UNIVERZA V LJUBLJANI
BIOTEHNIŠKA FAKULTETA

Damir MARKOVIĆ

DEJAVNIKI ANTIKSENOZE KOT MEHANIZEM ODPORNOSTI ZELJA (*Brassica oleracea* var. *capitata* L.) NA IZBRANE ŠKODLJIVE ŽUŽELKE

DOKTORSKA DISERTACIJA

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FACTORS OF ANTIXENOSIS AS MECHANISM OF CABBAGE (*Brassica oleracea* var. *capitata* L.) RESISTANCE AGAINST SELECTED INSECT PESTS

DOCTORAL DISSERTATION

Ljubljana, 2014

Doktorska disertacija predstavlja zaključek podiplomskega študija bioloških in biotehniških znanosti na znanstvenem področju agronomije. Poljski poskus je bil izveden na Laboratorijskem polju Biotehniške fakultete v Ljubljani, laboratorijski del poskusa pa v laboratorijih Katedre za fitomedicino, kmetijsko tehniko, poljedelstvo, pašništvo in travništvo na Oddelku za agronomijo Biotehniške fakultete in Katedre za tehnologije, prehrano in vino na Oddelku za živilstvo Biotehniške fakultete.

Na podlagi Statuta Univerze v Ljubljani ter po sklepu Senata Biotehniške fakultete in sklepa 9. seje Komisije za doktorski študij z dne 9.9.2010 je bilo potrjeno, da kandidat izpolnjuje pogoje za neposredni prehod na doktorski Podiplomski študij bioloških in biotehniških znanosti ter opravljanje doktorata znanosti na znanstvenem področju agronomija. Za mentorja je bil imenovan prof. dr. Stanislav Trdan.

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Datum zagovora:

Naloga je rezultat lastnega raziskovalnega dela. Izjavljam, da so vsa vključena znanstvena dela identična objavljenim verzijam oz. verziji, ki je sprejeta v objavo. Podpisani si strinjam z objavo svojega dela na spletni strani Digitalne knjižnice. Izjavljam, da je delo, ki sem ga oddal v elektronski obliki, identično tiskani verziji.

Damir Marković

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IJ sl

JI sl/en

AI Med leti 2006 in 2011 so na Laboratorijskem polju Biotehniške fakultete v Ljubljani potekali poljski poskusi, kjer smo na različnih genotipih zelja preučevali dejavnike naravne odpornosti in njihov vpliv na predstavnike dveh rodov škodljivih žuželk, kapusove bolhače (*Phyllotreta* spp.) in kapusove stenice (*Eurydema* spp.). V poskusih smo uporabili 20 genotipov zelja, ki smo ga posadili v 4 bloke. Obravnavani genotipi so glede na dolžino rastne dobe pripadali zgodnjim, srednje zgodnjim in srednje poznim sortam in hibridom. Ugotovili smo, da se dovzetnost posameznih genotipov zelja za prehranjevanje kapusovih bolhačev in kapusovih stenic med rastno dobo razlikuje. Kapusovi bolhači so se na zelju začeli pojavljati že v zadnji dekadi maja, medtem ko so kapusove stenice izkazale za bolj termofilne vrste. Obseg poškodb zaradi kapusovih bolhačev je bil na listih zelja veliko večji na belih genotipih, najvišje vrednosti indeksov poškodb pa smo ugotovili v prvi in drugi dekadi julija. Poškodbe kapusovih stenic smo veliko prej opazili na belih kot na rdečih genotipih zelja. Določanje antioksidativnega potenciala s pomočjo DPPH* je potrdilo veliko večjo vsebnost antioksidantov v rdečih genotipih zelja. Rezultati naše raziskave kažejo na to, da višja vrednost antioksidativnega potenciala negativno vpliva na obseg poškodb kapusovih stenic na zelju. Pomemben dejavnik naravne odpornosti zelja na napad preučevanih škodljivcev pa je tudi vsebnost epikutikularnega voska na listih. Ugotavljamo, da rdeči genotipi zelja vsebujejo več epikutikularnega voska kot beli, a ta dejavnik ne vpliva na večje razlike v občutljivosti genotipov zelja na napad kapusovih bolhačev in kapusovih stenic. Naša raziskava dokazuje, da so dejavniki antiksenoze pogojeni z genotipom zelja, posledično pa ugotavljamo, da antiksenoza zelja predstavlja potencial, ki ga velja v prihodnje v večji meri kot doslej izkoristiti v različnih načinih okoljsko sprejemljivejšega pridelovanja zelja in drugih kapusnic.

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PI FACTORS OF ANTIXENOSIS AS MECHANISM OF CABBAGE (*Brassica oleracea* var. *capitata* L.) RESISTANCE AGAINST SELECTED INSECT PESTS

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LA sl

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AB Between 2006 and 2011 we carried out field experiments at the Laboratory Field of the Biotechnical Faculty in Ljubljana in which we studied some factors of natural resistance in different cabbage genotypes and their influence on the representatives of two generations of insect pests, cabbage flea beetles (*Phyllotreta* spp.) and cabbage stink bugs (*Eurydema* spp.). In the experiments we used 20 cabbage genotypes which were planted in 4 blocks. In regard to the length of growth period, the studied genotypes belonged to early, mid-early and mid-late cultivars and hybrids. We found out that susceptibility of individual cabbage genotypes to the feeding of cabbage flea beetles and cabbage stink bugs varies during the growth period. Cabbage flea beetles began appearing on cabbage already in the last decade of May, while cabbage stink bugs proved to be more thermophilous species. The extent of damage done by cabbage flea beetles was on cabbage leaves considerably greater in white genotypes, while the highest values of damage indexes were established in the first and the second decade of July. The damage done by cabbage stink bugs was detected considerably earlier in white cabbage genotypes than in the red ones. Determining antioxidative potential with DPPH* confirmed greater antioxidant content in red cabbage genotypes. The results of our experiment point to the fact that higher value of antioxidative potential negatively influences the extent of damage on cabbage done by cabbage stink bugs. An important factor of cabbage's natural resistance to attacks of the studied harmful pests is also epicuticular wax content on leaves. We found out that the red cabbage genotypes contained more epicuticular wax than the white ones; however, this factor does not cause greater differences in susceptibility of cabbage genotypes to attacks by cabbage flea beetles and cabbage stink bugs. Our study proves that the antixenosis factors are conditioned by individual cabbage genotypes, we thus found out that antixenosis represents a potential which should be in future used more extensively in different ways of more environmentally acceptable production of cabbage and other brassicas.

KAZALO VSEBINE

KAZALO SLIK

KAZALO PRILOG

1 UVOD

Usmerjenost pridelovalcev hrane v čim večji pridelek in posledično večji tržni izplen je v svetu vplival na mnoge neželene učinke intenzivnega kmetijstva na okolje. Pridelovanje hrane v monokulturah je (bilo) praktično nemogoče brez uporabe fitofarmacevtskih sredstev, za katere je znano, da imajo poleg pozitivne vloge pri pridelavi hrane, krme in okrasnih rastlin tudi nekatere neželene učinke (Tahvanainen in Root, 1972; Bommarco in sod., 2011). Zavedanje potrošnikov in pridelovalcev o pomenu zdrave hrane in okolja, v katerem živimo, je imelo pomemben vpliv na vse bolj integrirano usmerjeno varstvo rastlin (George in sod., 2009).

Postopne spremembe podnebja, intenzivnejši prenos škodljivih organizmov med celinami pod vplivom globalizacije in velike gospodarsko-politične spremembe v zadnjih dvajsetih letih, so imele pomemben vpliv na evropsko (Iglesias in sod., 2012), s tem pa tudi na slovensko kmetijstvo. Čeprav smo se v Sloveniji s številčnimi pojavi škodljivcev na gojenih ali samoniklih rastlinah srečevali že v preteklosti (na primer koloradski hrošč in ameriški kapar sta celo grozila, da pridelava krompirja in nekaterih sadnih vrst v Sloveniji ne bo mogoča) (Janežič, 1951), pa so z (večkrat zelo strupenimi) insekticidi brez večjih težav zmanjšali njihovo številčnost in s tem omejili njihovo gospodarsko škodljivost.

Številčnost škodljivih žuželk so že v preteklosti uravnavali naravni sovražniki ali pa se je z delovanjem obrambnih mehanizmov rastlin vzpostavilo ustrezno ravnovesje med številčnostjo populacij škodljivcev in njihovih gostiteljev. S tem se je bilo mogoče izogniti večjim izgubam pridelka, še bolj pomembno pa je dejstvo, da je omenjeno ravnovesje omogočilo preživetje rastlin in njihovih »napadalcev«. Koncept uporabe obrambnih mehanizmov rastlin pri zmanjševanju škodljivosti žuželk predstavlja pomemben sestavni del integriranega varstva rastlin (v svetu znan po kraticah IPM, angl. Integrated Pest Management), seveda pa mora biti izbira sort z "vgrajenimi" obrambnimi mehanizmi prilagojena razširjenosti in gospodarskemu pomenu škodljivcev na določenem območju (Onstad, 2014). Naravna odpornost rastlin je eden od najučinkovitejših načinov zmanjševanja škodljivosti rastlinojedih žuželk, v kombinaciji s kemičnim in biotičnim načinom zatiranja škodljivcev pa omogoča učinkovito in okolju prijaznejše varstvo rastlin.

V zadnjih desetletjih je javno mnenje vse manj naklonjeno uporabi kemičnih sredstev za varstvo rastlin, saj jim večkrat pripisuje največji pomen pri onesnaževanju okolja (Gavrilescu, 2005), pa čeprav je znano, da imajo številni drugi dejavniki (promet, industrija idr.) zelo velik pomen pri onesnaževanju tal, vode, živeža... (Ajmone-Marsan in sod., 2008). Ker v takšnih primerih velikokrat tudi z argumenti o omejeni škodljivosti ali celo o neškodljivosti številnih insekticidov (in drugih fitofarmacevtskih sredstev) ne dosežemo prav veliko, predstavlja eno od alternativ za prihodnost tudi ustreznejša agrotehnika; z njo je namreč mogoče v precejšnji meri zmanjšati gospodarski pomen rastlinskih škodljivcev (Tshernyshev, 1995). Med pomembne agrotehnične ukrepe štejemo tudi izbiro na škodljive organizme odpornejših genotipov. Njihova odpornost na napad škodljivcev ima lahko podlago v različnih dejavnikih: dolžini rastne dobe (z izbiro ustreznega genotipa, v povezavi s časom sajenja ali setve, je mogoče preprečiti koincidenco občutljivega razvojnega stadija rastline in kalamitete škodljivcev), fizikalni odpornosti, kemični odpornosti itn. (Jakobsson, 2012).

Vsaka rastlinska vrsta ima edinstven obrambni sistem, ki ga sestavljajo tako morfološki kot fitokemični dejavniki (Ahuja in sod., 2010). Na žuželko ali drugo žival, ki se s takšno rastlino prehranjuje, delujejo ti dejavniki tako v fiziološkem smislu kot tudi v smislu obnašanja. Naravna odpornost rastlin je lahko pogojena s tremi mehanizmi, tj. antiksenozo, antibiozo in toleranco (Jyoti in sod., 2001). "Učinkovita" fitofagna žuželka mora imeti sposobnost, da po odkritju gostitelja na njem v čim krajšem času najde prehransko najustreznejši rastlinski organ ali njegov del. Pri tem lahko žuželko ovira antiksenoza, t.j. mehanizem, ki ga predstavljajo morfološki, fizikalni in strukturno biokemični dejavniki (lastnosti) gostitelja. Z njihovim »delovanjem« so žuželke ali drugi škodljivci ovirani pri parjenju, ovipoziciji in hranjenju (Trdan in sod., 2004).

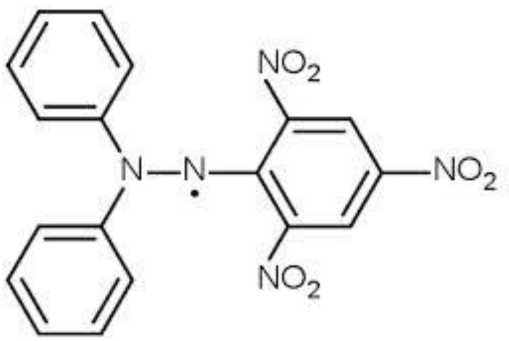
Pri antiksenozi gre za obliko odpornosti, pri kateri rastline odvračajo žuželke od rastlin in s tem preprečijo nastanek poškodb na rastlinah. Navadno je izražena kot nepreferenca žuželk do odpornih rastlin, v primerjavi z občutljivimi rastlinami. Deluje le na škodljivce, ki aktivno iščejo svojega gostitelja in je lahko dolgotrajna oblika odpornosti, če imajo škodljivci na voljo alternativne gostitelje, na katerih se razmnožujejo in prehranjujejo (Eigenbrode, 2002). Med najpomembnejšimi dejavniki antiksenoze so listne dlačice (trihomi) (Tian in sod., 2012), epikutikularni vosek (Stoner, 1992; Eigenbrode, 2004) in vsebnost različnih kemičnih snovi (sekundarni metaboliti, antioksidanti, sladkorji, aminokisline, fosfolipidi idr.) v rastlinah (Eigenbrode in Espelie, 1995; Singh in sod., 2006; Bjőrkman in sod., 2011; Krasavina in sod., 2014).

Antioksidanti so spojine, ki varujejo celice pred poškodbami, katere povzročajo prosti radikali. Imajo velik pomen na zdravje ljudi (Krinsky, 1989; Kaur in Kapoor, 2001). Glavna in osnovna vloga antioksidantov je preprečevanje tvorbe prostih radikalov. Antioksidante lahko razdelimo na primarne in sekundarne. Primarni oksidanti so tisti, ki lahko reaktivne radikale spremenijo v bolj stabilne produkte in s tem prekinejo verižno reakcijo avtooksidacije. Na drugi strani sekundarni antioksidanti reagirajo s kovinskimi ioni, ki katalizirajo oksidacijo. Med sekundarne antioksidante spadajo fenoli, derivati galne kisline in galna kislina, flavonoidi in dekatere druge naravne spojine (Štrukelj, 2012). Polifenoli, vitamin C, GSH (reducirana oblika glutationa), vitamin E in karotenoidi so najpomembnejši antioksidanti v rastlinskem svetu (Korošec, 2000).

Antioksidacijski potencial (antioksidativni potencial) je ovrednoten kot posledica vsebnosti polifenolov, predvsem nekaterih flavonoidov (flavonov, izoflavonov, flavonov, antocianinov, katehina in izokatehina) in v manjši meri vitaminov (Petrović, 2011). Višja kot je vsebnost polifenolov, višji je antioksidacijski potencial. Med polifenole, ki so največkrat zastopani v križnicah, uvrščamo flavonoide in hidroksicimetno kislino (Soengas in sod., 2012).

Problem pri določ http://www.arso.gov.si/evanju antioksidativne aktivnosti v kompleksnih matriksih predstavlja stabilnost antioksidantov. Izjemno nestabilen je vitamin C, ki se po homogenizaciji zelo hitro pretvarja v dehidroaskorbinsko kislino. Vitamin C lahko stabiliziramo z dodatkom reducenta ali metafosforne kisline med postopkom homogenizacije (Wechtersbach, 2005).

Antioksidativno aktivnost v živilih lahko določamo na dva načina, neposredno in posredno. Pri posrednih metodah merimo sposobnost antioksidantov za lovljenje tistih prostih radikalov, ki niso neposredno povezani z oksidacijskim razkrojem. Pri posrednem določanju uporabljamo obarvane proste radikale, kjer določamo sposobnost antioksidantov, da oddajo vodikove atome in ne neposredno antioksidativno aktivnost, čeprav sta ti dve lastnosti včasih neposredno povezani. Neposredne metode pa na splošno temeljijo na preučevanju vpliva dodanega antioksidanta na potek verižne oksidacije določenega substrata s prostimi radikali. V naši raziskavi smo uporabili posredno metodo za določevanje antioksidativne aktivnosti v živilu s prostim radikalom DPPH*. Metoda s prostim radikalom DPPH* spada med najstarejše posredne metode določevanja antioksidativne aktivnosti. Reakcije je pogojena z razmerjem med stabilnim prostim radikalom DPPH* in donorji vodika (Brand-Williams, 1995). ZA DPPH* je značilen velik molarni absorpcijski koeficient v vidnem delu spektra z maksimumom pri 517 nm. To pomeni, da koncentracijo radikala DPPH* lahko določamo spektrofotometrično. Mogoče pa je tudi določanje navedenega radikala z elektronsko spinsko resonanco (Roginsky in Lissi, 2005).



Slika 1: Strukturna formula DPPH* (2,2-difenil-1-pikrilhidrazl) uporabljen v naši raziskavi
Figure 1: Structurral class of DPPH* (2,2-diphenylpicrylhydrazyl), used in our research

Vpliv antocianinov na naravno odpornost rastlin je bil pojasnjen že v predhodnih raziskavah (Schaefer in Rolshausen, 2005; Lev-Yadun in Gould, 2009). Vsebnost antocianinov (ki so po svoji funkciji barvila) je zelo visoka v rdečem zelju (Simmonds, 2003; Xu in sod., 2010). Glede na raziskavo Wiczkowski in sod. (2014) naj bi rdeče zelje vsebovalo 9-24 različnih antocianinov. Antocianini lahko na več načinov sodelujejo pri obrambnih reakcijah rastlin. Lahko delujejo kot kemični repelenti oziroma je njihovo delovanje usmerjeno na vidni spekter škodljivih organizmov. Vsebnost antioksidantov se med posameznimi vrstami križnic razlikuje. Razlike obstajajo tudi med različnimi genotipi iste rastlinske vrste. Pomembno na vsebnost antioksidantov vpliva tudi tehnološka zrelost (Rokayya in sod., 2013). Skupaj s preostalimi flavonoidi naj bi bilo njihovo delovanje antibakterijsko, preprečevalo naj bi delovanje patogenih mikroorganizmov, med njimi tudi virusov. Njihovo antimikrobno delovanje je vseeno manj izrazito kot pri ostalih fenolih.

Med sekundarni metabolite uvrščamo tudi glukozinolate, ki s svojimi razgradnimi produkti vplivajo na naravno odpornost rastlin (Bohinc in Trdan, 2013). Glukozinolati oziroma β-tioglukozidni-N-hidroksisulfati spadajo v skupino sekundarnih rastlinskih metabolitov in jih glede na kemijsko zgradbo uvrščamo med tioglikozide (Fahey in sod., 2001). Glukozinolati so vrstno specifični. Njihova vsebnost v rastlinah je pogojena tudi z vrsto tal, razmerami med rastjo, vrsto rastlinskega tkiva, zdravstvenim stanjem rastlin (Bohinc, 2013).

