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ASSOCIATION BETWEEN ANTIOXIDATIVE POTENTIAL AND LEVEL OF INJURY CAUSED BY *EURYDEMA* SPP. FEEDING ON RED AND WHITE CABBAGE GENOTYPES

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Abstract - In a two-year field experiment, we studied the extent of damage caused by cabbage stink bugs (*Eurydema* spp.) on the frame leaves and the outer leaves of cabbage heads in relation to genotype color. We established that the extent of damage varied with genotype color. In both years of the experiment, the affinity of *Eurydema* spp. toward green genotypes was significantly greater on the first four assessment dates (from the third decade of May to the third decade of June), while on the remaining dates (from the third decade of July to the second decade of August) we did not establish any differences between the white and red genotypes. Cabbage stink bugs first appeared on white cabbage genotypes. The antioxidative potential conditioned by the content of anthocyanins was significantly higher on the red cabbage genotypes. Research shows that higher antioxidative potential is related to a lower extent of damage caused by *Eurydema* spp. The average value of antioxidative potential in the cabbage varieties was 0.58 mmol/100 g of the sample, while in the hybrids it was 0.47±0.01 mmol/100 g. We confirmed significant differences in values of antioxidative potential between red (0.68 mmol/100 g) and white (0.48 mmol/100 g) cabbage genotypes. Between mid-late (0.55 mmol/100 g) and mid-early (0.53 mmol/100 g) cabbage genotypes we did not establish differences in antioxidative potential levels, while the average value of this parameter in the early genotypes (0.46 mmol/100 g) was significantly low. We established that the color of plants (cabbage) represents one of the successful factors of antixenosis and has the potential for reducing the damage caused by cabbage stink bugs in environmentally acceptable systems of cabbage production.

Key words: antioxidative potential; cabbage; red genotypes; white genotypes; *Eurydema* spp.; antixenosis

INTRODUCTION

The factors of natural resistance of cabbage to harmful pests can be morphological, for example epicuticular wax (Trdan et al., 2009), or chemical, which includes glucosinolates and other types of secondary metabolites (Bohinc et al., 2012). When plants are under stress, important factors of their resistance are also antioxidants (Singh et al., 2006).

Phenolic molecules, vitamin C, folic acid, carotenoids and vitamin E (Soengas et al., 2011) thus play an important role in cabbage. Flavonoids are plant phenols (Dai and Mumper, 2010). Anthocyanins, which are usually mentioned as pigments (also in red cabbage) (Hatier and Gould, 2008) belong to flavonoids (Soengas et al., 2011) ,They condition the natural resistance of plants (Lev-Yadun and Gould, 2008), as they influence harmful pests' color perception.

The Brassicaceae are plant species that are important both from the agronomic (Font et al., 2005; Vaughn and Berhow, 2005; Cartea et al.,

2008) and economic (Vaughn and Berhow, 2005) perspectives. The production of cabbage, which is in Europe a very important field vegetable crop (Devetak et al., 2013), is exposed to many harmful pests (Bohinc and Trdan, 2012), including cabbage stink bugs (*Eurydema* spp.). By sucking the sap of older plants, this genus of univoltile harmful pests (Trdan et al., 2006) causes discoloration or bronze blisters, while on young plants the injuries are seen as white freckles (Trdan et al., 2006; Demirel, 2009; Eltez and Karsavuran, 2010). Among the alternative methods that have been proven successful in reducing the damage caused by cabbage stink bugs on cabbage, are trap cropping (Bohinc and Trdan, 2012) and the selection of cabbage genotypes that can considerably differ in epicuticular wax content, which is an important factor of natural resistance of this garden crop to cabbage bugs (Trdan et al., 2009).

The aim of our research was to establish the extents of damage caused by *Eurydema* spp. in white and red cabbage genotypes, the levels of antioxidative potential in these genotypes, and the correlations between antioxidative potential and the extent of damage caused by cabbage stink bugs.

