Pyrethroid insecticide resistance in British populations of the cabbage whitefly, *Aleyrodes proletella*. *Pest Manage. Sci*. **68**, 260-267. .
- Statgraphics Centurion XVI (2009). Statpoint Technologies Inc., Warrenton, USA
- Trdan, S., Žnidarčič, D., Zlatič, E. and J. Jerman (2004). Correlation between epicuticular wax content in the leaves of early white cabbage (*Brassica oleracea* L. var. *capitata*) and

- damage caused by *Thrips tabaci* Lindeman (Thysanoptera: Thripidae). *Acta Phytopathol. Entomol. Hung.* **39**, 173-185.
- Trdan S., Valič N. and D. Žnidarčič (2007). Field efficacy of deltamethrin in reducing damage caused by *Thrips tabaci* Lindeman (Thysanoptera: Thripidae) on early white cabbage. *J. Pest Sci.* **80**, 217-223.
- Trdan, S., Valič, N., Andjus, L., Vovk, I., Martelanc, M., Simonovska, B., Jerman, J., Vidrih, R., Vidrih, M. and D. Žnidarčič (2008). Which plant compounds influence the natural resistance of cabbage against onion thrips (*Thrips tabaci* Lindeman)? *Acta Phytopathol. Entomol. Hung.* **43**, 385-395.
- Trdan, S., Valič, N., Vovk, I., Martelanc, M., Simonovska, B., Vidrih, R., Vidrih, M. and D. Žnidarčič (2009). Natural resistance of cabbage against three insect pests. In: Integrated Protection of Field Vegetables. Proceedings of the meeting. *IOBC/wprs Bull.* **51**, 93-106.
- Ulmer, B., Gillot, C. and M. Erlandson (2001). Feeding preference, growth and development of *Mamestra configurata* (Lepidoptera: Noctuidae) on Brassicaceae. *Can. Entomol.* **133**: 509-519.
- Wang, T., Liang, H. and Q. Yuan (2012). Separation of sinigrin from Indian mustard (*Brassica juncea* L.) seed using macroporous ion-exchange resin. *Korean J. Chem. Eng.* **29**, 396-403.
- Zalokar, N. (2011). Resistance of cabbage (*Brassica oleracea* L. var. *capitata* L.) to attack of selected insect pests in field conditions. M.Sc. Thesis. University of Ljubljana, Biotechnical Faculty, Ljubljana, 1-149. [Slovenian]
- Xue, M., Pang, Y.H., Wang, H.T., Li, Q.T., and T.X. Liu (2010). Effects of four host plants on biology and food utilization of the cutworm, *Spodoptera litura*. *J. Insect Sci.* **10**, 1-14.