Kapusnice (Brassicaceae) so skupina rastlin, ki so pomembne tako z agronomskega (Font in sod., 2005; Vaughn in Berhow, 2005; Cartea in sod., 2008) kot z ekonomskega (Vaughn in Berhow, 2005) vidika. Kapusnice predstavljajo skupino zelenjadnic, ki je v Sloveniji najbolj razširjena (Ugrinović in sod., 2013). Čeprav je pridelava zelja v Sloveniji v zadnjih letih nekoliko upadla, se ta kapusnica pri nas še vedno goji na največjem deležu zemljišč pod vrtninami (442 ha oziroma 29,50 % njiv, namenjenih pridelavi zelenjadnic) (Popis tržnega vrtnarstva, 2010). Belo zelje predstavlja kar 80% vseh pri nas pridelanih kapusnic (Ugrinović in sod., 2013), med najpomembnejše regije pridelave kapusnic pa uvrščamo Podravje in Osrednjo Slovenijo, kjer pridelamo skoraj polovico vseh kapusnic. Druge vrste kapusnic so pri nas manj razširjene in zato tudi nekoliko manj preučene v občutljivosti na napad različnih vrst škodljivcev. Genotipe zelja lahko glede na čas spravila/pobiranja razdelimo v več skupin, kar smo upošteval v naši nalogi. Tako genotipe zelja, ki od presajanja do tehnološke zrelosti potrebujejo 55-70 dni, uvrščamo med zgodnje genotipe, genotipe, ki od presajanja do tehnološke zrelosti potrebujejo 80-90 dni uvrščamo med srednje zgodnje, genotipe, ki od presajanja do tehnološke zrelosti potrebujejo 110-140 dni, pa uvrščamo v skupino srednje poznih (Trdan in sod., 2009).

V našo raziskavo smo vključili 20 različnih genotipov zelja, in sicer 14 hibridov (zgodnji: 'Pandion F1', 'Sunta F1', 'Delphi F1', 'Tucana F1', 'Ixxion F1', 'Autumn queen F1', 'Destiny F1', 'Green rich F1'; srednje zgodnji: 'Red dinasty F1', 'Cheers F1', Fieldforce F1', 'Vestri F1'; srednje pozni: 'R1-Cross F1', 'Hinova F1') in 6 sort (zgodnja: 'Erfurtsko rdeče'; srednje zgodnja: 'Futoško'; srednje pozna: 'Kranjsko okroglo', 'Ljubljansko', 'Holandsko rdeče', 'Varaždinsko'). V več raziskavah je bilo ugotovljeno, da predstavlja izbor genotipa pomemben dejavnik zmanjševanja škodljivosti različnih vrst škodljivcev na zelju (Stoleru in sod., 2012).

Znano je, da obstajajo med različnimi vrstami kapusnic (ohrovt, cvetača, brokoli, kitajski kapus) precejšnje razlike v dovzetnosti na napad škodljivcev, manj pa je zaenkrat znano, kateri dejavniki determinirajo takšno različnost. Tobakov resar (*Thrips tabaci* Lindeman), kapusove stenice (*Eurydema* spp.), kapusovi bolhači (*Phyllotreta* spp.), kapusova hržica (*Contarinia nasturtii* [Kieffer]), mokasta kapusova uš (*Brevicoryne brassicae* [L.]), kapusov molj (*Plutella xylostella* [L.]), kapusov belin (*Pieris brassicae* [L.]), kapusov ščitkar (*Aleyrodes proletella* [L.]), brazdasti kljunotaj (*Ceutorrhynchus pleurostigma* [Marsham]) in še kakšna vrsta so pri nas med najbolj izpostavljenimi vrstami škodljivih žuželk na zelju (Trdan in sod., 2009), manj pa je znanega o tem, katere fizikalne pregrade ali kemične snovi v zelju in sorodnih rastlinskih vrstah so podlaga za zmanjševanje ali celo povečevanje njihovih populacij.

Naša raziskava se je osredotočala na dve skupini škodljivcev na zelju, kapusove stenice (*Eurydema* spp.) in kapusove bolhače (*Phyllotreta* spp.). Kapusove stenice so žuželke, ki se hranijo na zelju in drugih kapusnicah (Vrabl, 1992; Bohinc, 2013). Ličinke in odrasli osebki stenic izsesavajo liste zelja in drugih křižnic, pri čemer vanje vstavijo sesalo in z njim sesajo rastlinske sokove. Na listih se pojavijo značilne belkaste pege, ki se večajo. Ob njihovem močnem napadu se lahko posušijo celi listi ali (redkeje) cela rastlina. Na leto imajo le en rod, ki je škodljiv od maja do julija (Vrabl, 1992; Maceljski in sod., 1997; Pajmon, 1999). Na mladih rastlinah naredijo ti škodljivci največ škode, predvsem v času kalitve in po presajanju. Pri močnem napadu je lahko obseg poškodb velik tudi na starejših rastlinah (Bohinc in Trdan, 2013). Za zatiranje kapusovih stenic v Sloveniji nimamo registriranega insekticida (Seznam registriranih …, 2014). V Sloveniji je med kapusovimi stenicami najštevilčneje zastopana pisana stenica (*Eurydema ventrale*), kapusova stenica (*Eurydema oleracea*) se pojavlja v manjših populacijah.

Tudi kapusovi bolhači (*Phyllotreta* spp.) predstavljajo na križnicah gospodarsko pomemben rod škodljivih žuželk (Trdan in sod., 2005c; Bohinc in Trdan, 2013). Hrošči so značilne grizoče žuželke, ki s povzročanjem izjed na mladih rastlinah lahko povzročijo sušenje (Bohinc in Trdan, 2013), na starejših rastlinah pa navadno vplivajo na zaostajanje rastlin v rasti in razvoju, s tem pa tudi na manjše število semen in manjši pridelek (Tansey in sod., 2009). Zanje je značilno, da v srednji in v celinskem delu južne Evrope razvijejo en rod letno (Trdan in sod., 2005b) in da so v nekaterih območjih nekatere vrste že pridobile odpornost na insekticide (Turnock in Turnbull, 1994; Ekbom in Műller, 2011; Bohinc in Trdan, 2013). Na območju, na katerem je bila izvedena pričujoča raziskava, v Sloveniji, se na zelju najpogosteje pojavljajo vrste *Phyllotreta armoraciae* (Koch), *Phyllotreta sundulata* Kutschera, *Phyllotreta nemorum* L., *Phyllotreta striolata* (Fabricius), *Phyllotreta atra* (Fabricius) in *Phyllotreta vittula* (Redtenbacher) (Brelih in sod., 2003; Bohinc in Trdan, 2013).

Izbira pragov gospodarske škode temelji na podatkih, ki so navedeni v prispevku, ki so ga objavili Garbe in sod. (1996). V omenjeni raziskavi je meja, kjer je potrebno kemično zatirati vrsto *Psylliodes chrysocephala* L., pri 10 % poškodovane listne površine. Za ocenjevanje poškodb kapusovih bolhačev smo v naši raziskavi uporabili 5-stopenjsko lestvico EPPO (Guidelines …, 2002), medtem ko smo poškodbe kapusovih stenic ocenjevali po 6-stopenjski lestvici avtorjev Stonerjeve in Sheltona (1998). Več kot 25 % poškodovane listne površine zaradi hranjenja kapusovih bolhačev je na lestvici ovrednoteno z oceno 5, medtem ko je več kot 50 % poškodovane listne površine zaradi hranjenja kapusovih stenic ovrednoteno z oceno 6.

Število registriranih sintetičnih pripravkov, ki so namenjeni za zatiranje škodljivcev na zelju se v zadnjih letih zmanjšuje (Seznam registriranih …, 2014). Kljub temu pa študija o rabi fitofarmacevtskih sredstev navaja podatke, da pridelovalci v Sloveniji v povprečju zelje škropijo 2,1-krat, od tega z insekticidom 1,6-krat (Ugrinović in sod., 2013). V Sloveniji lahko za zatiranje kapusovih bolhačev, a le v setvenicah, uporabljamo insekticide lambda-cihalotrin, alfa-cipermetrin in azadirahtin A, medtem ko za zatiranje kapusovih stenic nimamo registriranega pripravka.

Alternativni načini zmanjševanja obsega poškodb kapusovih bolhačev pridobivajo na pomenu. Med uspešnejše alternativne metode uvrščamo uporabo privabilnih posevkov (Trdan in sod., 2005b; Bohinc in Trdan, 2013), poznejše termine setve oz. sajenja križnic, kolobar, talne prekrivke (Andersen in sod., 2006) ter izbiro ustreznih (naravno odpornejših) genotipov, saj je znano, da na manjši obseg poškodb na nekaterih sortah ali hibridih križnic vpliva tudi večja poraščenost rastlin s trihomi (Soroka in sod., 2011; Jaime in sod., 2013), večja vsebnost epikutikularnega voska (Trdan in sod., 2009) in vsebnost glukozinolatov (Bohinc in sod., 2013). Ugrinović in sod. (2013) navajajo, da bi morali nekatere preventivne ukrepe pri varstvu kapusnic pred škodljivimi organizmi v praksi veliko bolj pogosto in dosledno uporabljati. Med omenjenimi ukrepi izpostavlja doslednejše odstranjevanje plevelov ob robovih njiv (zmanjšanje napada listnih uši, bolhačev, sovk), pogostejše pregledovanje posevkov, spremljanje pojavljanja škodljivcev z vabami in pastmi in globoko zaoravanje rastlinskih ostankov. Premalo pa je po njihovem mnenju uporablja tudi biotično zatiranje škodljivcev kapusnic.

Trihomi delujejo na fitofagne škodljive organizme kot ovira pri njihovem prvem stiku z gostiteljem, na rastlinah pa ovirajo gibanje in prehranjevanje škodljivcev. Dolžina in gostota trihomov imata lahko velik pomen pri odlaganju jajčec na rastline, zanimivo pa je, da so si rezultati tovrstnih raziskav precej nasprotujoči; od takšnih, ki potrjujejo negativno korelacijo med dolžino (gostoto) trihomov in številom odloženih jajčec, do takšnih, ki trdijo obratno (Valverde in sod., 2001). Plast voska na površju epikutikule varuje rastline pred izsušitvijo, okužbami s povzročitelji bolezni in napadi škodljivcev. V kontekstu vpliva na rastlinske škodljivce epikutikularni vosek ovira njihovo prehranjevanje, še prav poseben vpliv pa ima pri žuželčji naselitvi rastlin, saj lahko deluje kot fagostimulant ali deterent. Delovanje epikutikularnega voska zato torej ni pomembno le v smislu fizikalne odpornosti, ampak lahko deluje tudi v "kemičnem smislu", kjer imajo lahko pomembno vlogo njegovi gradniki (alkani, maščobne kisline) (Stoner, 1992).

Znano je, da se hrošček iz družine Chrysomelidae, *Phaedon cochleariae* (F.), lažje giblje na tistih kapusnicah ali genotipih določene vrste kapusnic, ki imajo na površju listov manj epikutikularnega voska. Na genotipih z bolj gladkim površjem je njihovo gibanje oteženo. Stork (1980) navaja, da je na listih z manj epikutikularnega voska, večina hroščkov ostala na listih tudi, če je spremenil njihov naklon do 45^o, medtem ko je na gladkih listih pri istem naklonu večina osebkov padla z njih. Podobno je bilo ugotovljeno tudi za vrsto *Chrysolina fastuosa* Scopoli. Podobno vlogo kot gladko voskasto površje listov imajo tudi grandularni trihomi na listih, ki lahko ovirajo gibanje, ovipozicijo in prehranjevanje škodljivcev gojenih rastlin. Ugotovljeno je bilo, da gladko voskasto površje mladih listov varuje rastlino *Eucalyptus nitens* H. Deane & Maiden pred objedanjem lepenjca *Paropsis charybdis* Stål, zato se žuželka hrani predvsem s ravnimi starejšimi listi. V raziskavi so odrasli osebki stalno padali z mladih listov, po odstranitvi voska pa se to ni več dogajajo. Očitno je torej, da je gladkost epikutikularnega voska na površju mladih rastlin *E. nitens* najpomembnejši obrambni dejavnik rastlin pred preučevanim škodljivcem. Do podobnih ugotovitev so prišli tudi na relaciji *P. charybdis* in mladimi ter starejšimi listi rastline *E. Globulus* Labill.

Pomanjkanje znanja o alternativnih načinih zmanjševanja škodljivosti omenjenih organizmov lahko velikokrat pripelje do zmanjšanja gospodarnosti pridelave živeža. S tem namenom na Biotehniški fakulteti od začetka novega tisočletja preučujejo okoljsko sprejemljive načine zmanjševanja gospodarskega pomena škodljivcev, zlasti na zelju in čebuli. Tako so na primer ugotovili, da z izbiro ustreznega kultivarja zelja lahko vplivamo na manjšo škodljivost tobakovega resarja na tej vrtnini (Trdan in sod., 2005a), da lahko z večkratno aplikacijo naravnih insekticidnih pripravkov pri zatiranju kapusovih stenic (*Eurydema* spp.) dosežemo podoben učinek kot z manjkratno aplikacijo sintetičnih insekticidov (Trdan in sod., 2006a), preučevali so učinkovitost vmesnih rastlin (Trdan in sod., 2006b) in svetlo modrih lepljivih plošč (Trdan in sod., 2005c) pri zmanjševanju škodljivosti tobakovega resarja na čebuli ter smotrnost uporabe kitajskega kapusa za privabljanje kapusovih bolhačev, z namenom odvračanja od zelja (Trdan in sod., 2005b).

Nadaljevanje in nadgradnjo omenjenih raziskav predstavljajo temeljne študije naravne odpornosti zelja, katerih začetki na Biotehniški fakulteti datirajo v leto 2003. Tedaj so namreč potrdili negativno korelacijo med vsebnostjo epikutikularnega voska na listih zelja in obsegom poškodb zaradi tobakovega resarja na listih te vrtnine (Trdan in sod., 2004). V naši raziskavi obravnavamo dve skupini škodljivcev na zelju, ki v povezavi z naravno odpornostjo zelja še nista bili dovolj preučeni, spadajo pa pri nas med pomembnejše škodljive organizme na zelju. Kapusove stenice in kapusove bolhače smo izbrali zato, ker je nabor kemičnih pripravkov za njihovo zatiranje v Sloveniji močno omejen in je zato izbira ustreznega genotipa, s poudarkom na njihovi kemični sestavi, pomemben biotični dejavnik odpornosti (občutljivosti) rastlinske vrste na izbranega škodljivca (Ciepiela in sod.., 1999).

Vsesplošna zastopanost površinskih voskov pri kopenskih rastlinah je trden dokaz, da imajo ti v življenju rastlin velik pomen. Kutikularni voski so hidrofobna bariera na površju rastlin in njihova osnovna funkcija je, da rastlino varujejo pred preveliko izgubo vode. Poleg tega so obramba pred okužbami z bakterijami in povzročitelji glivičnih bolezni, pred abiotičnim stresom, kakršna sta suša in UV sevanje. Epikutikularni voski so pomembni tudi pri vzpostavljanju stika med rastlinami in žuželkami, tako, da jih privlačijo ali odganjajo (Eigenbrode in Espelie, 1995). Voski se med rastlinskimi vrstami zelo razlikujejo, prav tako med tkivi in organi posamezne rastline. Vsebnost voskov in njihova sestava sta odvisna tudi od okoljskih dejavnikov. Razmere relativno visoke vlažnosti, npr. v tkivnih kulturah, zavirajo tvorbo voskov, fotoperioda pa vpliva na dolžino verig sestavin voskov. Kljub njihovemu velikemu pomenu v življenju rastlin in številnim študijam o sestavi voskov, je malo znanega o iniciaciji tvorbe voska in o tem, kako je njihova tvorba pogojena z dejavniki razvoja in okolja (Bjőrkman in sod., 2011).

Rastlinski škodljivci in njihovi naravni sovražniki se morajo učinkovito pritrditi na površje rastlin (Eigenbrode in sod., 1996), kar pomeni, da se morajo pritrditi na vosek. Kutikula primarnih organov višjih rastlin sestoji iz polimerne ovojnice kutikule in kutikularnih voskov, topnih v organskih topilih, kot sta npr. heksan in diklormetan. Voski so po definiciji kompleksne mešanice alifatskih spojin z zelo dolgimi verigami, vključno s primarnimi alkoholi (n-alkan-1-oli), aldehidi, maščobnimi kislinami (n-alkanojske kisline) in alkil estri (večinoma imajo verige s sodim številom členov) ter ogljikovodiki, sekundarnimi alkoholi in ketoni (večinoma imajo verige z lihim številom členov) (Jeffree, 1986; Juniper, 1995; Riederer in Markstädter, 1996). Preprečevanje izhlapevanja vode iz kutikule je najpomembnejša funkcija površinskih voskov (Buschhaus in Jetter, 2012), vendar pa njihova kemična kompleksnost nakazuje tudi na nekatere druge funkcije. Epikutikularni voski prekrivajo kutikulo in se kemično razlikujejo od voskov v kutikuli (Jetter in sod., 2000). Del rastlinskih kutikularnih voskov je zunaj kutikularne ovojnice (matriks) in je tako neposredno na površju rastlin. Zaradi specifične lokacije (meja med rastlino in njenim okoljem) imajo epikutikularni voski zelo verjetno tudi številne ekološke funkcije, npr. varovanje rastlin pred elektromagnetnim sevanjem (Day in sod., 1992), zmanjševanje okužb rastlin s strani patogenov (Carver in sod., 1990), imajo pa tudi funkcijo semiokemikalij v relaciji do škodljivih žuželk (Bjőrkman in sod., 2011).

Vendar pa gladkost epikutikularnih voskov ni vedno podlaga za rezistenco rastlin na herbivorne žuželke in druge škodljivce (na primer pršice). Nasprotno s pričakovanji, ki temeljijo na prejšnjih primerih, nekateri avtorji ugotavljajo, da so lahko genotipi rastlin z manjšo vsebnostjo voskov relativno odporne na škodljivce, v primerjavi z bolj voskastimi genotipi (Eigenbrode in Espelie, 1995). Takšni primeri so znani pri soji, čebuli, sirku, pšenici, križnicah (Eigenbrode in sod., 2000) in grahu. Med takšne žuželke ne štejemo hroščev, ampak večkrat precej manj gibljive listne uši, resarje ali manjše gosenice. Balint in sod. (2013) ugotavljajo, da debelina epikutikularnega voska kot takšna ne predstavlja pomembnega dejavnika naravne odpornosti pred napadom tobakovega resarja.