MATERIALS AND METHODS

Study site, material and field experiment

A field experiment was done in 2010 and 2011 at the Experimental Field of Biotechnical Faculty in Ljubljana (altitude 296.4 m, 46° 2' 58" N, 14° 28' 28" E), Slovenia. The experimental plots (20) were allocated randomly within each of four blocks. Each plot was 1.20 m long and planted with seedlings of the same cabbage variety. The experiment consisted of twenty cabbage genotypes, which were divided into two groups (red and white), depending on plant color. The seedlings were transplanted by hand into the field on 26th April 2010 and on 3rd May 2011). Transplants were grown in a greenhouse in plant trays with commercial compost, and fed and irrigated according to standard practices (Trdan et al., 2008).

There were 14 hybrids (mid-late: 'R1-cross F1', 'Hinova F1'; mid-early: 'Red dynasty F1', 'Cheers F1', 'Fieldforce F1' and 'Vestri F1'; early: 'Pandion F1', 'Sunta F1', 'Delphi F1', 'Tucana F1', 'Ixxion F1', 'Autumn queen F1', 'Destiny F1', 'Green Rich F1') and 6 non-hybrid varieties (mid-late: 'Kranjsko okroglo' [as 'Kranjsko'], 'Holandsko rdeče' [as 'Holandsko'], 'Varaždinsko'; mid-early: 'Futoško' and early: 'Erfurtsko rdeče' ['Erfurtsko']). There were 17 white genotypes ('Autumn queen F1', 'Cheers F1', 'Delphi F1', Destiny F1', 'Fieldforce F1', 'Futoško', 'Green rich F1', 'Hinova F1', 'Ixxion F1', 'Kranjsko okroglo', 'Ljubljansko', 'Pandion F1', 'R1 Cross F1', 'Sunta F1', 'Tucana F1', 'Varaždinsko' in 'Vestri F1') and 3 red ones ('Erfurtsko', 'Holandsko', and 'Red dinasty F1'). The length of growth period of the mid-late cabbage genotypes was 110-140 days, in the mid-early ones it was 80-90 days, and in early ones, it was 55-70 days.

The experiment was carried out in a 110.4 m^2 plot. The field was divided into four blocks, 1.10 x 24 m each. The plants were grown using standard practices, except that no insecticide was applied; a more detailed description is available in Trdan et al. (2007).

Field evaluation and observations

The damage caused by cabbage stink bugs on 20 cabbage genotypes and the growth stages of plants were assessed at 10-day intervals. In both years of the experiment, we assessed damage on nine different points during the growth period. The assessment was thus carried out in the last ten days of May (May 3), in the first ten days of June (June 1), June 2 represents the second 10 days of June, June 3 represents the third 10 days of June, July 1 represents the first ten days of July, July 2 represents the second ten days of July, July 3 represents the third ten days of July. The assessment carried out in the first ten days of August was marked as August 1, while the assessment carried out in the second ten days of the same month was marked as August 2.

The damage caused by cabbage stink bugs on the frame leaves and the outer leaves of the cabbage heads was assessed by the 6-grade visual scale, which was originally intended for assessing the damage caused by onion thrips (*Thrips tabaci* L.) (Stoner and Shelton, 1988). Mark 1 thus means undamaged leaves, mark 2 means less than 1% of damaged leaf surface, mark 3 means from 2 to 10% of damaged leaf surface, mark 4 means from 11 to 25% of damaged leaf surface, mark 5 means from 26 to 50% of damaged leaf surface, and mark 6 means more than 50% of damaged leaf surface. The said visual scale was for the needs of assessing the extent of damage caused by cabbage stink bugs on cabbage used previously by Trdan et al. (2006) and Bohinc et al. (2012).

Growth stages of the cabbage were identified using the BBCH scale for leafy vegetables that form heads (Feller et al., 1995; Growth stages of mono- and dicotyledonous plants, 2001) (Tables 1-2).

Laboratory study

When in the first year of the experiment the cabbage genotypes reached technological maturity, the outer leaves were stored in special plastic bags. Using a sharp knife we measured 10 g of individual genotypes and put them into plastic 50 mL centrifuge tubes. Fifteen g of 2% solution of metaphosphoric acid was added into the centrifuge tubes and homogenized by Ultra-Turax for 5 min. After homogenization, the samples were frozen and stored at -20°C until the analyses.