Odpornost rastlin z gladkim voskastim površjem nadzemnih delov rastlin še ni dovolj razumljena, zelo verjetno pa je ne bo mogoče pojasniti le z laboratorijskimi raziskavami ali raziskavami v rastlinjaku, ampak so v ta namen potrebni tudi poljski poskusi. Raziskovalna dejavnost, na kateri je zasnovan del pričujoče doktorske naloga, je namenjena tudi preučevanju tega problema.

Seznam genotipov zelja, ki se priporočajo za pridelavo v Evropi, je relativno velik, podatki o njihovi ustreznosti za prehranjevanje kapusovih bolhačev in kapusovih stenic pa so redki. Zato je bil namen naše raziskave tudi preučiti afiniteto kapusovih bolhačev in kapusovih stenic do 20 genotipov zelja med rastno dobo, da bi lažje določili genotip(e) zelja, ki bi bili na preučevane škodljivce najbolj naravno odporni in posledično ustrezni za pridelavo v okoljsko sprejemljivih načinih pridelave zelja.

Pred raziskavo smo postavili naslednje hipoteze:

- predpostavljamo, da se obseg poškodb zaradi hranjenja kapusovih stenic in kapusovih bolhačev razlikuje med genotipi zelja;

- predpostavljamo, da je epikutikularni vosek pomemben dejavnik odpornosti zelja na napad kapusovih stenic in kapusovih bolhačev, da se vsebnost epikutikularnega voska med preučevanimi genotipi razlikuje, s tem pa tudi njihova ustreznost za pridelavo v območjih, kjer predstavljajo omenjeni škodljivci pomemben biotični dejavnik, ki omejuje gospodarnost pridelave zelja;

- predpostavljamo, da se antioksidacijski potencial razlikuje med posameznimi genotipi zelja, posledično se razlikujejo tudi povezave med vrednostjo antioksidacijskega potenciala in indeksom poškodb, nastalih zaradi hranjenja kapusovih stenic in kapusovih bolhačev na zelju;

- predpostavljamo, da je dolžina rastne dobe kultivarjev zelja pomemben dejavnik naravne odpornosti zelja pri napadu kapusovih bolhačev in kapusovih stenic.

2 ZNANSTVENI ČLANKI

2.1 DOVZETNOST 20 GENOTIPOV ZELJA NA NAPAD KAPUSOVIH BOLHAČEV V POLJSKIH RAZMERAH

MARKOVIĆ, Damir, BOHINC, Tanja in TRDAN Stanislav
Susceptibility of 20 cabbage genotypes to flea beetles attack under field conditions.
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V dveletnem poljskem poskusu (2010-2011) smo preučevali obseg poškodb kapusovih bolhačev (*Phyllotreta* spp.) na 20 genotipih zelja. Ugotovili smo, da se obseg poškodb med genotipi razlikuje. Prve izjede na listih smo ugotovili konec maja, največji povprečni obseg poškodb pa smo zabeležili v prvi polovici julija. V obeh letih poskusa smo na zgodnjih in srednje zgodnjih genotipih največji obseg poškodb potrdili konec maja, medtem ko so bili srednje pozni genotipi za poškodbe kapusovih bolhačev najbolj dovzetni v začetku julija. Gospodarski prag škodljivosti (10 % poškodovane listne površine) je bil v obeh letih poskusa prvikrat presežen v prvi polovici junija (Jun 1 - Jun 2), drugič pa v prvi polovici julija (Jul 1 - Jul 2), ko se pojavi prvi rod hroščev. V začetku julija so bile povprečne dnevne temperature najvišje, kar je še dodatno vplivalo večjo številčnost škodljivcev. Naša raziskava dokazuje, da obseg poškodb narašča do vključno druge dekade julija, nato zaradi zmanjšanja števila hroščev pride tudi do zmanjšanja obsega poškodb. Ugotovili smo, da so zgodnji hibridi zelja veliko bolj dovzetni za poškodbe od kapusovih bolhačev kot sorte in da ima izbira genotipa zelja velik pomen v okoljsko sprejemljivih načinih pridelovanja kapusnic, zato temu agrotehničnemu ukrepu v kombinacijami z drugimi alternativnimi načini zatiranja preučevanih škodljivcev v prihodnje pripisujemo večji pomen kot ga ima danes.

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Susceptibility of 20 cabbage genotypes to flea beetles attack under field conditions

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Abstract

In our two-year field experiment (2010-2011), we studied the extent of damage done by flea beetles (*Phyllotreta* spp.) on 20 different genotypes of cabbage. We established that the extent of damage differed among the genotypes. The first feeding tracks on leaves were detected in the end of May, while the highest average extent of damage was recorded in the first half of July. The early, the mid-early and the mid-late genotypes of cabbage manifested the highest extent of damage in the second decade of July in both years of the experiment. The economic threshold (10% of damaged leaf surface) was in the first year of the experiment in the early genotypes exceeded for the first time in the second decade of June, while it was in both years exceeded for the second time in all groups of genotypes in the second decade of July, when the next-generation beetles in the research area usually emerged. In the beginning of July, the average daily temperatures were the highest, which further increased the numbers of beetles. Our study proves that the extent of damage on cabbage leaves caused by flea beetles increases up to the second decade of July, then it decreases to some extent due to a sharp decrease in the number of flea beetles. We have found out that in early growth stages hybrids of cabbage are more susceptible to attacks by flea beetles than varieties, and that the selection of a cabbage genotype is of great importance in environmentally acceptable systems of production of the vegetable, while this agrotechnical measure combined with other alternative methods can in future become even more important.

Key words: Antixenosis, cabbage flea beetles, cabbage, *Phyllotreta* spp.

Introduction

Flea beetles (*Phyllotreta* spp.) represent an economically important genus of harmful insects, which damage the Cruciferae [3, 20]. In the Middle Europe and in the continental parts of southern Europe, they characteristically develop one generation per year [20], and in some areas certain species have already developed resistance to insecticides [4, 9, 26]. In the area in which our study was carried out, in Slovenia, the most frequently appearing species on cabbage are *P. armoraciae* (Koch), *P. sundulata* Kutschera, *P. nemorum* L., *P. striolata* (Fabricius), *P. atra* (Fabricius) and *P. vittula* (Redtenbacher) [3, 5].

Beetles are characteristically plant-eating insects, which make holes on young plants and can thus bring about withering [24], while on older plants they usually cause uneven growth of the plants and reduced quantity of seed or marketable product [17]. There are several ways to reduce the extent of damage done by flea beetles on the Cruciferae. Besides treating seeds of the Cruciferae with insecticides [9], the alternative (environmentally acceptable) methods for suppression are coming to the fore due to the appearance of resistance and the decreasing number of permitted insecticides [7, 19]. Methods, which have been proven relatively successful are trap crops [3, 20], delayed planting or sowing of the Brassicas, crop rotation, using covers [1], and the selection of a genotype, as it is known that the extent of damage on some varieties or hybrids of the Brassicas can be reduced by the density of trichomes on plants [11, 15], higher contents of epicuticular wax [25], and proportions of glucosinolates [4].

The list of cabbage genotypes, which are recommended for production in Europe, is relatively long, while the information about their appropriateness in regard to the feeding of flea beetles is scarce. Consequently, the purpose of our study was to investigate the flea beetle's affinity for 20 cabbage genotypes during the growth period, so we could more easily identify those cabbage genotypes, which have the highest natural resilience to the studied harmful pest, and are thus appropriate for environmentally-acceptable production of cabbage.

Materials and Methods

Study site, material and field experiment: The two year field experiment was conducted at the Laboratory Field of the Biotechnical Faculty in Ljubljana (46°04' N latitude, 14°31' E longitude, 300 m above sea level). 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 in three groups (early [55-70 days], mid-early [80-90 days], and mid-late [110-140 days]) depending on the length of growth period.

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 varieties (mid-late: 'Kranjsko okroglo' [as 'Kranjsko'], 'Holandsko rdeče' [as 'Holandsko'], 'Varaždinsko'; mid-early: 'Futoško' and early: 'Erfurtsko rdeče' ['Erfurtsko']).

The seedlings were transplanted by hand into the field on the 26th of April 2010 and on the 3rd of May 2011. Transplants were grown in a greenhouse in plant trays with commercial compost and fed and irrigated according to the standard practices [24].

The experiment was performed using a plot of 110.4 m^2. The field was divided into four blocks of dimensions of 1.10 × 24 m. The plants were grown using the standard cultural practices, except that no insecticide was applied. A more detailed description of cultural practices is available in the study of Trdan *et al.* [22].

Field observations and evaluation: The damage caused by cabbage flea beetles was assessed at approximately 10 day intervals. In both years of the experiment, results were collected on 9 different dates: May 3 - corresponding to the last 10 days of May; Jun 1 - corresponding to the first 10 day of June; Jun 2 - corresponding to the second 10 days of June; Jun 3 - the last 10 days of June; Jul 1 - the first 10 days of July; Jul 2 - corresponding to the second 10 days of July; Jul 3 - the last 10 days of July; Aug 1 - the first 10 days of August; and Aug 2 - corresponding to the second 10 days of August.

The damage caused by cabbage flea beetles was evaluated using the 5 grade EPPO scale [13]. Cabbage plants were evaluated on a scale from 1 (presenting no damage) to 5 (more than 25% leaf area eaten). A more detailed description is presented by Bohinc and Trdan [3].

The growth stages of cabbage genotypes were identified using the BBCH scale for leafy vegetables (forming heads) [10, 12]. The BBCH scale for leafy vegetables (forming heads) includes the main stages, as follows: (0) germination, (1) leaf development (main shot), (4) development of harvestable vegetative plant parts and (5) inflorescence emergence.

The assessments of economic damage threshold are based on the data published by Garbe *et al.* [8]. The said study sets the limit of 10% damaged leaf surface, above which the species *Psylliodes chrysocephala* L. should be chemically suppressed. The crops were harvested on three different dates; in 2010 on the 11th of July, the 29th of July and the 21st of August, in 2011 on the 13th of July, the 28th of July and the 24th of August.

Statistical analysis: Analysis of variance (ANOVA) was used to assess differences between cabbage genotypes in regard to flea beetles 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 analyse the impact of different factors on the damage level. The differences ($P<0.05$) between the mean values were identified using Student-Newman-Keuls multiple range test. We calculated correlations between the indices of feeding damage caused by cabbage flea beetles and growth stages of 20 cabbage genotypes. The correlation coefficients were calculated using the data of all 9 field evaluations at the same matrix. All the statistical analyses were performed using Statgraphics Centurion XVI [16]. The data are presented as untransformed means ± SE.

Results and Discussion

Levels of injuries caused by cabbage flea beetles in 2010: The results our study show that the average extent of damage done on cabbage leaves by flea beetles (*Phyllotreta* spp.) was influenced by the assessment date (ANOVA, F=56.27, Df=8; $P<0.0001$; KW test, H=331.68; Df=8; $P<0.0001$) and the genotype of cabbage (ANOVA, F=6.16, Df=1; $P<0.0001$; KW test, H=2.89; Df=8; P=0.0049), while the extent of damage among the studied groups (in relation to the length of the growth period) did not differ (ANOVA, F=1.65; Df=2; P=0.1926; KW test, H=2.92, Df=1.65; P=0.1926). We established that the extent of damage was influenced also by the plants' growth stage (ANOVA, F=26.22, Df=16, $P<0.0001$; KW test, H=321.15, Df=16, $P<0.0001$), and that the extent of damage in the early (ANOVA, F=19.15, Df=5, $P<0.0001$; KW test, H=92.25, Df=5, $P<0.0005$), the mid-early (ANOVA, F=26.32, Df=5, $P<0.0001$) and the mid-late genotypes (ANOVA, F=68.80, Df=8, $P<0.0001$; KW test, H=316.16, Df=8, $P<0.0001$) was influenced by the assessment date.

The general extent of damage varies from 2.10±0.03 on the first date of assessment (May 3) to 2.85±0.03 in the first ten days of July (Jul 1). In the second ten days of July (Jul 2), the average extent of damage was 3.17±0.05, on the last tree assessment dates, Jul 3 (2.89±0.09), Aug 1 (2.89±0.09), and Aug 2 (2.90±0.09), the intensity of feeding did not change significantly (Fig. 1).

On the second date of assessment (Jun 1), we observed the lowest extent of damage on the early (BBCH 19-45) (2.52±0.05) and the mid-late (2.58±0.08) (BBCH 14-19) genotypes of cabbage. In the second ten days of June (Jun 2), the extent of damage was on the early genotypes (BBCH 42-47) significantly highest (3.18±0.08). Among the early genotypes (Table 1), 'Green rich F1' (2.66±0.10) (BBCH 43-44), 'Pandion F1' (2.83±0.10) (BBCH 47-48),

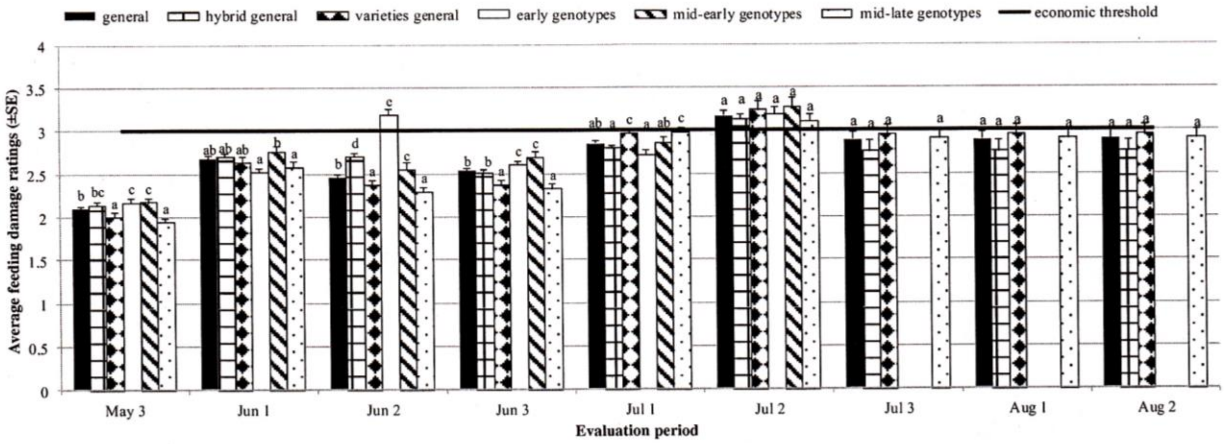

Figure 1. The average indices (±SE) of feeding damage caused by cabbage flea beetles during the growth season 2010.
The average values belonging to a specific dates of evaluation, followed by the same lowercase letter are not significantly different according to Student-Newman-Keuls multiple range test ($P<0.05$). The bars represent the SE of the mean. The black bold horizontal line represents hypothetical economic threshold of the flea beetle harmfulness.

Table 1. The average indices (±SE) of feeding damage caused by cabbage flea beetles during the growth season 2010 among different cabbage genotypes.

	May 3	Jun 1	Jun 2	Jun 3	Jul 1	Jul 2	Jul 3	Aug 1	Aug 2
'Autumn queen F1'	1.92±0.01B^{a}	2.92±0.10^{BCd}	2.25±0.10^{Bb}	2.33±0.10^{Bb}	2.58±0.20^{Ac}	3.00±0.02^{Cd}			
'Delphi F1'	2.92±0.01^{Fd}	2.83±0.02^{Bc}	2.08±0.10^{A2}	2.50±0.20^{BCb}	2.75±0.10^{ABbc}	2.75±0.10^{Bbc}			
'Destiny F1'	2.25±0.01^{CDb}	2.67±0.30^{Bc}	2.50±0.20^{CDc}	2.08±0.10^{ABa}	3.00±0.00^{Ccd}	3.15±0.20^{ABCDcd}			
'Green rich F1'	2.08±0.02^{BCa}	2.66+0.10^{Bb}	2.66±0.10^{CDb}	2.50±0.20^{BCb}	2.50±0.20^{Ab}	3.75±0.10^{Ec}			
'Ixxion F1'	2.25±0.10^{CDa}	2.33±0.10^{Aa}	2.42±0.20^{Ca}	2.42±0.20^{BCa}	2.75±0.10^{ABab}	3.25±0.10^{Db}			
'Pandion F1'	2.50±0.10^{Ea}	2.58±0.20^{ABab}	2.83±0.10^{Db}	3.00+0.00^{Dc}					
'Sunta F1'	2.75±0.10^{Ea}	3.00±0.10^{Cb}	3.00±0.00^{Eb}	2.92±0.10^{CDab}					
'Tucana F1'	2.08±0.20^{BCa}	2.67±0.30^{BCabc}	2.75±0.20^{CDb}	2.92±0.10^{CDc}					
'E rano'	1.75±0.10^{Aba}	2.75±0.20^{BCc}	2.17±0.10^{Ab}	2.75±0.10^{Cc}	2.75±0.10^{ABc}	2.83±0.30^{ABCDc}			
'Cheers F1'	2.25±0.10^{CDa}	2.66±0.10^{Bh}	2.25±0.10^{Ba}	2.75±0.10^{Cb}	2.75±0.10^{ABb}	3.00±0.02^{Cc}			
'Field force F1'	2.33±0.10^{Da}	3.08±0.20^{BCbc}	2.75±0.30^{CDb}	2.92±0.10^{CDb}	3.25±0.10^{Dc}				
'Red dynasty F1'	1.83±0.10^{Ba}	2.33±0.14^{Ab}	2.33±0.14^{Bb}	2.42±0.15^{BCb}	2.75±0.13^{ABc}	3.33±0.14^{Dd}			
'Vestri F1'	2.00±0.00^{Ca}	2.92±0.03^{BCd}	2.58±0.09^{Cb}	2.50±0.25^{BCbc}	2.75±0.10^{ABc}	3.00±0.09^{Ce}			
'Futoško'	2.42±0.20^{CDa}	2.75±0.18^{BCab}	2.83±0.11^{Db}	2.83±0.17^{CDb}	2.75±0.13^{ABab}	3.75±0.25^{Ec}			
'Hinova F1'	1.91±0.10^{Ba}	2.33±0.14^{Ab}	2.50±0.15^{Cbc}	2.00±0.00^{Aa}	2.75±0.13^{ABc}	2.75±0.13^{Bc}	2.75±0.13^{Bc}	2.75±0.13^{Bc}	2.75±0.13^{Bc}
'R1 cross F1'	1.92±0.20^{ABCa}	2.83±0.13^{BCb}	2.00±0.12^{Aa}	2.08±0.08^{Aa}	2.92±0.20^{Bb}	3.00±0.20^{CDb}			
'Holandsko'	1.75±0.01^{Aa}	2.42±0.15^{Ac}	2.41±0.15^{Cc}	2.17±0.11^{ABb}	3.00±0.00^{Cd}	2.50±0.15^{Ac}	2.50±0.15^{Ac}	2.50±0.15^{Ac}	2.50±0.15^{Ac}
'Kranjsko'	2.00±0.00^{BCa}	2.42±0.15^{Ab}	2.00±0.00^{Aa}	2.63±0.15^{Cbc}	3.00±0.00^{Cd}	2.83±0.11^{ABc}	2.83±0.11^{Bc}	2.83±0.11^{Bc}	2.83±0.11^{Bc}
'Ljubljansko'	2.00±0.00^{BCa}	2.67±0.18^{Bc}	2.17±0.11^{ABb}	2.58±0.15^{BCc}	3.00±0.00^{Cd}	3.50±0.26^{DEe}	3.50±0.26^{Ce}	3.50±0.26^{Ce}	3.50±0.26^{Ce}
'Varaždinsko'	2.08±0.20^{BCa}	2.83±0.24^{BCc}	2.67±0.20^{CDbc}	2.50±0.15^{BCb}	3.00±0.00^{Cc}	4.00±0.21^{EFd}	4.00±0.21^{Dd}	4.00±0.21^{Dd}	

The average values belonging to a specific dates of evaluation followed by the same uppercase letter are not significantly different according to Student-Newman-Keuls multiple range test (P<0.05). The average values belonging to a specific genotype during the growth season followed by the same lowercase letter are not significantly different according to Student-Newman-Keuls multiple range test (P<0.05). The bars represent the SE of the mean.

'Sunta F1' (3.00±0,00) (BBCH 47-48) and 'Tucana F1' (2.75±0.20) (BBCH 47-48) stand out in regard to the extent of damage. The extent of damage done by the species from the genus *Phyllotreta* in the said interval was on the mid-late genotypes distinctly the lowest (2.29±0.06). 'Kranjsko' (2.00±0.00) (BBCH 19-42) and 'R1 cross F1' (41-42) (2.00±0.00) are the genotypes on which the extent of damage was the lowest. At the first assessment in July (Jul 1), the highest extent of damage was noted on the selected genotypes (2.96±0.04) (BBCH 41-48) and the mid-late genotypes (2.98±0.05) (BBCH 41-47). The extent of damage did not differ among individual groups of genotypes in the second ten days of July (Jul 2). On the last three assessment dates in the first year of the experiment, no significant differences could be detected in the extent of damage done by *Phyllotreta* spp. The early and the mid-early geno-types reached technological ripeness on the last three assessment dates.

Levels of injuries caused by cabbage flea beetles in 2011: In the second year of our experiment, we established that the extent of damage on cabbage leaves during the growth period differed (ANOVA, F=57.41; Df=8; P<0.0001; KW test, H=342.55, Df=8, P<0.0001). The extent of damage on cabbage differed also among the studied genotypes (ANOVA, F=7.15, Df=1, P=0.0075; KW test, H=3.16, Df=1, P=0.0066), as well as among the growth stages of cabbage (ANOVA, F=22.43, Df=16, P<0.0001; KW test, H=288.17, Df=16, P<0.0001). However, we did not establish any significant differences in the extent of damage among individual groups (in relation to the length of the growth period) (ANOVA, F=1.81, Df=2, P=0.1635; KW test, H=1.88, Df=2, P=0.3893). The extent of damage done by flea beetles was in the early genotypes influenced by the assessment date (ANOVA, F=28.79, Df=5, P<0.0001; KW test, H=105.58, Df=5, P<0.0001), while the influence of the assessment date was detected also in the mid-early (ANOVA, F=21.19, Df=5, P<0.0001; KW test, H=80.13, Df=5, P<0.0001) and the mid-late genotypes of cabbage (ANOVA, F=29.15, Df=8, P<0.0001; KW test, H=174.63, Df=8, P<0.0001).

At the first date of assessment (May 3), over 2% of damaged leaf surface (2.10±0.03) was observed on cabbage leaves, and the extent of damage began increasing in the beginning of July (Jul 1) (2.87±0.03) to reach the peak on the second assessment date in July (Jul 2) (3.20±0.05). The general extent of damage was the same (2.89±0.10) at the last two assessment dates, in the first (Aug 1) and the second (Aug 2) ten days of August (Fig. 2).

On the first date of assessment, the extent of damage was lowest in the mid-late genotypes (BBCH 13-19) (1.94±0.05), among which stands out the variety 'Holandsko' (BBCH 16-19) (1.75±0.10). On the first date of assessment, the average extent of damage on the selected varieties was 2.00±0.05 (BBCH 13-19), while the extent of damage on the hybrids was 2.15±0.05 (BBCH 19-41). On the second date of assessment (Jun 1), we did not establish any differences in the average extent of damage on the hybrids (2.72±0.05) (BBCH 19-45), the varieties (2.86±0.09) (BBCH 14-42), the early genotypes (2.74±0.06) (BBCH 41-48), the mid-early genotypes (2.78±0.08) (BBCH 42-45) and the mid-late genotypes (2.78±0.08) (BBCH 41-44). On the third assessment date (Jun 2), we established the highest extent of damage on the mid-late genotypes (2.77±0.08) (BBCH 19-43). The mid-late genotypes were most susceptible to damage also on the fourth (Jun 3) (2.66±0.03) (BBCH 41-43) and the fifth assessment date (Jul 1) (2.99±0.05) (BBCH 41-47). Among

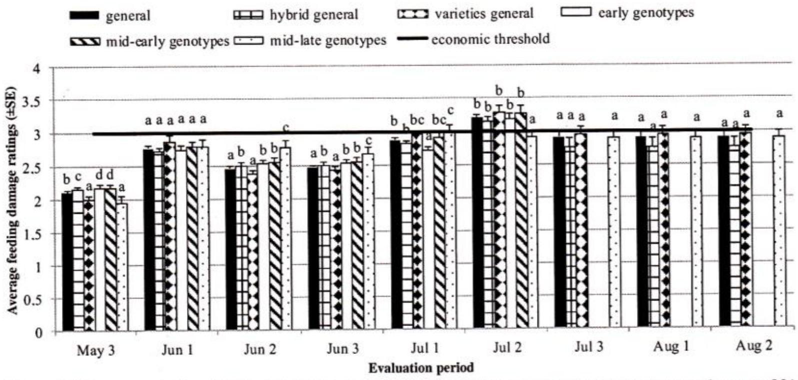

Figure 2. The average indices (±SE) of feeding damage caused by cabbage flea beetles during the growth season 2011. The average values belonging to a specific dates of evaluation followed by the same lowercase letter are not significantly different according to Student-Newman-Keuls multiple range test (P<0.05). The bars represent the SE of the mean. The black bold horizontal line represents the hypothetical economic threshold of the flea beetle harmfulness.

the mid-late genotypes, at the said assessment date stand out 'R1-cross F1' (2.92±0.19) (BBCH 42-44), 'Holandsko' (3.00±0.00) (BBCH 42-43), 'Kranjsko' (3.00±0.00) (BBCH 19-42), 'Ljubljansko' (3.00±0.00) (BBCH 42-44) and 'Varaždinsko' (3.25±0.03) (BBCH 42-44). On the next assessment date (Jul 2), the lowest extent of damage was recorded on the selected mid-late genotypes (2.90±0.10) (BBCH 42-47). Among the mid-late genotypes in the second ten days of July (Jul 2), 'Varaždinsko' (4.00±0.21) stands out in regard to the extent of damage. On the remaining three assessment dates, no differences among the studied groups were detected (Table 2).

The correlation between feeding damage caused by cabbage flea beetles and the growth stage of 20 cabbage genotypes: There is a moderate correlation between the extent of damage done by *Phyllotreta* spp. and the growth stages of the hybrid 'Autumn queen F1', both in the first (r=0.46) and the second year (r=0.45). A moderate correlation was established between the extent of damage and the growth stages of the genotypes 'Delphi F1' (2010, r=0.49; 2011, r=0.44), 'Green rich F1' (2010, r=0.58; 2011, r=0.60), 'Erfurtsko' (2010, r=0.45), 'Fieldforce F1' (2010, r=0.42), 'Red dynasty F1' (2010, r=0.59; 2011, r=0.58), 'Vestri' (2010, r=0.49), 'Kranjsko' (2010, r=0.50), 'Ljubljansko' (2010, r=0.50; 2011, r=0.51), 'Varaždinsko' (2010, r=0.57; 2011, r=0.52). The remaining relations are presented in the Table 3.

The results of our field experiment prove that the flea beetle's preference for different genotypes of cabbage varies. The said finding is congruent with the past studies, in which Bohinc and Trdan [3], Bohinc *et al.* [4] and Brown *et al.* [6] established that the flea beetle's preference for different genotypes of cabbage and for different species of the Cruciferae differed. As Bohinc and Trdan [3], Trdan *et al.* [23,24] say for Slovenia, and Toshova *et al.* [21] for Bulgaria, flea beetles begin appearing outdoors already in the second half of May, while they are most numerous in July [3,23], when beetles of a new generation appear. Consequently, in the beginning of July, there is a distinct increase in the extent of damage on outer leaves and surfaces of cabbage heads.

The economic threshold, 10% of damaged leaf surface [8], was in the first year of the experiment for the first time exceeded in the second decade of June on the early genotypes when the plants were in the phase of cabbage head formation. The hybrid 'Sunta F1' was among the early genotypes in the said period the most susceptible to damage.

It is known that higher average daily temperatures bring about high numbers of adult flea beetles [3,21]. This was confirmed also in our study, as we in both years established the highest average daily temperature (2010: 25.9°C, 2011: 23.2°C) in the second decade of July, which was exactly at the time when we observed the highest average extent of damage on outer leaves and surfaces of cabbage heads of the studied cabbage genotypes, and consequently recorded the harmfulness above the economic threshold.

While in both years of the experiment the threshold of harmfulness on the early genotypes in the first decade of July was not reached, the opposite held true for the extent of damage on the mid-late genotypes, especially for the variety 'Varaždinsko', whose average index of damage in the said period was 3.25. In the first and in the second decade of July the early genotypes of cabbage were already nearing technological ripeness, while the mid-late genotypes were in the phase of head formation and were thus more susceptible to feeding [2,3].

It has been discovered by Prešeren [14] that the varieties 'Varaždinsko' and 'Futoško' were in her several-year study the most susceptible to feeding by beetles from the genus *Phyllotreta*. Among the reasons for her finding, which are confirmed also by the results of our study, we can point out the interesting opinion of Tenczar and Krischik [18], that flea beetles are much more susceptible to varieties, which are present in the environment longer. The fact that in the second year of the experiment we noted higher average indexes of damage on cabbage leaves can also be a result of the absence of crop rotation, as this measure is often said to be one of the most important alternative measures for reducing damage done on the Brassicas by flea beetles [1].

Our study points out to the fact that plants in early growth stages are more susceptible to attacks by flea beetles, as well as to the fact that the extent of damage until the middle of July increases, and then decreases to some extent. The latter is a consequence of the flea beetle's bionomy as it is known in the country where the research was carried out.

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2.2 POVEZAVA MED ANTIOKSIDATIVNIM POTENCIALOM IN OBSEGOM POŠKODB KAPUSOVIH STENIC (*Eurydema* spp.) NA RAZLIČNIH GENOTIPIH ZELJA

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V dveletnem poljskem poskusu smo preučevali obseg poškodb kapusovih stenic (*Eurydema* spp.) na listih zelja glede na barvo genotipov. Ugotovili smo, da se obseg poškodb razlikuje glede na barvo genotipa. V obeh letih poskusa je bila afiniteta kapusovih stenic do zelenih genotipov signifikatno višja v prvih štirih terminih ocenjevanja (zadnja dekada maja - zadnja dekada junija), medtem ko v terminih ocenjevanja med tretjo dekado julija in drugo dekado avgusta nismo ugotovili razlik med belimi in rdečimi genotipi. Poškodbe kapusovih stenic smo najprej opazili na belih genotipih. Antioksidativni potencial, ki je pogojen z vsebnostjo antocianinov, je bil sigfikantno višji na rdečih genotipih zelja. Rezultati naše raziskave kažejo, da je višji antioksidativni potencial negativno vplival na obseg poškodb kapusovih stenic. Ugotovili smo, da je antioksidativni potencial višji na sortah (0,58±0,02 mmol/100g) kot na hibridih (0,47±0,01 mmol/100 g). Naša raziskava prav tako potrjuje višje antioksidativni potencial na rdečih (0,67±0,03 mmol/100 g) kot na belih genotipih zelja (0,48±0,01 mmol/100 g). Med srednje poznimi genotipi (0,55±0,02 mmol/100 g) in srednje zgodnjimi genotipi (0,53±0,02 mmol/100 g) nismo ugotovili razlik v vrednosti antioksidacijskega potenciala, medtem ko je bila vrednost antioksidacijskega potenciala v zgodnjih genotipih zelja signifikatno najnižja (0,46±0,01 mmol/100 g). Ugotavljamo, da je barva rastlin pomemben dejavnik naravne odpornosti zelja na napad kapusovih stenic in posledično predstavlja enega od načinov zmanjševanja obsega poškodb kapusovih stenic v okoljsko sprejemljivih načinih pridelovanja zelja.

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 the two-year field experiment we studied the extent of damage done by cabbage stink bugs (*Eurydema* spp.) on outer leaves and surface of cabbage heads in relation with the colour of a genotype. We established that the extent of damage varied with the colour of a genotype. 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 decades 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 the red genotypes. Cabbage stink bugs first appeared on white cabbage genotypes. The study of antioxidative potential established differences between the white and the red genotypes. The antioxidative potential conditioned by the content of anthocyanins was significantly higher on the red cabbage genotypes. The research shows that higher antioxidative potential is related to a lower extent of damage done by *Eurydema* spp. The average value of antioxidative potential was in the cultivars 0.58 mmol/100 g of the sample, in the hybrids it was 0.47±0.01 mmol/100 g. We confirmed significant differences in values of antioxidative potential also 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 lowest. We established that the colour of plants (cabbage) represents one of the successful factors of antixenosis and has the potential for reducing the harm done 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 include, besides others, glucosinolates and other types of secondary metabolites (Bohinc et al., 2012). When plants are under stress, an important factor 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. Among plant phenols are also flavonoids (Dai and

Mumper, 2010). Anthocyanins, which are usually mentioned as pigments (also in red cabbage) (Hatier and Gould, 2008) and belong to flavonoids (Soengas et al., 2011), condition also natural resistance of plants (Lev-Yadun and Gould, 2008), as they influence harmful pest's colour perception.

The Brassicaceae are plant species which are important both from agronomic (Font et al., 2005; Vaughn and Berhow, 2005; Cartea et al., 2008) and economic (Vaughn and Berhow, 2005) perspective. The production of cabbage, which is in Europe a very important garden crop (Devetak et al., 2013), is exposed to many harmful pests (Bohinc and Trdan, 2012), among others also to cabbage stink bugs (*Eurydema* spp.). The said genus of univoltile harmful pest (Trdan et al., 2006) by sucking sap of older plants cause 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 which have been proven successful in reducing the damage done by cabbage stink bugs on cabbage we point out trap cropping (Bohinc and Trdan, 2012) and the selection of cabbage genotypes, as they 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 purpose of our research was to find out how the extent of damage done by *Eurydema* spp. differs in the white and the red cabbage genotypes, what are the levels of antioxidative potential in these genotypes, and what are the correlations between antioxidative potential and the extent of damage caused by cabbage stink bugs' sucking.

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 in two groups (red and white) depending on plant colour. 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 dinasty 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 performed using a plot of 110.4 m^2. The field was divided into four blocks of dimensions of 1.10 x 24 m. The plants were grown using standard cultural practices, except that no insecticide was applied. More detailed description about cultural practices is available in Trdan et al. (2007).

Field evaluation and observations

The damage done 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 9 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 which was carried out in the first ten days of August was marked as August 1, while the assessment which was carried out in the second ten days of the same month was marked as August 2.

The damage done by cabbage stink bugs on outer leaves and cabbage heads was assessed by the 6-grade visual scale, which was originally intended for assessing the damage done by onion thrips (*Thrips tabaci* L.) (Stoner and Shelton, 1988). The mark 1 thus means undamaged leaves, the mark 2 means less than 1 % of damaged leaf surface, the mark 3 means from 2 to 10 % of damaged leaf surface, the mark 4 means from 11 to 25 % of damaged leaf surface, the mark 5 means from 26 to 50 % of damaged leaf surface, and the mark 6 means more than 50 % of damaged leaf surface. The said visual scale was for the needs of assessing the extent of damage done 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, we stored outer leaves in special plastic bags. By a sharp knife we measured 10 g of individual genotypes and put them into plastic 50 mL centrifuge tubes. 15 g of 2 % solution of metaphosphoric acid was added into the centrifuge tubes and homogenised by Ultra-Turax for 5 minutes. After the homogenisation we froze the samples and stored them at -20 °C until the analyses.

The frozen samples in 2 % metaphosphoric acid were melted and centrifuged for 5 minutes at 4000 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 in an eppendorf tube 60 µl of methanol and 1.5 ml of DPPH solution. 60 µl of the studied genotypes sample and 1.5 ml of DPPH solution were mixed. The samples of each genotype were analysed in three parallels. In the blind experiment we mixed 60 µl of a sample with 1.5 ml of methanol. The mixtures were well mixed, decanted into cuvettes, we determined the absorption by a spectrophotometer (Hewlett-Packard, model: HP model 8453, USA) at 517 nm after 15 minutes. The antioxidative potential is presented as mmol/100 mg of the sample (hereafter mmol/100 mg).

For monitoring antioxidative potential we used the method DPPH. The radical DPPH absorbs light at 517 nm. DPPH was prepared fresh each time. In 100 ml flask we put 4 mg of DPPH (2.2-diphenyl-1- picrylhydrazyl) (Sigma-Aldrich, Germany) with 20 ml of methanol (Merck, Germany) and mixed so it dissolved completely. We added methanol until the absorption of the solution was 1.

Data analysis

The experimental results were statistically evaluated with the program Statgraphics Centurion XVI (2009). The 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 analyse 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 analyse 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 feeding

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 colour (ANOVA, F=6.23, Df=1, P=0.0126; KW test, H=8.25, Df=0.0040) and the 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 cultivar 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 (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 between cabbage genotypes of different colours 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) (Figure 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 colour of 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 the cultivar or the hybrid (ANOVA, F=2.02, Df=1, P=0.1549; KW test, H=2.76, Df=1, P=0.0964) and the length of growth period (ANOVA, F=1.31, Df=2, P=0.2714).