The frozen samples in 2% metaphosphoric acid were melted and centrifuged for 5 min at 4 000 rpm. The upper phase was filtered through a filter (17 mm syr filter CA 0.45 µm) into vials (PK 100 1.5 ml ABC vial clear glass W/PATCH 6 mm ID. 11.6 x 32 mm). For the reference value (RF), we mixed 60 µl of methanol and 1.5 ml of DPPH solution in an Eppendorf tube. Sixty µl of the studied genotypes sample and 1.5 ml of DPPH solution were mixed. Three samples of each genotype were analyzed in parallel. In the blind experiment, we mixed 60 µl of a sample with 1.5 ml of methanol. The mixtures were mixed thoroughly and absorption was measured at 517 nm after 15 min (Hewlett-Packard spectrophotometer: HP model 8453, USA). The antioxidative potential is presented as mmol/100 mg of the sample. The antioxidative potential was assessed by the DPPH method.

Data analysis

The experimental results were statistically evaluated with the program Statgraphics Centurion XVI (2009). Analysis of variance (ANOVA) was used to assess differences between cabbage cultivars due to *Eurydema* spp. feeding. Before the analysis, each variable was tested for the homogeneity of treatment variances (Bartlett's test), and the data found to be non-homogenous were modified to log (Y) prior to the ANOVA. Kruskal-Wallis tests were also applied to analyze the impact of different factors on the damage level on cabbage caused by cabbage stink bugs. ANOVA was also used to identify differences between antioxidative potential among different cabbage genotypes. Duncan's tests were used to analyze the differences between different cabbage genotypes. We calculated the correlation between the average index of damage on the last date of evaluation and the average level of antioxidative potential.

RESULTS

The extent of damage on cabbage leaves caused by cabbage stink bugs

The results of the general statistical analysis show that the average extent of damage on cabbage leaves caused by cabbage stink bugs sucking in the first year of the experiment depended on the date of assessment (ANOVA, $F=70.37$, $Df=8$, $P<0.0001$; KW test, $H=463.11$, $Df=8$; $P<0.0001$) and the plants' growth stage (ANOVA, $F=51.17$, $Df=16$, $P<0.0001$; KW test, $H=594.15$, $Df=16$, $P<0.0001$). The extent of damage differs also in relation to the color (ANOVA, $F=6.23$, $Df=1$, $P=0.0126$; KW test, $H=8.25$, $Df=0.0040$) and cabbage genotype (ANOVA, $F=4.58$, $Df=19$, $P<0.0001$; KW test, $H=88.21$, $Df=19$, $P=<0.0001$). However, we did not detect the influence of variety or hybrid on the extent of damage (ANOVA, $F=0.54$, $Df=1$, $P=0.4608$; KW test, $H=1.13$, $Df=1$, $P=0.2862$); the same holds true for the length of growth period

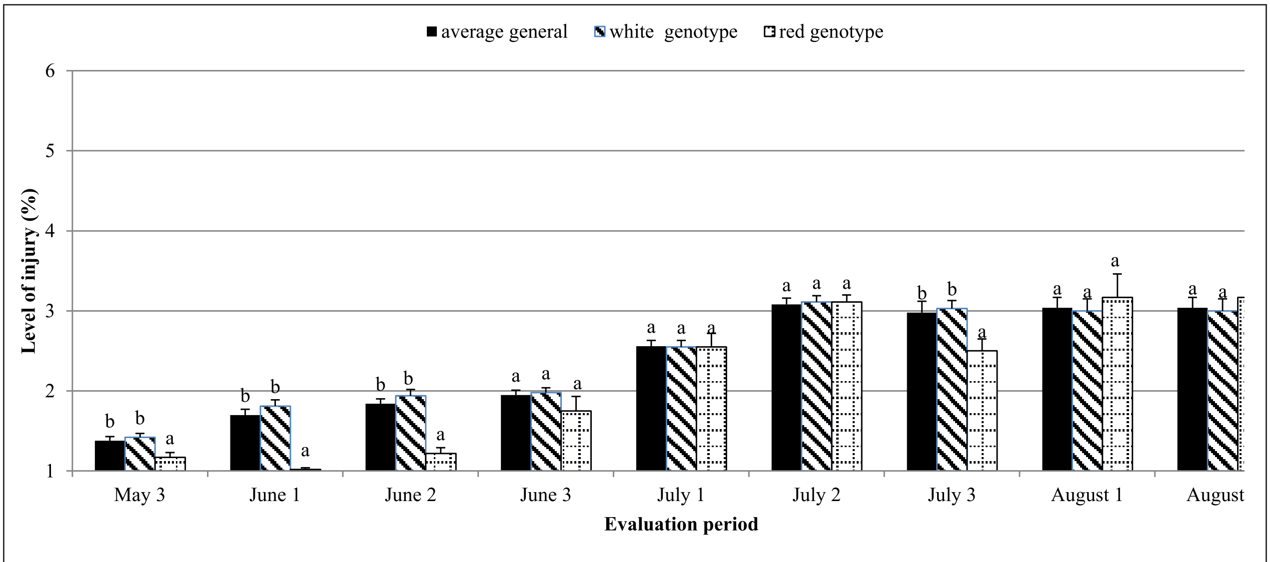

Fig. 1. Level of injury caused by *Eurydema* spp. on different groups of cabbage genotypes in 2010.

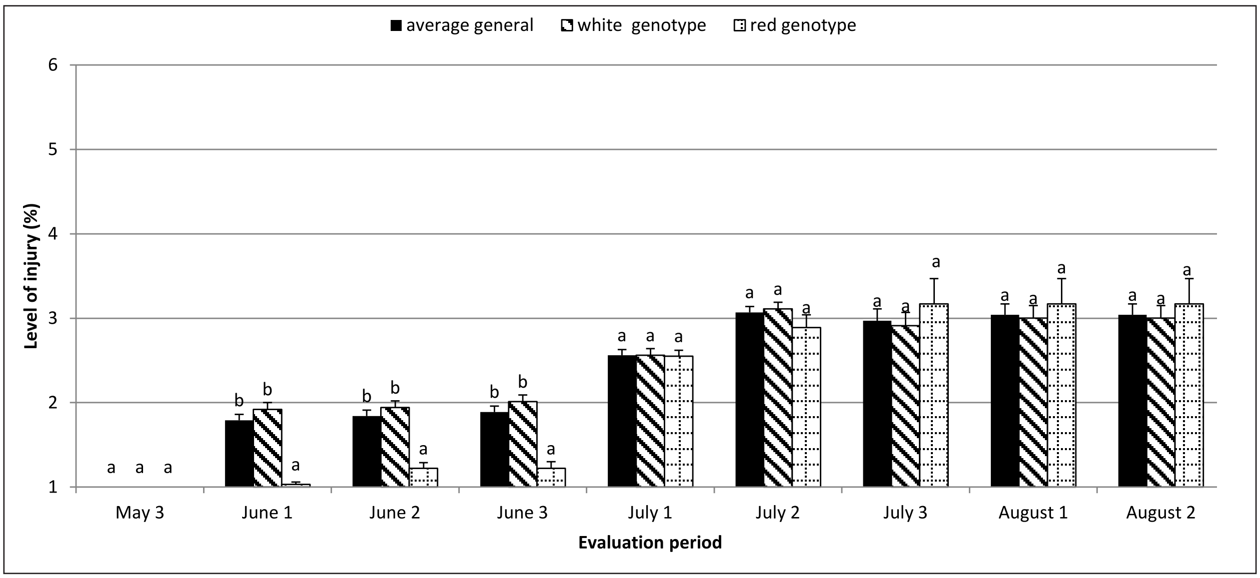

Fig. 2. Level of injury caused by *Eurydema* spp. on different groups of cabbage genotypes in 2011.

(ANOVA, F=0.63, Df=2, P=0.5310; KW test, H=1.46, Df=2, P=0.4798).