At the first date of assessment in the second year of the experiment we did not detect any damage done 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 cultivars at the third (1.94±0.08) and the fourth (2.01±0.08) date of assessment, while no significant differences between both colour groups were observed at later dates (Figure 2).

Antioxidative potential in white and red genotypes

In view of the results of our research we can say that antioxidative potential in cabbage depends on the genotype's colour (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 the genotypes (ANOVA, F=3.50; Df=19, P<0.0001; KW test, H=44.24, Df=19, P=0.00087). We also found out that the antioxidative potential content differed between the hybrids and the cultivars (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 cultivars 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 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 cultivars '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 cultivar 'Varaždinsko' (0.51±0.03 mmol/100 g of fresh sample). The lowest antioxidative potential was established in the v 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 significantly negative correlation between the levels of antioxidative potential in outer leaves and the extent of damage caused by cabbage stink bugs sucking in 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 cultivar 'Red dinasty 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 cultivar 'Kranjsko'. In other genotypes we established significant low or very low correlations, even insignificant correlations (Table 3).

DISCUSSION

With our research we wished to add the plants' colour 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). We can say we reached the desired purpose.

Cabbage stink bugs (*Eurydema* spp.), which are in the wider region of the said research univoltile insects (Trdan et al., 2006), in our experiment 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 said harmful 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 dinasty F1' and 'Holandsko') did not exceed the index 1.75 or 1 % of damage leaf surface.

Some past studies mention the length of 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 pest did not confirm this. Pronounced changes in the rise of the extent of damage were in both years of the experiment observed in the second decade of July, which coincides with the appearance of the bug larvae *Eurydema ventrale* Kolenti and *Eurydema oleracea* (L.) (Trdan et al., 2006; Bohinc and Trdan, 2012).

Since anthocyanins are the substances which to the largest extent influence the colour of the red cabbage genotypes (Soengas et al., 2011), we can assume that anthocyanins importantly influence also the antioxidative potential of cabbage (Li et al., 2012). Because we did not determine antioxidative potential of cabbage during the growth period, the said statement cannot be confirmed. The results of related studies show that the selection of a cabbage genotype (Peńas et al., 2012) is an important factor of bioactive substance content, also anthocyanins.

The results of our research – the analysis of antioxidative potential content at technological maturity – show that the content of these bioactive substances is at that time still high. Ribera *et al*. (2010) say that highbush blueberry (*Vaccinium corymbosum* L.) has the highest antioxidative potential at technological maturity, which we cannot say for the cabbage in our research. Lee et al. (2007) and Soengas et al. (2011) established that antioxidative potential is the highest in the red cabbage genotypes, the conclusion we made on the basis of our research's results. In regard to high levels of antioxidative potential, the red cultivars 'Erfurtsko' and 'Holandsko' stand out, 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

Antioxidative potential in our research turned out to be an important factor of natural resistance of cabbage to attacks by cabbage stink bugs. Among the antioxidants present in cabbage we cannot point to the one which to the largest extent determine the feeding of *Eurydema* spp., yet we can point out the general positive significance of antioxidants in plant protection and human diet. The red cabbage cultivars 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 cultivars, such as 'Erfurtsko' and 'Holandsko', due to their lesser susceptibility to damage done by *Eurydema* spp. and higher antioxidant content represent a potential in environmentally acceptable systems for the Brassicaceae. Our research thus proves that the colour of plants is a factor which distinctly affects the value of antioxidative potential. In cases when we select to grow a cultivar, the value of the said parameter is even higher.

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Table 1. Growth stages of individual 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			

Table 2. Growth stages of individual 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'	19-42	42-42	42-43	42-45	46-49	49-49			
'Cheers F1'	19-41	41-41	41-44	42-44	45-48	49-49			
'Delphi F1'	19-41	41-43	41-45	43-48	44-49				
'Destiny F1'	19-41	41-42	41-44	43-45	46-47	49-49			
'Erfurtsko'	19-19	19-42	19-42	19-42	43-44	43-47	49-49		
'Fieldforce F1'	19-41	41-45	45-48	47-49	49-49				
'Futoško'	14-19	41-41	19-42	42-44	42-48	49-49			
'Green rich F1'	19-41	42-44	42-44	43-44	46-47	49-49			
'Hinova F1'	19-19	19-41	19-41	42-43	42-45	45-45	47-47	48-48	49-49
'Holandsko'	16-19	19-41	19-42	42-44	43-44	46-47	46-47	48-49	49-49
'Ixxion F1'	19-41	41-42	41-42	43-44	43-44	43-48	49-49		
'Kranjsko'	13-19	14-41	19-41	19-42	41-42	41-45	45-49	47-49	49-49
'Ljubljansko'	16-19	16-41	19-42	42-44	44-46	47-48	48-49	48-49	49-49
'Pandion F1'	41-41	43-45	45-48	49-49					
'R1 cross F1'	19-19	41-41	41-42	42-44	43-47	49-49			
'Red dinasty F1'	19-19	19-42	19-42	42-43	42-47	49-49			
'Sunta F1'	19-41	42-44	43-48	49-49					
'Tucana F1'	41-41	42-44	46-48	47-47	49-49				
'Varaždinsko'	19-19	19-42	19-43	42-44	44-47	46-46	47-48	49-49	
'Vestri F1'	19-19	41-42	41-43	43-44	43-44	45-47	49-49		

Table 3. Correlation between the antioxidative potential and the level of *Eurydema* spp. 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 dinasty 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

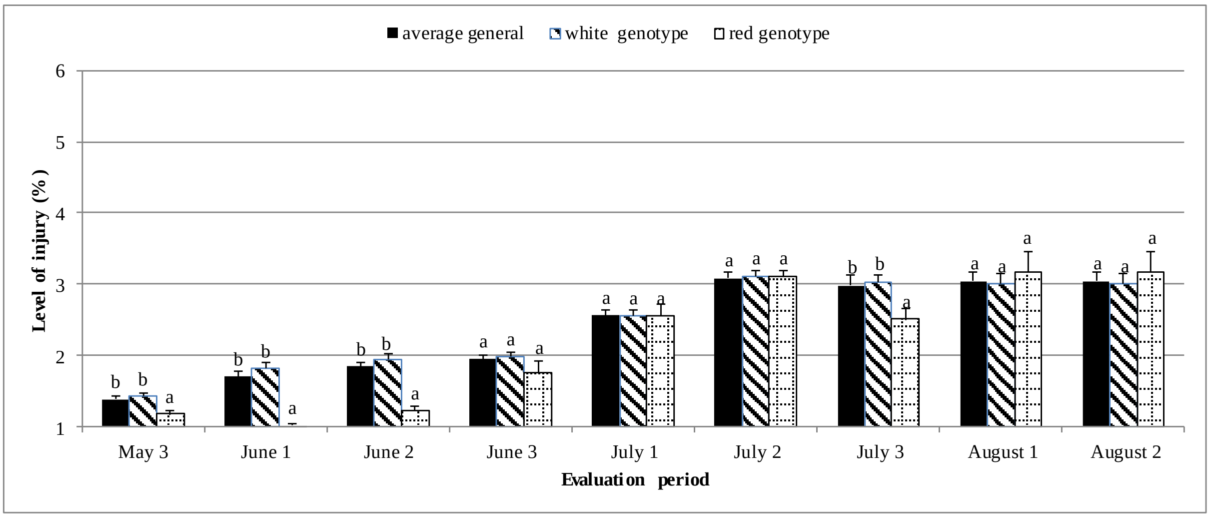

Fig. 1. Level of injury caused by *Eurydema* spp. on different groups of cabbage genotypes in 2010.

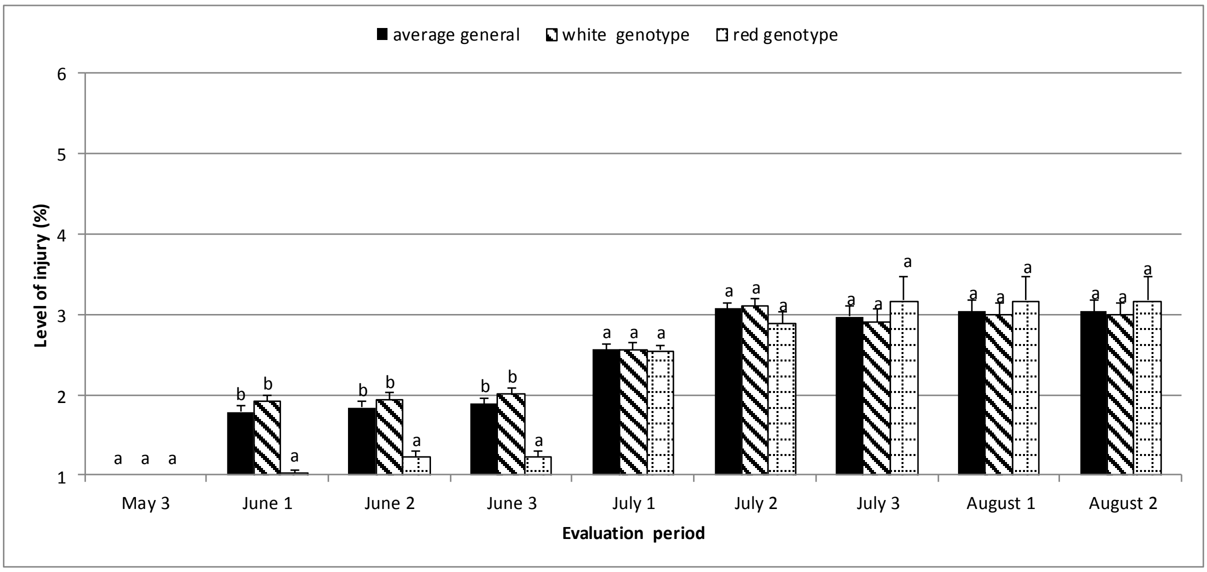

Fig. 2. Level of injury caused by *Eurydema* spp. on different groups of cabbage genotypes in 2011.

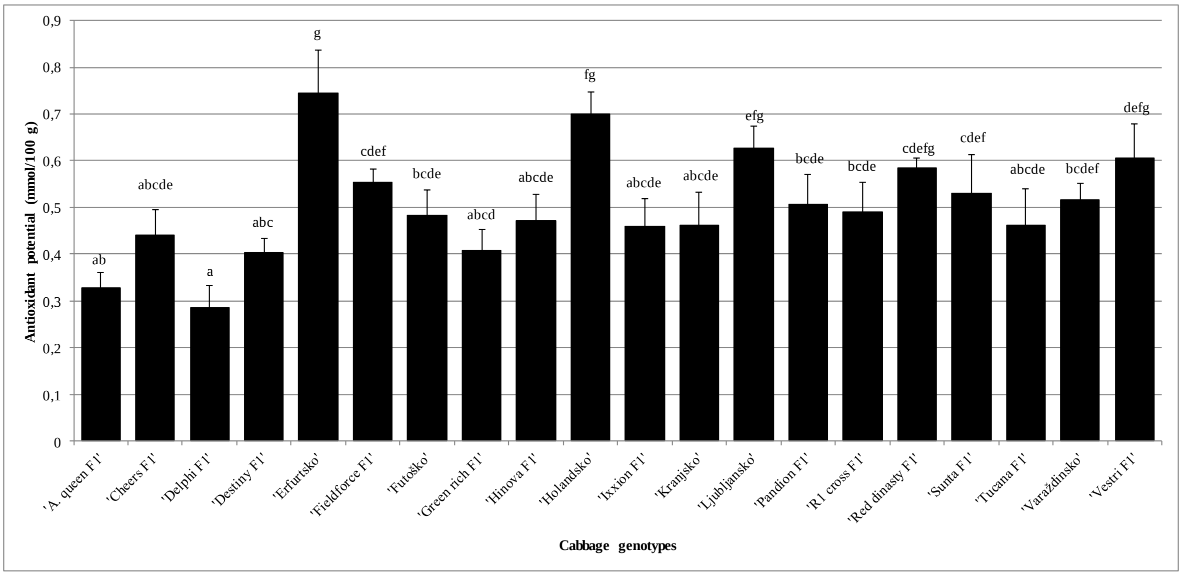

Fig. 3. Antioxidative potential among 20 different cabbage genotypes in 2010.

2.3 EPIKUTIKULARNI VOSEK KOT DEJAVNIK ANTIKSENOZE ZELJA NA NAPAD KAPUSOVIH BOLHAČEV IN KAPUSOVIH STENIC

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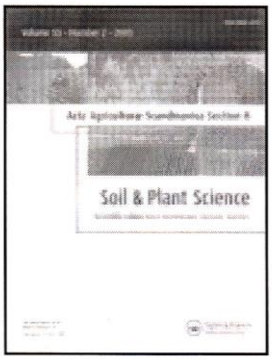
Namen naše raziskave je bil ugotoviti pomen epikutikularnega voska v 8 različnih genotipih zelja (4 beli in en rdeč hibrid, 2 rdeči in ena bela sorta), kot dejavnika naravne odpornosti zelja na napad kapusovih bolhačev (*Phyllotreta* spp.) in kapusovih stenic (*Eurydema* spp.). Omenjeni skupini škodljivcev spadata med pomembnejše škodljivce zelja v južni Evropi. S tem razlogom, in ker je naš cilj zmanjšati uporabo sintetičnih insekticidov, smo poljski poskus izvedli v letih 2006-2008. Ugotovili smo, da se vsebnost epikutikularnega voska razlikuje med posameznimi genotipi, posledično pa obstajajo med različnimi genotipi razlike v obsegu poškodb zaradi hranjenja kapusovih bolhačev. Najvišji obseg poškodb smo v prvem letu poskusa ugotovili na hibridu ‘Cheers F1’ (1,68±0,05), tudi v drugem letu poskusa pa je po največjem obsegu poškodb (2,87±0,13) izstopal isti genotip. Obseg poškodb na listih zaradi sesanja kapusovih stenic je bil v obeh letih poskusa najvišji na hibridih 'Destiny F1', 'Cheers F1' in 'Vestri F1'. Vsebnost epikutikularnega voska je bila višja pri rdečih genotipih zelja. Pri skoraj vseh preučevanih genotipih zelja smo potrdili negativno korelacijo med vsebnostjo epikutikularnega voska in obsegom poškodb zaradi kapusovih bolhačev. Zato ugotavljamo, da je epikutikularni vosek pomemben dejavnik naravne odpornosti zelja na napad kapusovih bolhačev, kar posledično vpliva na to, da so genotipi z višjo vsebnostjo epikutikularnega voska ustreznejši za implementacijo v okoljsko sprejemljive načine pridelave zelja.



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Leaf epicuticular wax as a factor of antixenotic resistance of cabbage to cabbage flea beetles and cabbage stink bugs attack

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ORIGINAL ARTICLE

Leaf epicuticular wax as a factor of antixenotic resistance of cabbage to cabbage flea beetles and cabbage stink bugs attack

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The aim of present research was to establish the role of epicuticular wax content in eight cabbage genotypes (four white hybrids and one red hybrid, two red varieties and one white variety) in the context of its natural resistance to attack cabbage flea beetles (*Phyllotreta* spp.) and cabbage stink bugs (*Eurydema* spp.), which are among the most important cabbage pests in southern Europe. For this reason and for the purpose of diminishing the use of synthetic insecticides against the cabbage pests the field experiments in 2006 and 2008 were conducted. We found out that individual cabbage genotypes – they had different epicuticular wax content – differ in regard to their susceptibility to attacks by the studied groups of harmful insect pests. The highest susceptibility to attacks by *Phyllotreta* spp. was confirmed for the hybrid 'Cheers F1', in the first year (1.68 ± 0.05), as well as in the second year of the experiment (2.87 ± 0.13). Cabbage stink bugs in both years of the experiment caused the highest extent of injuries on the hybrids 'Destiny F1', 'Cheers F1', and 'Vestri F1'. In both years we found higher epicuticular wax content in red cabbage genotypes. In almost all studied genotypes we found a pronounced negative correlation between the content of epicuticular wax and the extent of injuries done by both groups of harmful pests. We have established that epicuticular wax is an important factor of cabbage's antixenotic resistance to attacks by cabbage flea beetles and cabbage stink bugs, and that the cabbage genotypes with higher content of this substance are consequently more suitable for environmentally acceptable manners of cabbage production.

Keywords: antixenosis; cabbage; leaf epicuticular wax; *Phyllotreta* spp.; *Eurydema* spp.

Introduction

Cabbage is a very important field vegetable crop in Europe because it represents an important source of income for a large number of producers (Trautwein 2005), and has many beneficial effects in human diet (Martinez-Villaluenga et al. 2012). In the Old continent, especially in its south and central parts, the cabbage is attacked by a large number of different pests (Reddy 2011). The majority of the insect pests on cabbage, which are economically important in these areas, belong to four orders: Lepidoptera (Trdan et al. 2008a; Devetak et al. 2013), Coleoptera (Trdan et al. 2005b; Žnidarčič et al. 2008), Thysanoptera (Trdan et al. 2005a) and Heteroptera (Trdan et al. 2006).

The number of insecticides available for controlling insect pests is reducing gradually (Souza et al. 2013). Because of this there are now no chemical products available for controlling some pests in many European countries (Neumeister 2007). The lack of knowledge about alternative methods of reducing pest damage may have adverse effects on the economics of food production.

In nature, plants have evolved various defence strategies to fend off herbivorous attackers either directly or indirectly (Ali et al. 2011). Many plants release volatiles in response to herbivore attack (Laznik & Trdan 2013). According to Chacón et al. (2012), host plant resistance can also interfere with biological control if morphological or physiological traits harm both the pests and its natural enemies. Positive interactions between host plants resistance and biological control are possible as well – but these are typically mediated by host density (Oriani et al.

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sampled the early genotypes first, followed by the mid-early and mid-late genotypes. We chose 15 leaves at random from each genotype in each block, and these leaves were taken from the middle of the rosette (avoiding internal and external leaves). Each day we sampled three varieties. Once in the laboratory, we placed the leaves on newspaper for several hours so as to lose some turgidity. The leaves (45 per variety) were marked first and then photographed with digital camera Canon EOS 350D. Then the leaves were weighed.