The average extent of damage on the white cabbage genotypes was 2.16±0.03, while the average extent of damage on the red genotypes was 1.96±0.07. The extent of damage on the first date of assessment was on the white genotypes 1.42±0.05, while on the red genotypes it was significantly lower (1.17±0.06). On the second date of assessment (June 1) we again recorded significant differences between the white (1.81±0.08) and the red genotypes (1.02±0.02); there were also differences in susceptibility of the white (1.94±0.08) and the red (1.22±0.07) genotypes on the third date of assessment. On the remaining dates of assessment, we established significant differences

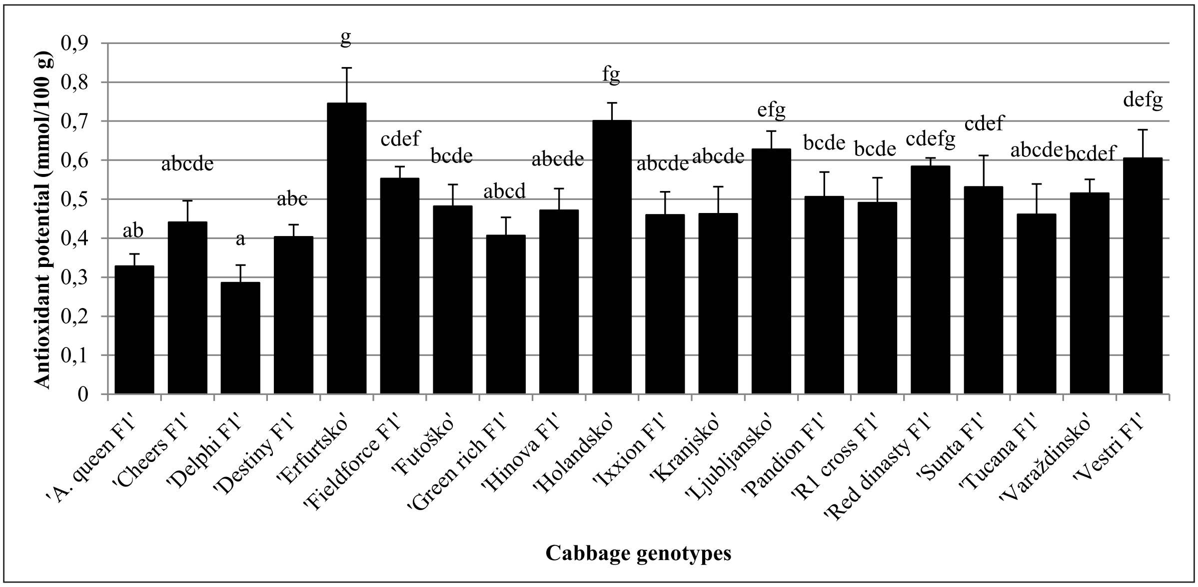

Fig. 3. Antioxidative potential among 20 different cabbage genotypes in 2010.

between cabbage genotypes of different colors only in the 3rd decade of July, when the average index of damage on the red genotypes (3.03±0.10) was significantly lower than on the white genotypes (2.50±0.15) (Fig. 1).

In the second year of the experiment the extent of damage was influenced by the date of assessment (ANOVA, F=92.73, Df=8, P<0.0001; KW test, H=599.27, Df=8, P<0.0001) and the growth stage (ANOVA, F=59.25, Df=16, P<0.0001; KW test, H=664.75, Df=16, P<0.0001) of cabbage. The extent of damage on leaves was influenced also by the color of the cabbage (ANOVA, F=9.28, Df=1, P=0.0023; KW test, H=10.31, Df=1, P=0.0013) and the genotype (ANOVA, F=4.22, Df=19, P<0.0001; KW test, H=75.96, Df=19, P=9.13*10^{-9}), while we detected no influence of variety or hybrid (ANOVA, F=2.02, Df=1, P=0.1549; KW test, H=2.76, Df=1, P=0.0964) and length of growth period (ANOVA, F=1.31, Df=2, P=0.2714).