We extracted the epicuticular wax in a laminar flow cabinet. We poured 300 ml of organic solvent dichloromethane (CH_2Cl_2) into a large glass vessel. We treated each sample (1 variety and 1 replication – 15 leaves) separately. The leaves were added to the glass vessel and homogenised for two minutes using a blender. We decanted the extract of 15 leaves (=1 sample) into an Erlenmeyer flask and gradually added 25 g of anhydrous sodium sulphate (Na_2SO_4). The contents of Erlenmeyer flask were stirred periodically. The Erlenmeyer flask was covered with aluminium foil and left in the laminar flow cabinet over night so that all the water in the sample was taken up by the salt. The next day we passed the extract through filter paper and the filtrate was transferred to a rotary evaporator flask. If the procedure was not completed in one day, we put the Erlenmeyer flasks into a refrigerator. Using the rotary evaporator, we removed the dichloromethane (pressure 850 mbar, temperature 35°C) so that only a few millilitres of solvent remained in the flask. We transferred the small quantity of the solvent containing the epicuticular wax into creo test tubes that have been weighed and marked previously. The cryogenic tubes were left open in a laminar flow cabinet so that the solvent would evaporate completely and only the epicuticular wax remained. We weighed the samples and then stored them in a refrigerator at 4°C.

We analysed the photographs of the leaves using ImageAnalyzer software. With this procedure we measured the surface area of each cabbage leaf in order to calculate the quantity of wax per unit area of leaf surface. This part of the research was conducted in the Entomological Laboratory of the Department of Agronomy at the Biotechnical Faculty, Ljubljana, Slovenia.

Data analysis

The differences of injuries extent due to the feeding on cabbage leaves were analysed using general ANOVA (one-way). Prior to the 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 tests were also applied to analyse the impact of different factors on the damage level. The differences ($P < 0.05$) between the mean values were identified using Student-Newman–Keuls multiple range test. The differences between values of epicuticular wax were calculated by analysing the variance (ANOVA) and Duncan's test of multiple comparison ($P < 0.05$). We calculated correlations using linear regression analysis ($y = kx + n$) between the epicuticular wax content of an individual genotype and the level of injury caused by *Phyllotreta* spp. and *Eurydema* spp. Prior to statistical analysis, the experimental results were statistically evaluated by the program Statgraphics Centurion (2009). Data are presented as untransformated means + SE.

Results

Level of injury caused by Phyllotreta *spp.*

We found out that the extent of injuries done by cabbage flea beetles in the first year of the experiment significantly differed between individual cabbage genotypes (ANOVA, $F = 9.29$, df = 7, $P < 0.0001$; KW test, $H = 63.72$, df = 7; $P < 0.0001$). Such differences were established also between the white and the red genotypes (ANOVA, $F = 51.96$, df = 1, $P < 0.0001$; KW test, $H = 45.72$, df = 1, $P < 0.0001$), while the length of growth period did not significantly influence the extent of injuries (ANOVA, $F = 0.80$, df = 2, $P = 0.4482$; KW test, $H = 0.4708$, df = 2, $P = 0.5003$). Significant differences in the extent of injuries were established also between cabbage hybrids and varieties (ANOVA, $F = 3.93$, df = 1, $P = 0.0474$; KW test, $H = 3.68$, df = 1; $P = 0.0356$) in the experiment. The average extent of injuries on hybrids was 1.46 ± 0.02, while the extent of injuries on varieties was lower (1.38 ± 0.04). The extent of injuries was in the first year of the experiment lowest in the genotypes 'Erfurtsko' (1.24 ± 0.06),'Holandsko pozno' (1.27 ± 0.05) and 'Red dinasty F1' (1.21 ± 0.05), while the highest extent of injuries was established in genotypes 'Cheers F1' (1.68 ± 0.05) and 'Varaždinsko' (1.61 ± 0.06). On average the extent of injuries in white cabbage genotypes did not exceed 2% of damaged leaf surface, while the extent of injuries on red genotype was even lower.

The analysis of the experiment's results in 2008 confirms that the extent of injuries done by cabbage flea beetles significantly differed between individual cabbage genotypes (ANOVA, $F = 4.14$, df = 7, $P = 0.0004$; KW test, $H = 25.89$, df = 7; $P = 0.0005$) and between the white and the red cabbage genotypes (ANOVA, $F = 1.30$, df = 1, $P = 0.0488$; KW test, $H = 0.39$, df = 1, $P = 0.0329$). The extent of injuries was significantly influenced also by the length of growth period (ANOVA, F = 3.14, df = 2, P = 0.0362; KW test, H = 6.0208, df = 2, P = 0.0492). Significant differences in the extent of injuries were established also between the hybrids and the varieties (ANOVA, F = 8.83, df = 1, P = 0.0384; KW test, H = 5.68, df = 1; P = 0.0444). The extent of injuries done by *Phyllotreta* spp. was higher in the hybrids (2.03 ± 0.11), while it was lower in the varieties (1.93 ± 0.02). 'Cheers F1' was the genotype in which we recorded the highest extent of injuries in the second year of the experiment (2.87 ± 0.13). No significant differences in the extent of injuries between the remaining genotypes were established. The remaining average values are presented in Figure 1.

Level of injuries caused by Eurydema *spp.*

The results of our research showed that the affinity of *Eurydema* spp. for individual genotypes in the first year experiment significantly varied (ANOVA, F = 8.87, df = 7, P < 0.0001; KW test, H = 51.96, df = 7; P < 0.0001). We found out that the extent of injuries between the cabbage genotypes differed also due to the length of growth period (ANOVA, F = 10.72, df = 2, P < 000.1; KW test, H = 19.13, df = 19.14, P < 0.0001), due to the colour of plants (ANOVA, F = 28.83, df = 1, P < 0.0001; KW test, H = 19.40, df = 1, P < 0.0001), and that there was a significant difference in preference of cabbage stink bugs for the hybrids or the varieties (ANOVA, F = 16.16, df = 1, P < 0.0001; KW test, H = 18.49, df = 1, P < 0.0001).

In the first year of the experiment the extent of injuries was highest in the genotypes 'Cheers F1' (2.31 ± 0.10), 'Destiny F1' (2.20 ± 0.08) and 'Vestri F1' (2.27 ± 0.10), while in 'Erfurtsko' (1.72 ± 0.09), 'Hinova F1' (1.71 ± 0.08), 'Holandsko' (1.72 ± 0.08), 'Red dinasty' (1.78 ± 0.08) and 'Varaždinsko' (1.91 ± 0.08) we recorded the lowest extent of injuries. The extent of injuries done by *Eurydema* spp. in the first year of the experiment on the hybrids exceeded 2% of damaged leaf surface, while the extent of injuries on the varieties was 1.78 ± 0.05. More than 2% of damaged leaf surface was established in the white varieties, while the extent of injuries in the red varieties was 1.75 ± 0.05.

The analysis of the experiment's results in Year 3 showed that the extent of injuries significantly differed between individual genotypes (ANOVA, F = 4.14, df = 7, P = 0.0004; KW test, H = 25.54, df = 7, P = 0.0005), while the preference of cabbage stink bugs was significantly influenced also by the colour of plants (ANOVA, F = 9.68, df = 1, P = 0.0128; KW test, H = 8.53, df = 1, P = 0.0467) and the length of growth period of the studied genotypes (ANOVA, F = 5.67, df = 2, P = 0.0044; KW test, H = 10.68, df = 2, P = 0.0047). In 2008, we established significant differences in the extent of injuries caused by sucking by cabbage stink bugs also between the hybrids and the varieties (ANOVA, F = 4.78, df = 1, P = 0.0488; KW test, H = 5.42, df = 1, P = 0.0337). The extent of injuries caused by *Eurydema* spp. was in 2008 highest in the genotype 'Cheers F1' (1.13 ± 0.19), while no damage was detected in the genotype 'Hinova F1'. The remaining values are shown in Figure 2.

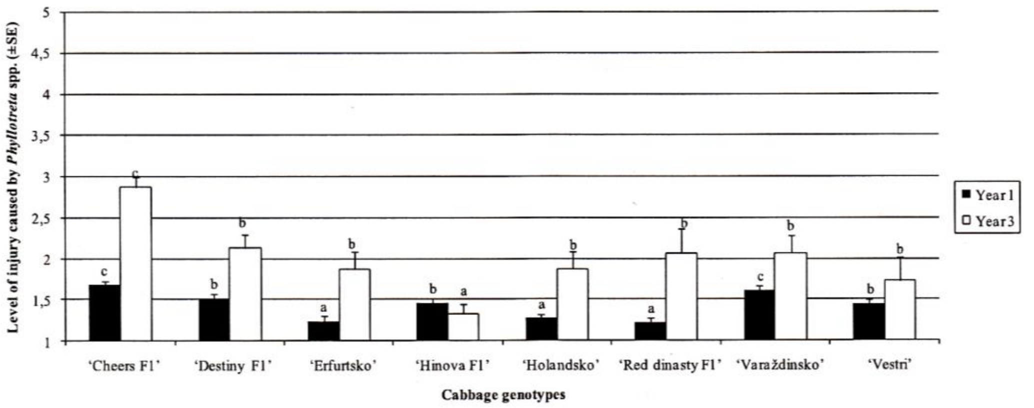

Figure 1. Level of injuries caused by *Phyllotreta* spp. on eight different cabbage genotypes in 2006 and 2008. Average values belonging to a specific genotype, followed by the same lowercase letter, are not significantly different according to Student-Newman–Keuls multiple range test (P < 0.05). The bars represent the SE of the mean.

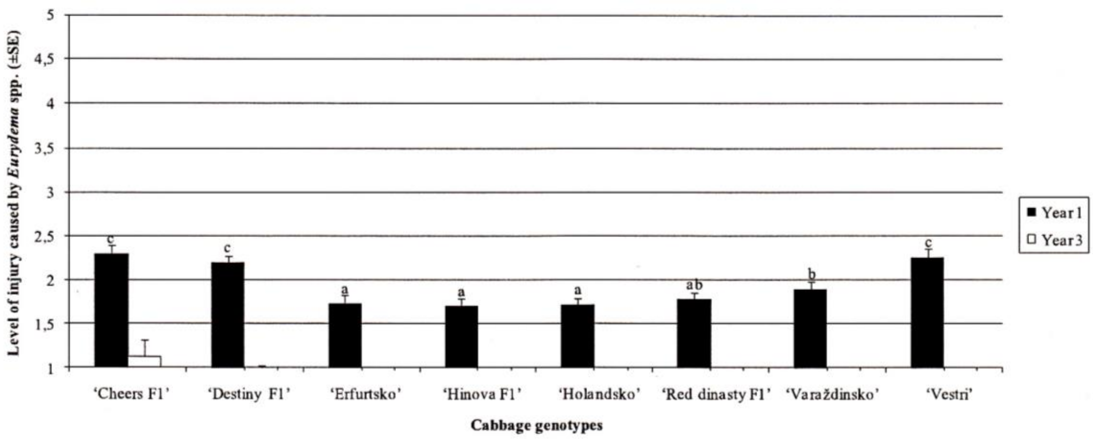

Figure 2. Level of injuries caused by *Eurydema* spp. on eight different cabbage genotypes in 2006 and 2008. Average values belonging to a specific genotype, followed by the same lowercase letter, are not significantly different according to Student-Newman–Keuls multiple range test ($P < 0.05$). The bars represent the SE of the mean.

Epicuticular wax content in the leaves of cabbage

In the first year (Year 1) of the experiment, we found out that the epicuticular wax content significantly differed between the studied genotypes (ANOVA, $F = 2.75$, df = 7, $P = 0.0499$; KW test, $H = 13.34$, df = 7, $P = 0.0429$). No significant influence of the length of growth period on the epicuticular wax content was detected (ANOVA, $F = 0.09$, df = 2, $P = 0.9134$; KW test, $H = 0.1111$, df = 2, $P = 0.9458$). The wax content was in the first year of the experiment significantly conditioned by the colour of a genotype (ANOVA, $F = 17.84$, df = 1, $P = 0.0005$; KW test, $H = 10.23$, df = 1, $P = 0.0014$). The average epicuticular wax content in the white genotypes was 33.53 ± 2.35 μg cm^{-2}, while the wax content in the red genotypes was 49.91 ± 3.20 μg cm^{-2}. The epicuticular wax content was among the studied genotypes lowest in the variety 'Varaždinsko' (26.50 ± 2.64 μg cm^{-2}), while it was highest in 'Red dinasty F1' (48.79 ± 1.25 μg cm^{-2}), 'Erfurtsko' (49.25 ± 14.59 μg cm^{-2}) and 'Holandsko' (51.71 ± 6.95 μg cm^{-2}).

In third year (Year 3), we found out that the length of growth did not significantly influence the epicuticular wax content in cabbage (ANOVA, $F = 1.16$, df = 2, $P = 0.3302$; KW test, $H = 1.67$, df = 2, $P = 0.4330$). The results of our research show that the epicuticular wax content significantly differed also due to the colour of genotypes (ANOVA, $F = 4.96$, df = 1, $P = 0.0349$; KW test, $H = 3.41$, df = 1, $P = 0.0447$). However, we confirmed significant differences in the epicuticular wax content between different cabbage genotypes (ANOVA, $F = 4.77$, df = 7, $P = 0.0027$; KW test, $H = 18.22$, $P = 0.0109$). The epicuticular wax content was thus lowest in the variety 'Varaždinsko' (16.24 ± 0.00 μg cm^{-2}), while it was significantly highest in the hybrid 'Red dinasty F1' (42.75 ± 4.79 μg cm^{-2}) and in the variety 'Erfurtsko' (39.57 ± 4.67 μg cm^{-2}). The average epicuticular wax content in white genotypes was 29.23 ± 2.62 μg cm^{-2}, while it was 38.30 ± 2.02 μg cm^{-2} in the red genotypes (Figure 3).

Correlation between the epicuticular wax content and the extent of injuries caused by **Phyllotreta** *spp. and* **Eurydema** *spp.*

The results of our research show that in the first year of the experiment the epicuticular wax content on cabbage leaves negatively influenced the feeding of the studied groups of harmful pests. In the first year of the experiment we thus found out that there were significant very strong correlations between the extent of injuries caused by *Eurydema* spp. and the epicuticular wax content in the genotypes 'Hinova F1' ($r = -0.98$), 'Varaždinsko' ($r = -0.97$) and 'Vestri F1' ($r = -0.99$). A significant negative correlation between the extent of injuries caused by *Phyllotreta* spp. and the epicuticular wax content was established also in the genotypes 'Red dinasty F1' ($r = -0.99$) and 'Varaždinsko' ($r = -0.95$).

The analysis of the data acquired in 2008 also indicates a negative influence of the epicuticular wax content on the feeding of *Euydema* spp. and *Phyllotreta* spp. on cabbage leaves. A significantly negative influence of the wax content on the feeding of *Eurydema* spp. ($r = -0.89$) and *Phyllotreta* spp. ($r = -0.99$) was established in 'Red dinasty F1'. A pronounced negative influence ($r = -0.85$) of the epicuticular wax content was established in the variety 'Varaždinsko'. No correlation between the extent of injuries caused

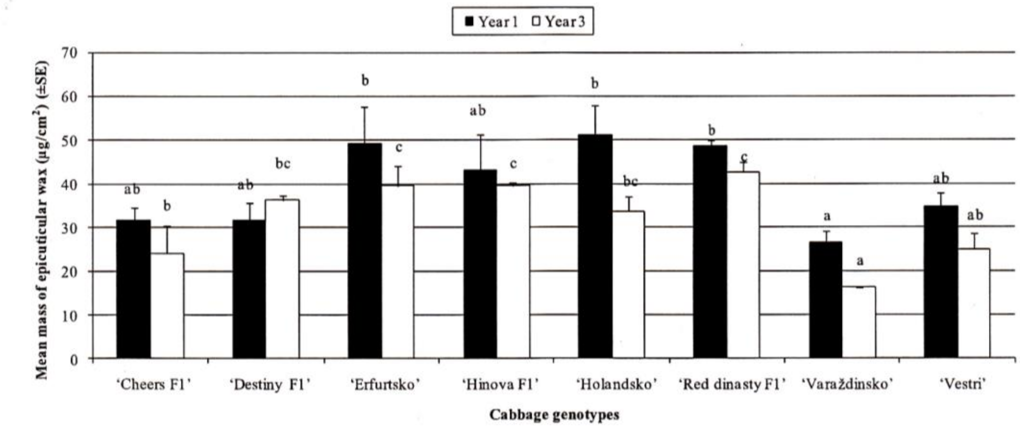

Figure 3. Mean epicuticular wax content on the leaves of eight different cabbage genotypes (μg cm^{-2}) in 2006 and 2008. Average values belonging to a specific genotype, followed by the same lowercase letter, are not significantly different according to Duncan's multiple range test ($P < 0.05$). The bars represent the SE of the mean.

by *Eurydema* spp. and the epicuticular wax content was established in 2008 in the hybrid 'Cheers F1', we also established no correlation between the extent of injuries caused by *Phyllotreta* spp. and the epicuticular wax content. The remaining values are presented in Table 1.

Discussion

On the basis of the results of our research we have found out that the susceptibility of individual cabbage genotypes to attacks by cabbage flea beetles and cabbage stink bugs varies. The fact that the extent of injuries caused by *Phyllotreta* spp. and *Eurydema* spp. varies among individual cabbage genotypes was established also by Žnidarčič et al. (2008), Trdan et al. (2009) and Bohinc and Trdan (2012).

Differences in the extent of injuries caused by the feeding of cabbage flea beetles among individual groups (in regard to the length of growth period) of cabbage genotypes as reported by Trdan et al. (2009), Bohinc and Trdan (2012) and Bohinc et al. (2013a) were not confirmed in our research. Preference of cabbage stink bugs for individual groups of genotypes, however, was more pronounced. In regard to the extent of injuries in our research the group of mid-early genotypes thus stands out, which is not congruent with the findings of some past studies (Trdan et al. 2006; Bohinc & Trdan 2012). The reasons for this can be found in the specifics of individual factors of antixenosis, such as glucosinolates (Bohinc et al. 2012), the colour of plants (Peñas et al. 2011; Soengas et al. 2011; Li et al. 2012), as well as the epicuticular wax content on cabbage

Table 1. Correlation between level of injuries caused by *Phyllotreta* spp. and *Eurydema* spp. and EW (epicuticular wax) value ($P < 0.05$ Duncan's multiple range test) in eight different cabbage genotypes in 2006 and 2008.

	Year 1						Year 3					
	Eurydema spp.			*Phyllotreta* spp.			*Eurydema* spp.			*Phyllotreta* spp.		
	r	*a*	*b*	*r*	*a*	*b*	*r*	*a*	*b*	*r*	*a*	*b*
'Cheers F1'	−1	2.81	−0.02	−1	3.16	−0.04	x	x	x	−1.0	3.57	−0.07
'Destiny F1'	−0.97	4.56	−0.08	−0.15	1.47	−0.02	−0.57	2.48	−0.04	−0.81	5.64	−0.07
'Erfurtsko'	−0.88	1.38	−0.04	−0.31	1.16	−0.04	−0.76	20.84	−0.53	−0.48	11.22	−0.21
'Hinova F1'	−0.98*	1.59	−0.02	−1.0	2.83	−0.01	−1.0	7.33	−0.15	x	x	x
'Holandsko'	−0.51	2.30	−0.05	−0.83	3.10	−0.03	−0.61	2.12	−0.02	−0.51	3.84	−0.04
'Red dinasty F1'	−0.88	5.82	−0.09	−0.99*	3.27	−0.02	−0.89*	4.52	−0.09	−0.99*	11.78	−0.21
'Varaždinsko'	−0.97*	3.25	−0.03	−0.95*	3.08	−0.06	−0.85*	5.61	−0.22	−1.00	16.63	−0.88
'Vestri'	−0.99*	8.88	−0.21	−0.12	1.76	−0.01	−0.37	2.14	−0.01	−0.88	4.44	−0.03

r, correlation coefficient; *a*, intercept; *b*, slope; x, not able to calculate correlation; $*P < 0.05$.

leaves (Stoner 1992; Trdan et al. 2009), which was very precisely analysed in our research.

As already stated by Trdan et al. (2004, 2009) and established also by our research, the epicuticular wax content differs between individual cabbage genotypes. Thus the red genotypes stand out in regard to the highest content of the studied factor of antixenosis, in our research these were the varieties 'Holandsko' and 'Erfurtsko' and the hybrid 'Red dinasty F1'. The epicuticular wax content was in both years of the experiment lowest in the mid-late white genotype 'Cheers F1', which can be related to the greatest susceptibility to injuries in both years of the experiment or the distinctively highest extent of injuries. The results of our research confirm the finding by Bodnaryk (1992), namely that epicuticular wax on leaves is an important factor of antixenotic resistance of cabbage to attacks by cabbage flea beetles, the same holds true for cabbage stink bugs, which was established in a research carried out by Trdan et al. (2009).

Susceptibility of genotypes to the feeding by *Phyllotreta* spp. and *Eurydema* spp. is species specific. The intensity of the feeding of cabbage flea beetles (*Phyllotreta* spp.) was in both years of the experiment highest in the hybrid 'Cheers F1', while the intensity of the feeding by cabbage stink bugs (*Eurydema* spp.) was in both years of the experiment highest in the hybrids 'Cheers F1', 'Vestri F1' and 'Destiny F1'.

The results of our research indicate a negative influence of the epicuticular wax content on the feeding of the studied groups of harmful pests, for this reason we maintain that epicuticular wax represents an important factor of natural resistance of cabbage to attacks by cabbage flea beetles and cabbage stink bugs, cabbage genotypes with higher content of this substance are consequently more appropriate for environmentally acceptable manners of cabbage production in regions where these two groups of harmful organisms represent important factors which reduce the quality and quantity of cabbage harvest.

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3 RAZPRAVA IN SKLEPI

3.1 RAZPRAVA

V Sloveniji se vse več pridelovalcev vrtnin odloča za okolju prijaznejšo pridelavo, ki vključuje uporabo manj strupenih sintetičnih kemičnih sredstev za varstvo rastlin, manjše število škropljenj ali kar njihovo popolno opustitev. Pomembno vlogo pri odločanju za enega od teh načinov pridelave ima tudi izbira ustreznih agrotehničnih ukrepov, s katerimi lahko vplivamo na zmanjšanje škodljivosti nekaterih fitofagnih žuželk. Med pomembne agrotehnične ukrepe štejemo tudi izbiro genotipa, ti pa se med seboj razlikujejo v dolžini rastne dobe, kemičnih in mehanskih lastnostih rastlin, vsi ti in mnogi drugi dejavniki pa pogojujejo tudi odpornost rastlin na škodljive organizme. V naši raziskavi smo se osredotočili na ovrednotenje nekaterih parametrov naravne odpornosti različnih genotipov zelja na napad predstavnikov dveh rodov škodljivih žuželk, da bi s tem pridobili podatke, ki bi bili uporabni v okoljsko sprejemljivih načinih pridelovanja kapusnic.

Preučevanje antiksenoze različnih genotipov zelja na napad odraslih osebkov kapusovih bolhačev (*Phyllotreta* spp.) in odraslih osebkov in ličink kapusovih stenic (*Eurydema* spp.) je potekalo med leti 2006 in 2011, kjer smo v različno zastavljenih poskusih ovrednotili delovanje (učinkovitost) izbranih parametrov naravne odpornosti zelja.

Poljski poskus (v letih 2010 in 2011), na katerem je temeljila naša raziskava, dokazuje, da se preferenca kapusovih bolhačev do posameznih genotipov zelja razlikuje. To sta sicer v eni od prehodnih sorodnih raziskav že potrdila Bohinc in Trdan (2012). Kot navajajo Trdan in sod. (2008), Toshova in sod. (2009) ter Bohinc in Trdan (2013) se kapusovi bolhači (*Phyllotreta* spp.) na omenjenem geografskem območju začnejo pojavljati že v drugi polovici maja. V naši raziskavi je bil v letih 2010 in 2011 obseg poškodb na listih zelja zaradi hranjenja odraslih osebkov kapusovih bolhačev najvišji na zgodnjih in srednje zgodnjih genotipih. Večjo dovzetnost zgodnjih genotipov za poškodbe kapusovih bolhačev v svoji raziskavi potrjuje tudi Bohinčeva (2013). Odrasli osebki kapusovih bolhačev so bili v naši raziskavi najštevilčnejši v drugi polovici julija, kar so v preteklih raziskavah ugotavljali tudi Trdan in sod. (2008) ter Bohinc in Trdan (2013). Omenjeni rezultati nam omogočajo potrditev naše prve hipoteze.

V naši raziskavi je bil v omenjenem časovnem intervalu v letu 2010 obseg poškodb največji pri vseh preučevanih skupinah genotipov zelja, hibridih generalno (3,13±0,1), sortah generalno (3,23+0,11), zgodnjih genotipih (3,19+0,08), srednje zgodnje genotipih (3,27+0,11) in srednje poznih genotipih (3,11+0,09). V letu 2011 smo v drugi polovici julija, tako v skupinah, kjer smo upoštevali hibride generalno (3,15+0,06) kot tudi v skupini, kjer smo upoštevali vse sorte (3,28±0,11), v skupini zgodnjih genotipov (3,18+0,08), srednje zgodnjih genotipov (3,27+0,11) in srednje-poznih genotipov (2,90±0,1) ugotovili največji obseg poškodb.

Gospodarski prag škodljivosti je bil v prvem letu poskusa prvič presežen v sredini junija (Jun 2) na zgodnjih genotipih zelja, ko so bile rastline v fazi razvijanja vegetativnih delov ustreznih za pridelek. Hibrid 'Sunta F1' je bil v omenjenem obdobju med zgodnjimi genotipi najbolj dovzeten za poškodbe kapusovih bolhačev. Glede na to, da so kapusovi bolhači največkrat navedeni kot gospodarsko pomembni škodljivci mladih rastlin kapusnic (Andersen in sod., 2006; Tansey in sod., 2009), vpliva poškodb na rast rastlin omenjenega genotipa nismo ugotovili.

Znano je, da višja povprečna dnevna temperatura stimulativno vpliva na razvoj preučevane skupine škodljivcev (Toshova in sod., 2009; Bohinc in Trdan, 2013). Povprečna dnevna temperatura je v omenjenem časovnem intervalu znašala 20,7°C (Arhiv …, 2014), kar je bilo v primerjavi z istim obdobjem v letu 2011 (19,6°C) bistveno topleje. Povprečne dnevne temperature so bile v obeh letih poskusa najvišje v juliju (Jul 1 – Jul 2), kar sovpada z najvišjim obsegom poškodb kot tudi preseženim gospodarskim pragom škodljivosti.

Obdobje, kjer je bil gospodarski prag škodljivosti znova dosežen, se znova pojavi v juliju (obdobje Jul 1 in Jul 2). Medtem, ko generalno gledano, poškodbe na zgodnjih genotipih v obeh letih poskusa v prvi dekadi julija niso bile gospodarsko pomembne, pa je obseg poškodb na srednje poznih genotipih, 'Kranjsko' (leto 2010: 3,00±0,00; leto 2011: 3,00±0,00), 'Ljubljansko' (leto 2010: 3.00±0.00; leto 2011: 3,00±0,00) in 'Varaždinsko' (leto 2010: 3,00±0,00; leto 2011: 3,25±0,11) presegel gospodarski prag škodljivosti. V obdobju Jul 1 in Jul 2 so se zgodnji genotipi že bližali tehnološki zrelosti oziroma so že bili v tehnološki zrelosti, medtem ko so bili srednje pozni genotipi v fazi razvijanja vegetativnih delov ustreznih za pridelek, in s tem bolj dovzetnosti za prehranjevanje (Bohinc in Trdan, 2012, 2013).

Prešernova (2011) v njeni magistrski nalogi navaja, da sta bili v njeni večletni raziskavi za prehranjevanje hroščev iz rodu *Phyllotreta* najbolj občutljivi sorti 'Varaždinsko' in 'Futoško'. Podobno ugotavljata Tenczar in Krischik (2007), ki navajata, da so kapusovi bolhači veliko bolj dovzetni za sorte, ki se v pridelavi pojavljajo dlje. Rezultati naše raziskave kažejo, da so bili ob prvem pojavu kapusovih bolhačev v začetku rastne dobe napadu najbolj izpostavljeni zgodnja genotipa 'Sunta F1' in 'Destiny F1' in srednje zgodnja genotipa 'Futoško' in 'Fieldforce F1', medtem ko smo obsežnejše poškodbe v juliju ugotovili na srednje pozni sorti 'Varaždinsko'.

Z našo raziskavo smo na seznam dejavnikov naravne odpornosti zelja (vsebnost epikutikularnega voska (Trdan in sod., 2009), glukozinolatov (Bohinc in sod., 2012) poskušali uvrstiti tudi antioksidativni potencial. Kapusove stenice (*Eurydema* spp.), ki veljajo za univoltilne vrste (Trdan in sod., 2006a), so se na območju naše raziskave prvič pojavile v zadnji dekadi maja oziroma prvi dekadi junija, kar je v skladu s preteklimi trditvami trditvami Trdana in sod. (2006a) ter Bohinčeve in Trdana (2012). Razlike v obsegu poškodb zaradi sesanja kapusovih stenic so bile med rdečimi in belimi genotipi zelja najbolj očitne v obdobju prvega pojava škodljivcev. Tako je bil v letu 2010 v prvem terminu ocenjevanja (May 3) obseg poškodb na rdečih genotipih zelja 1,17±0,06, v drugem letu poskusa pa so se poškodbe kapusovih stenic na rdečih genotipih zelja pojavile šele v tretjem terminu ocenjevanja (June 2). V prvem terminu ocenjevanja v letu 2011 nismo ugotovili poškodb niti na belih genotipih zelja.

Časovno obdobje, v katerem je obseg poškodb na belih genotipih večji kot na rdečih, je v obeh letih poskusa razpotegnjeno od prve dekade junija (June 1) do zadnje dekade istega meseca (June 3). Obseg poškodb na izbranih rdečih genotipih zelja ('Erfurtsko', 'Red dinasty F1' in 'Holandsko') v omenjenem časovnem intervalu ni presegel 1 % poškodovane listne površine.

Dosedanje raziskave kot pomemben dejavnik občutljivosti zelja na napad škodljivih žuželk navajajo tudi dolžino rastne dobe posameznih genotipov (Bohinc in Trdan, 2011). Naša raziskava vpliva dolžine rastne dobe na dovzetnost zelja na napad kapusovih stenic ni potrdila, medtem ko se dovzetnost genotipov za poškodbe kapusovih bolhačev glede na dolžino rastne dobe spreminja. Izrazite spremembe v naraščanju obsega poškodb smo v obeh letih poskusa opazili v drugi dekadi julija, kar sovpada s pojavom prvega rodu odraslih osebkov vrst *Eurydema ventrale* in *Eurydema oleracea* (Bohinc in Trdan, 2012). V letu 2010 je bil v omenjenem časovnem intervalu obseg poškodb na listih skoraj identičen na belih (2,55±0,08) in rdečih genotipih (2,55±0,08), medtem ko smo v naslednjem letu na rdečih genotipih zelja (2,98±0,15) ugotovili nižji obseg poškodb kot na belih genotipih (3,11±0,07). Če povzamemo, je bil obseg poškodb zaradi kapusovih stenic v letu 2010 med najnižjimi na zgodnji rdeči sorti, 'Erfurtsko rdeče' (1,75±0,11), medtem ko je bil v letu 2011 obseg poškodb najmanjši na genotipu 'Erfurtsko rdeče' (1,58±0,10). Naše četrte hipoteze zato ne moremo v celoti potrditi.

Glede na to, da so antocianini tisti dejavnik, ki daje barvo rdečim genotipom zelja (Soengas in sod., 2011), lahko domnevamo, da so omenjeni pigmenti v naši raziskavi v največji meri določali antioksidativni potencial zelja (Li in sod., 2012). Manjšo oviro v zgornji trditvi e med rastno dobo. Vendar pa na drugi strani Ribera in sod. (2010) za vrsto *Vaccinium co*predstavlja dejstvo, da smo antioksidativni potencial analizirali le ob tehnološki zrelosti in n*rymbosum* navajajo, da je v njej antioksidativnii potencial najvišji ravno v tehnološki zrelosti. Ena od novejših raziskav je pokazala, da je izbira genotipa (Peńas in sod., 2012) pomemben dejavnik, od katerega je odvisna predvsem vsebnost bioaktivnih substanc, tudi antocianinov.

Rezultati naše raziskave, kjer smo v zelju analizirali vrednost antioksidativnega potenciala ob tehnološki zrelosti, kažejo na to, da je vsebnost teh bioaktivnih substanc v preučevani vrtnini visoka tudi ob koncu rastne dobe. Zelje na splošno uvrščamo v tisto skupino vrtnin, ki zaradi posebne kemične sestave pozitivno vpliva na človeško zdravje.

Že Lee in sod. (2007) ter Soengas in sod. (2011) navajajo, da je antioksidativni potencial najvišji v rdečih genotipih zelja, kar velja tudi za našo raziskavo. Naša tretja hipoteza, da je antioksidativni potencial različen med posameznimi genotipi, tako drži. Po vrednosti antioksidativnega potenciala sta izstopali sorti 'Erfurtsko' (0,75±0,09 mmol/100 g) in 'Holandsko pozno' (0,70±0,04 mmol/100 g). Omenjeni sorti zelja se v pridelovalnih sistemih zelja v Sloveniji pojavljajo že precej časa. Tako lahko poleg vpliva antioksidativnega potenciala na škodljive organizme izpostavimo vpliv na ljudi, ki pa je definitivno pozitiven (Medoua in sod., 2009). Po vrednosti antioksidativnega potenciala izstopata hibrida 'Autumn queen F1' (0,33±0,03 mmol/100 g) in 'Delphi F1' (0,29±0,04 mmol/100 g), ki pa veljata za novejša bela genotipa zelja.

Masa epikutikularnega voska in njegov vpliv na obseg poškodb kapusovih bolhačev in kapusovih stenic sta bili predmet naše raziskave v letih 2006 in 2008. Ugotovili smo, da je obseg poškodb kapusovih bolhačev različen glede na hibrid/sorto. V obeh letih smo ugotovili večjo dovzetnost hibridov za poškodbe teh gospodarsko pomembnih žuželk. V povprečju smo v letu 2006 na hibridih zelja določili indeks poškodb 1,46±0,02, medtem ko je bil ta v letu 2008 2,03±0,11. V obeh letih poskusa smo najvišjo dovzetnost za prehranjevanje kapusovih bolhačev ugotovili pri hibridu 'Cheers F1'. V letu 2006 je bil povprečni indeks poškodb na tem hibridu 1,68±0,05, v letu 2008 pa 2,87±0,13. Na genotipu 'Cheers F1' smo v letih 2006 in 2008 ugotovili tudi največji obseg poškodb zaradi kapusovih stenic. Tako smo v prvem letu poskusa na omenjenem genotipu zelja določili povprečni indeks poškodb 2,31±0,10, v letu 2008 pa 1,13±0,19.

Rezultati naše raziskave kažejo, da je v prvem letu poskusa vsebnost epikutikularnega voska na listih zelja negativno vplivala na prehranjevanje preučevanih skupin škodljivcev. S tem smo potrdili našo drugo hipotezo. Tako smo v prvem letu poskusa ugotovili, da obstajajo signifikatno zelo močne negativne korelacije med obsegom poškodb vrst iz rodu *Eurydema* in vsebnostjo epikutikularnega voska pri genotipih 'Hinova F1' (r=-0,98), 'Varaždinsko' (r=-0,97) in 'Vestri F1' (r=-0,99). Zelo močno signifikantno negativno korelacijo med obsegom poškodb kapusovih bolhačev in vsebnostjo epikutikularnega voska pa smo ugotovili še pri genotipih 'Red dinasty F1' (r=-0,99) in 'Varaždinsko' (r=-0,95).

Analiza rezultatov, pridobljenih v letu 2008, prav tako kaže na negativen vpliv vsebnosti epikutikularnega voska na prehranjevanje žuželk iz rodov *Euydema* in *Phyllotreta* na listih zelja. Signifikanten negativni vpliv vsebnosti voska na prehranjevanje kapusovih stenic (r=-0,89) in kapusovih bolhačev (r=-0,99) smo ugotovili pri hibridu 'Red dinasty F1'. Izrazit negativen vpliv (r=-0,85) vsebnosti epikutikularnega voska smo nadalje ugotovili tudi pri sorti 'Varaždinsko'. Povezave med obsegom poškodb vrst iz rodu *Eurydema* in vsebnostjo epikutikularnega voska v letu 2008 pri hibridu 'Cheers F1' nismo ugotovili, prav tako kot tudi nismo ugotovili povezave med obsegom poškodb kapusovih bolhačev in vsebnostjo epikutikularnega voska.

Raziskava, ki smo jo izvajali med leti 2006 in 2008, vpliva dolžine rastne dobe izbranih osmih genotipov zelja na obseg poškodb vrst iz rodov *Phyllotreta* in *Eurydema* ni potrdila. Z analizo naših rezultatov ne moremo nedvoumno izpostaviti genotipe zelja glede na dolžino rastne dobe, ki bi po obsegu poškodb zaradi kapusovih bolhačev izstopali, kot to navajajo nekatere minule raziskave (Trdan in sod., 2005b, 2009; Bohinc in Trdan, 2012; Bohinc in sod., 2013). Dobro pa smo preučili preferenco kapusovih stenic do izbranih genotipov zelja. V naši raziskavi so bo večjem obsegu poškodb izstopali srednje zgodnji genotipi, kar ni v skladu z dosedanjimi raziskavami (Trdan in sod., 2006a). Razloge za to lahko iščemo v drugih dejavnikih naravne odpornosti (vsebnost glukozinolatov, flavonoidov,….), ki se pogojeni z delovanjem biotskih in abiotskih dejavnikov (Li in sod., 2013; Bohinc in sod., 2013).