On the first date of assessment in the second year of the experiment we did not detect any damage caused by cabbage stink bugs on cabbage leaves. However, the white genotypes in comparison to the red ones (1.03±0.03) were already at the second date of assessment significantly more susceptible to attacks by cabbage stink bugs (1.92±0.08). We also detected significantly higher extent of damage on white varieties on the third (1.94±0.08) and fourth (2.01±0.08) date of assessment, while no significant differences between both color groups were observed at later dates (Fig. 2).

Antioxidative potential in white and red genotypes

In view of the results of our research we conclude that the antioxidative potential in cabbage depends on the genotype's color (ANOVA, F=25.93, Df=1; P<0.0001; KW test, H=18.70; Df=1; P<0.0001), the length of growth period (ANOVA, F=2.48, Df=2; P=0.0452; KW test, H=7.81, Df=2, P=0.0201) and genotype (ANOVA, F=3.50; Df=19, P<0.0001; KW test, H=44.24, Df=19, P=0.00087). We also established that the antioxidative potential content differed between the hybrids and the varieties (ANOVA, F=13.42, Df=1; P=0.0004; KW test, H=10.82, Df=1, P=0.0010). On average, the antioxidative potential in the varieties was significantly higher (0.59±0.03 mmol/100 g) than in the hybrids (0.47±0.01 mmol/100 g). The average level of antioxidative potential was

Table 1. Growth stages of 20 cabbage genotypes on the assessment dates in 2010

	May 3	June 1	June 2	June 3	July 1	July 2	July 3	August 1	August 2
'A. queen F1'	19-19	41-41	41-43	42-45	46-49	49-49			
'Cheers F1'	19-41	41-41	41-43	42-44	45-48	49-49			
'Delphi F1'	19-41	41-42	41-42	43-45	41-47	49-49			
'Destiny F1'	19-41	41-42	41-44	43-45	46-47	49-49			
'Erfurtsko'	19-19	19-42	19-41	43-44	43-47	49-49			
'Fieldforce F1'	19-41	41-45	45-48	47-49	49-50				
'Futoško'	14-19	41-41	42-42	42-45	42-48	49-49			
'Green rich F1'	19-41	42-44	42-44	43-44	46-47	50-50			
'Hinova F1'	19-19	19-41	19-41	42-43	42-45	47-48	48-49	49-49	49-49
'Holandsko'	19-19	19-41	19-42	42-43	42-45	46-49	47-49	48-49	49-49
'Ixxion F1'	19-41	41-42	42-44	43-48	43-48	49-49			
'Kranjsko'	13-19	14-19	19-19	19-42	41-42	41-45	46-46	47-47	48-49
'Ljubljansko'	16-19	14-41	19-42	42-44	44-46	47-48	47-49	48-48	48-48
'Pandion F1'	41-41	43-45	43-45	49-49					
'R1 cross F1'	19-19	41-41	41-42	42-44	43-47	49-49			
'Red dinasty F1'	19-19	19-42	19-41	42-43	42-47	49-49			
'Sunta F1'	19-41	42-44	43-48	49-49					
'Tucana F1'	41-41	42-44	46-48	49-49	49-49				
'Varaždinsko'	19-19	19-42	19-43	42-44	44-47	49-49			
'Vestri F1'	19-19	41-42	41-43	43-44	45-47	47-49			

in the mid-late genotypes 0.55±0.01 mmol/100 g, in the mid-early ones 0.53±0.01 mmol/100 g, while it was on average the lowest in the early genotypes (0.46±0.02 mmol/100 g). The average level of antioxidative potential was in the white cabbage genotypes 0.48±0.01 mmol/100 g, while in the red genotypes it was 0.68±0.04 mmol/100 g.

Among the red cabbage genotypes we established the highest antioxidative potential in the varieties 'Erfurtsko' (0.74±0.09 mmol/100 g of fresh sample) and 'Holandsko' (0.70±0.05 mmol/100 g of fresh sample), among the white genotypes it was observed in the variety 'Varaždinsko' (0.51±0.03 mmol/100 g of fresh sample). The lowest antioxidative potential was established in the hybrids 'Autumn queen F1' (0.33±0.03 mmol/100 g) and 'Delphi F1' (0.29±0.04 mmol/100 g). The same parameter of natural resistance of cabbage was in the red genotypes significantly higher (0.67±0.03 mmol/100 g) than in the white ones (0.47±0.01 mmol/100 g).

The influence of antioxidative potential on feeding by cabbage stink bugs

In 16 out of 20 cabbage genotypes we established a significantly negative correlation between the antioxidative potential in the outer leaves and the extent of damage caused by cabbage stink bugs sucking in the outer leaves and cabbage heads. A very strong and significant negative correlation between the said parameters was observed in the hybrid 'Delphi F1', a strong correlation was observed in the hybrid 'Vestri F1' and the variety 'Red dynasty F1', while a moderate correlation was observed in the hybrids 'Autumn queen F1', 'Cheers F1', 'Green Rich F1', 'Sunta F1' and 'Tucana F1', as well as in the variety 'Kranjsko'. In other genotypes, we established significant low or

Table 2. Growth stages of 20 cabbage genotypes on the assessment dates in 2011

	May 3	June 1	June 2	June 3	July 1	July 2	July 3	August 1	August 2
'A. queen F1'	19-19	41-41	41-43	42-45	46-49	49-49			
'Cheers F1'	19-41	41-41	41-43	42-44	45-48	49-49			
'Delphi F1'	19-41	41-42	41-42	43-45	41-47	49-49			
'Destiny F1'	19-41	41-42	41-44	43-45	46-47	49-49			
'Erfurtsko'	19-19	19-42	19-41	43-44	43-47	49-49			
'Fieldforce F1'	19-41	41-45	45-48	47-49	49-50				
'Futoško'	14-19	41-41	42-42	42-45	42-48	49-49			
'Green rich F1'	19-41	42-44	42-44	43-44	46-47	50-50			
'Hinova F1'	19-19	19-41	19-41	42-43	42-45	48-48	48-49		
'Holandsko'	19-19	19-41	19-42	42-43	42-45	46-49	47-49		
'Ixxion F1'	19-41	41-42	42-44	43-48	43-48	49-49			
'Kranjsko'	13-19	14-19	19-19	19-42	41-42	41-49	44-49		
'Ljubljansko'	16-19	14-41	19-42	42-44	44-46	47-48	47-49		
'Pandion F1'	41-41	43-45	43-45	49-49					
'R1 cross F1'	19-19	41-41	41-42	42-44	43-47	49-49			
'Red dinasty F1'	19-19	19-42	19-41	42-43	42-47	49-49			
'Sunta F1'	19-41	42-44	43-48	49-49					
'Tucana F1'	41-41	42-44	46-48	49-49	49-49				
'Varaždinsko'	19-19	19-42	19-43	42-44	44-47	49-49			
'Vestri F1'	19-19	41-42	41-43	43-44	45-47	47-49			

very low correlations, even insignificant correlations (Table 3).

DISCUSSION

With our research we wanted to add plant color to the list of relatively well-researched factors of natural resistance of cabbage to attacks by harmful insects – the epicuticular wax content (Trdan et al., 2009) and glucosinolates (Bohinc et al., 2012). Cabbage stink bugs (*Eurydema* spp.) are in univoltile insects (Trdan et al., 2006). They appeared in the last decade of May (2010) or in the first decade of June (2011), which corresponds to the finding by Bohinc and Trdan (2012). Among the red and the white genotypes of cabbage, we detected the greatest differences in the extent of damage on leaves when the pest first appeared and in the two decades that followed. In this period (until the end of June), we established significantly more damage in the white genotypes than in the red ones, and the extent of damage on the red genotypes ('Erfurtsko', 'Red dynasty F1' and 'Holandsko') did not exceed the index 1.75 or 1% of damaged leaf surface.

Earlier studies mention the length of the growth period as an important factor of the extent of damage on cabbage (Bohinc et al., 2013), yet our research and the selected group of harmful pests did not confirm this. A pronounced rise in the extent of damage was observed during both years of the experiment in the second decade of July, which coincided with the appearance of the bug larvae *Eurydema ventrale* Kolenati and *Eurydema oleracea* (L.) (Trdan et al., 2006; Bohinc and Trdan, 2012).

Since anthocyanins are the substances that exert the largest influence on the color of red cabbage

Table 3. Correlation between the antioxidative potential and the level of *Eurydema* spp. damage in 20 different cabbage genotypes in 2010.

	r	model
'Autumn queen F1'	-0.44*	Ŷ=4.87-4.69*aop
'Cheers F1'	-0.67*	Ŷ=5.70-2.71*aop
'Delphi F1'	-0.99*	Ŷ=8.96-19.11*aop
'Destiny F1'	-0.32*	Ŷ=4.83-4.54*aop
'Fieldforce F1'	-0.05*	Ŷ=4.85-0.03*aop
'Futoško'	-0.40*	Ŷ=5.07-1.54*aop
'Green Rich F1'	-0.50*	Ŷ=5.15-1.75*aop
'Hinova F1'	-0.02	Ŷ=4.56-0.13*aop
'Ixxion'	-0.21*	Ŷ=4.21-1.19*aop
'Kranjsko'	-0.64*	Ŷ=5.91-4.14*aop
'Ljubljansko'	-0.05	Ŷ=3.65-0.23*aop
'Pandion F1'	-0.15	Ŷ=3.94-1.02*aop
'R1 Cross F1'	-0.19	Ŷ=4.51-2.05*aop
'Sunta F1'	-0.59*	Ŷ=5.84-2.11*aop
'Tucana F1'	-0.63*	Ŷ=5.90-4.14*aop
'Varaždinsko'	-0.23*	Ŷ=5.94-5.39*aop
'Vestri F1'	-0.74*	Ŷ=3.86-2.26*aop
'Red dynasty F1'	-0.83*	Ŷ=11.79-15.33*aop
'Holandsko'	-0.30*	Ŷ=1.10-1.36*aop
'Erfurtsko'	-0.21*	Ŷ=2.16-0.23*aop

genotypes (Soengas et al., 2011), we can assume that anthocyanins also have important influence on the antioxidative potential of cabbage (Li et al., 2012). However, since we did not determine the antioxidative potential of cabbage during the growth period, we cannot confirm this. The results of related studies show that the selection of cabbage genotype (Peñas et al., 2012) is an important factor of bioactive substance content, also anthocyanins.

The results of our analysis of the antioxidative potential at technological maturity show that the content of these bioactive substances is high at this time. Ribera et al. (2010) reported that highbush blueberry (*Vaccinium corymbosum* L.) has the highest antioxidative potential at technological maturity, which we cannot claim for the cabbage examined in our research. Lee et al. (2007) and Soengas et al. (2011) established that the antioxidative potential is highest in the red cabbage genotypes, the conclusion we made on the basis of our results. With regards to high levels of antioxidative potential, the red varieties 'Erfurtsko' and 'Holandsko' stand out, and they have been present in the systems of cabbage production in Slovenia for several years. Beside the influence of antioxidative potential on harmful organisms, we can thus also point out the influence on people, which is proven to be positive (Medoua et al., 2009).

CONCLUSIONS

The antioxidative potential was observed as an important factor of natural resistance of cabbage to attacks by cabbage stink bugs. Among the antioxidants present in cabbage, we cannot single out a compound that affects the feeding of Eurydema spp., yet we can stress the general positive significance of antioxidants in plant protection and human diet. The red cabbage varieties in the period from the first appearance of Eurydema spp. (from the end of May to the beginning of June) to the end of June turned out to be much less susceptible to damage than the white genotypes; the old varieties, such as 'Erfurtsko' and 'Holandsko', due to their lower susceptibility to damage caused by Eurydema spp. and higher antioxidant content represent a potential in environmentally acceptable systems for the Brassicaceae. Our research shows that the color of plants is a factor that distinctly affects their antioxidative potential.

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