Kot navajajo že Trdan in sod. (2004, 2009) in kot ugotavljamo v naši raziskavi, se masa epikutikularnega voska med genotipi zelja razlikuje. Po večji masi so tako v naši raziskavi izstopali rdeči genotipi zelja, signifikatno največjo vsebnost voska pa smo ugotovili pri genotipih 'Holandsko', 'Erfurtsko' in 'Red dinasty F1'. Masa epikutikularnega voska je bila v obeh letih poskusa najnižja pri belem genotipu 'Cheers F1', kar lahko povežemo z največjo dovzetnostjo za poškodbe v obeh letih poskusa oziroma izrazito najvišjim obsegom poškodb.

3.2 SKLEPI

Med leti 2006 in 2011 smo v različnih poljskih poskusih ugotavljali pomen nekaterih dejavnikov antiksenoze na obseg poškodb kapusovih bolhačev (*Phyllotreta* spp.) in kapusovih stenic (*Eurydema* spp.) na 20 različnih genotipih zelja. Izbrane genotipe zelja smo med seboj primerjali iz različnih vidikov, in sicer: primerjava med sorto in hibridom, primerjava med rdečimi in belimi genotipi, primerjava med zgodnjimi, srednje zgodnjimi in srednje poznimi genotipi. Tako je poskus potekal na 14 hibridih (zgodnji: 'Pandion F1', 'Sunta F1', 'Delphi F1', 'Tucana F1', 'Ixxion F1', 'Autumn queen F1', 'Destiny F1', 'Green rich F1'; srednje zgodnji: 'Red dinasty F1', 'Cheers F1', Fieldforce F1', 'Vestri F1'; srednje-pozni: 'R1-Cross F1', 'Hinova F1') in 6 sortah (zgodnja: 'Erfurtsko rdeče'; srednje zgodnje: 'Futoško'; srednje pozne: 'Kranjsko okroglo', 'Ljubljansko', 'Holandsko rdeče', 'Varaždinsko') zelja.

Poškodbe kapusovih bolhačev smo v letih 2010 in 2011 ugotovili že ob našem prvem ocenjevanju v zadnji dekadi maja. Omenjene ugotovitve se skladajo z znanimi informacijami o bionomiji preučevane skupine škodljivcev pri nas. Poškodbe vrst iz rodu *Phyllotreta* smo v prvem terminu ocenjevanja opazili na vseh izbranih genotipih, vendar je bil med njimi obseg poškodb različen. Pri mladih rastlinah (BBCH 19-41) smo v obeh letih poskusa najnižji obseg poškodb ugotovili na sorti 'Erfurtsko rdeče'. Gospodarski prag škodljivosti je bil prvič presežen na zgodnjih genotipih v fazi razvijanja vegetativnih delov ustreznih za pridelek. Ugotavljamo, da je dolžina rastne dobe posameznih genotipov zelja pomemben dejavnik dovzetnosti te vrtnine na napad kapusovih bolhačev.

Že v predhodnih prispevkih avtorji poročajo o tem, da je številčnost kapusovih bolhačev in s tem posledično obseg poškodb najvišji v drugi polovici julija, kar smo z našo raziskavo samo še nadgradili. Ugotavljamo, da kapusovi bolhači s prehranjevanjem ne povzročajo gospodarsko pomembnih posledic. Znano namreč je, da so kapusovi bolhači predvsem pomembni škodljivci mladih rastlin zelja. Rezultati naše raziskave izpostavljajo dejstvo, da so rastline nižjih razvojnih stadijev zelja občutljivejše na napad kapusovih bolhačev, kot tudi to, da se obseg poškodb med rastno dobo povečuje. Kapusove stenice so se v naši raziskavi prvič pojavile v začetku junija, ugotavljamo pa, da je izbira genotipa zelja pomemben dejavnik pri zmanjševanju obsega poškodb zaradi kapusovih stenic. Primerjava obsega poškodb med belimi in rdečimi genotipi je pokazala, da je bil v času, ko so se kapusove stenice prvič pojavile, obseg poškodb višji na belih genotipih. Ugotavljamo, da se je sorta 'Erfurtsko rdeče' v obeh letih poskusa (2010 in 2011) izkazala za najmanj dovzetno za poškodbe kapusovih bolhačev. Dolžina rastne dobe genotipov zelja se ni pokazala za pomemben dejavnik naravne odpornosti zelja na napad vrst iz rodu *Eurydema* spp. V vseh letih raziskava se je barva posameznih genotipov pokazala kot pomembnejši dejavnik naravne odpornosti zelja.

Vsebnost antioksidativnega potenciala je pogojena tudi z barvo genotipov. Višje vrednosti smo ugotovili pri rdečih genotipih. Rastlinska barvila (antocianini) so samo ene izmed substanc, ki določajo antioksidativni potencial. Uporabljena metoda analize s pomočjo radikala DPPH* je pokazala, da je omenjena vrednost veliko višja v rdečih genotipih. Z višanjem anitoksidativnega potenciala se znižuje obseg poškodb *Euydema* spp.. Kar smo ugotovili tudi v naši raziskavi.

Analiza površine epikutikularnega voska je pokazala, da so znova rdeči genotipi zelja tisti, ki izstopajo. Signifikatno večje vrednosti smo v obeh letih poskusa pridobili na rdečih genotipih. Splet različnih parametrov naravne odpornosti dokazuje, da so rdeči genotipi veliko bolj odporni na poškodbe stenic in bolhačev. Težko bi rekli, da je naravna odpornost rastlin pogojena samo z vsebnostjo epikutikularnega voska. Celoten del sestavljanke tvori tako vsebnost epikutikularnega voska, vsebnost antioksidantov, itd…Poleg vpliva na škodljive organizme, pa ima večja vsebnost antioksidantov v zelju tudi pozitiven vpliv na prehrano človeka.

Naša raziskava ugotavlja, da vsebnost epikutikularnega voska v genotipih zelja (na njihovih vehah) pomemben dejavnik naravne odpornosti rastlin. Da za določen genotip velja, da je njegova odpornost pogojena samo z površino epikutikularnega voska je malo verjetno. Z veliko verjetnostjo težje izpostavimo tisti genotip zelja, katerega odpornost je pogojena samo s povoščenostjo listov. Splet preostalnih dejavnikov antiksenoze dovoljuje trditev, da naravna odpornost rastlin predstavlja pomemben dejavnik v tržno ekološko naravnanih sistemih pridelovanja kapusnic. Kljub temu, da nekatere raziskave poročajo o stimulativnem vplivu epikutikularnega voska na prehranjevanje; pa naša raziskava potrjujejo negativen vpliv. Dokazali smo, da je izbira sorte pomemben dejavnik, ki omogoča ob upoštevanju preostalih alternativnih metod v varstvu kapusnic uspešen nadzor populacije kapusovih bolhačev in kapusovih stenic.

4 POVZETEK (SUMMARY)

4.1 POVZETEK

Med leti 2006 in 2011 smo v več poljskih poskusih preučevali naravno odpornost zelja (*Brassica oleracea* var. *capitata*) na napad predstavnikov dveh rodov škodljivih žuželk, kapusovih bolhačev (*Phyllotreta* spp.) in kapusovih stenic (*Eurydema* spp.). V poskusih, ki so bili izvedeni na Laboratorijskem polju Biotehniške fakultete v Ljubljani, smo posadili 20 genotipov zelja. V letih 2006 in 2008 smo preučevali pomen epikutikularnega voska kot dejavnika naravne odpornosti zelja na napad predstavnikov obeh rodov škodljivih žuželk, v letih 2010 in 2011 pa odpornost genotipov zelja na napad kapusovih bolhačev v poljskih razmerah in razlike v antioksidativnem potencialu različnih genotipov zelja kot dejavnika odpornosti na napad kapusovih stenic. Da bi lažje ovrednotili obseg poškodb preučevanih skupin škodljivcev in razlike med njimi smo preučevane genotipe zelja razdelili v 3 skupine, in sicer glede na dolžino rastne dobe, to je glede na čas od sajenja do tehnološke zrelosti. V poskus smo vključili 14 hibridov in 6 sort, ki so pripadali tako belim kot rdečim genotipom.

V poskus smo vključili 8 zgodnjih hibridov ('Pandion F1', 'Sunta F1', 'Delphi F1', 'Tucana F1', 'Ixxion F1', 'Autumn queen F1', 'Destiny F1', 'Green rich F1', 4 srednje zgodnje hibride ('Red dinasty F1', 'Cheers F1', Fieldforce F1', 'Vestri F1'), dva srednje pozna hibrida ('R1-Cross F1', 'Hinova F1') in eno zgodnja sorto ('Erfurtsko rdeče'), eno srednje zgodnjo sorto ('Futoško') in 4 srednje pozne sorte ('Kranjsko okroglo', 'Ljubljansko', 'Holandsko rdeče', 'Varaždinsko'). Omenjene genotipe zelja smo posadili v 4 gredice oziroma bloke. Znotraj posameznega bloka je posamezen genotip zelja predstavljalo 9 rastlin z gostoto sajenja 8,2 rastline/m^2. Obseg poškodb na listih zelja smo v letih 2010 in 2011 ocenjevali v 10-dnevnih intervalih, v letih 2006 in 2008 pa smo izvedli dve oziroma eno ocenjevanje poškodb. Obseg poškodb na listih zelja zaradi grizenja kapusovih bolhačev smo ocenjevali s 5-stopenjsko vizualno lestvico (Guidelines …, 2002), obseg poškodb zaradi sesanja kapusovih stenic pa s 6-stopenjsko lestvico Stonerjeve in Sheltona (1988).

Rezultati naše raziskave so pokazali, da obstaja preferenca preučevanih skupin škodljivcev do posameznih genotipov zelja. Kapusovi bolhači so se začeli pojavljati že v prvem terminu ocenjevanja, tj. konec maja. V prvem terminu ocenjevanja v letu 2010 smo največji obseg poškodb zaradi grizenja kapusovih bolhačev ugotovili na zgodnjih (2,17±0,05) in srednje zgodnjih genotipih zelja (2,17±0,05), tudi v drugem letu pa smo ugotovili podobno. Gospodarski prag škodljivosti kapusovih bolhačev je bil prvič presežen v drugi dekadi junija na zgodnjih genotipih zelja, to je bilo v času, ko so omenjeni genotipi začeli razvijati vegetativne dele, ustrezne za pridelek. Preseženemu gospodarskemu pragu škodljivosti v omenjenem časovnem intervalu ne pripisujemo večjega pomena, saj je znano, da je večji obseg poškodb zaradi kapusovih bolhačev na listih zelja gospodarsko škodljivejši pri mladih rastlinah.

Naša raziskava potrjuje ugotovitve nekaterih predhodnih raziskav, da sta številčnost odraslih osebkov kapusovih bolhačev in posledično obseg poškodb največja v drugi dekadi julija. Takrat je bil gospodarski prag škodljivosti presežen tako pri zgodnjih, srednje zgodnjih in srednje poznih genotipih zelja. Omenjeno je veljalo za leti 2010 in 2011. Tako smo v letu 2010 v omenjenem časovnem intervalu na zelju generalno ugotovili obseg poškodb, ki je znašal 3,17±0,05. Na hibridih generalno je obseg poškodb znašal 3,13±0,10, na sortah generalno 3,23±0,11, na zgodnjih genotipih 3,19±0,08, na srednje-zgodnjih genotipih 3,27±0,11 in na srednje-poznih genotipih 2,98±0,05. V skupini zgodnjih genotipov smo v omenjenem časovnem intervalu v letu 2011 ugotovili obseg poškodb, ki je znašal 3,18±0,08. Obseg poškodb na srednje-poznih genotipih ni presegel povprečnega indeksa 3, kar pomeni, da je bil pri slednji skupini genotipov zelja prag gospodarske škodljivosti dosežen, ne pa presežen. Če zanemarimo razdelitev genotipov v skupine, ugotavljamo, da sta bili v obeh letih poskusa v drugi dekadi julija najbolj poškodovani dve sorti zelja, in sicer 'Varaždinsko' in 'Futoško'. Navedeni sorti sta izkazali največjo dovzetnost za kapusove bolhače tudi ob prvem ocenjevanju v obeh letih poskusa. Med genotipi, kjer smo ugotovili najnižji obseg poškodb v prvem terminu ocenjevanja lahko v obeh letih poskusa izpostavimo 'Erfurtsko' , 'Holandsko pozno' in 'Red dinasty F1'.

Naša raziskava potrjuje predhodno dokazano dejstvo, da so posamezne skupine genotipov zelja različno dovzetne za poškodbe kapusovih stenic. Ob prvem pojavu stenic (obdobje od zadnje dekade maja do prve dekade junija) smo poškodbe najprej opazili na belih genotipih zelja. Obseg poškodb kapusovih stenic na belih genotipih je v prvem terminu ocenjevanja v letu 2010 znašal 1,42±0,05, v letu 2011 pa poškodb kapusovih stenic skoraj ni bilo. V letu 2010 je bil obseg poškodb na rdečih genotipih v prvem terminu ocenjevanja znatno nižji (1,17±0,06). Naša raziskava kaže, da je lahko pomemben dejavnik dovzetnosti zelja za kapusove stenice tudi dolžina rastne dobe te vrtnine, čeprav tega v raziskavi preučevanja antiksenotičnega vpliva epikutikularnega voska, ki smo jo izvedli med leti 2006 in 2008, nismo potrdili. Domnevamo, da so v slednji raziskavi bolj do izraza prišli drugi dejavniki. Zato lahko ugotovimo, da obseg poškodb kapusovih stenic variira tako glede na dolžino rastne dobe zelja kot tudi glede na razvojni stadij te vrtnine.

Z metodo, ki temelji na DPPH* radikalu, smo v vehah zelja ob tehnološki zrelosti določevali vrednost antioksidativnega potenciala. Ugotovili smo, da se vrednosti antioksidativnega potenciala med posameznimi genotipi razlikujejo in da več dejavnikov določa vsebnost antioksidativnega potenciala. Sorte zelja, vključene v našo raziskavo, imajo veliko višjo vrednost antioksidativnega potenciala (0,59±0,03 mmol/100 g) kot hibridi (0,47±0,01 mmol/100 g). Beli genotipi zelja so imeli povprečno vrednost antioksidativnega potenciala 0,47±0,04 mmol/100 g, rdeči genotipi pa 0,67±0,03 mmol/100 g). Z rezultati naše raziskave smo potrdili, da je antioksidativni potencial pomemben dejavnik naravne odpornosti rastlin. Tako smo pri vseh obravnavanih genotipih ugotovili negativen vpliv na prehranjevanje kapusovih stenic. Vsebnost antioksidativnega potenciala je na prehranjevanje kapusovih stenic najbolj negativno vplivala na hibridu 'Red dinasty F1', kjer smo ugotovili, da je bil Spearmanov koeficient korelacije (r) -0,83.

Pri osmih genotipih zelja smo v letih 2006 in 2008 določevali maso epikutikularnega voska ob tehnološki zrelosti in njegov vpliv na prehranjevanje izbranih skupin škodljivcev. Zna podlagi rezultatov naše raziskave ugotavljamo, da je vsebnost epikutikarnega voska signifikatno najvišja na rdečih genotipih, kjer smo v povprečju v letu 2006 ugotovili 49,91±3,20 µg voska cm^{-2}, medtem ko so beli genotipi v povprečju vsebovali 33,53±2,35 µg voska cm^{-2}. V letu 2008 pa smo na belih genotipih ugotovili 29,33±2,62 µg voska cm^{-2}, na rdečih genotipih pa 38,30±2,02 µg voska/cm^{2}. V prvem letu poskusa (26,50±2,64 µg cm^{2}) in v letu 2008 (16,24±0,00 µg cm^{2}) smo najmanjšo maso epikutikularnega voska ugotovili na genotipu 'Varaždinsko'. Prav tako smo pri večini izbranih genotipov ugotovili izrazit negativen vpliv površine epikutikularnega voska na prehranjevanje kapusovih bolhačev in kapusovih stenic.

Rezultati naša raziskave dokazujejo, da sta antioksidativni potencial in epikutikularni vosek pomembna dejavnika naravne odpornosti zelja na napad kapusovih bolhačev in kapusovih stenic in da v povezavi z drugimi alternativnimi metodami varstva zelja pred škodljivimi žuželkami predstavljajo dejavnika, ki ju velja v prihodnje (pri izbiri naravno odpornejših genotipov) upoštevati v okoljsko sprejemljivih sistemih pridelovanja zelja.

4.2 SUMMARY

Between the years 2006 and 2011 we studied in several field experiments the natural resistance of cabbage (*Brassica oleracea* var. *capitata*) to attacks by the representatives of two generations of harmful insects, cabbage flea beetles (*Phyllotreta* spp.) and cabbage stink bugs (*Eurydema* spp.). In the experiments, which were carried out at the Laboratory field of the Biotechnical Faculty in Ljubljana, we planted 20 cabbage genotypes. In the years 2006 and 2008 we studied the significance of epicuticular wax as a factor of natural resistance of cabbage to attacks by the representatives of both genera of harmful insects, while in the years 2010 and 2011 we studied resistance of cabbage genotypes to attacks by cabbage flea beetles in field conditions, and differences in antioxidative potential of different cabbage genotypes as a factor of resistance to attacks by cabbage stink bugs. In order to facilitate the evaluation of the extent of damage done by the studied groups of harmful pests and differences between them, we divided the studied cabbage genotypes into 3 groups, namely in regard to the length of growth period, i.e. the time between planting and technological maturity. The experiment included 14 hybrids and 6 cultivars which were both white and red genotypes.

The experiment included 8 early hybrids ('Pandion F1', 'Sunta F1', 'Delphi F1', 'Tucana F1', 'Ixxion F1', 'Autumn queen F1', 'Destiny F1', 'Green rich F1', 4 mid-early hybrids ('Red dinasty F1', 'Cheers F1', Fieldforce F1', 'Vestri F1'), two mid-late hybrids ('R1-Cross F1', 'Hinova F1') and one early cultivar ('Erfurtsko rdeče'), one mid-early cultivar ('Futoško') and 4 mid-late cultivars ('Kranjsko okroglo', 'Ljubljansko', 'Holandsko rdeče', 'Varaždinsko'). The said cabbage genotypes were planted in 4 small beds or blocks. Individual cabbage genotypes within individual blocks were represented by 9 plants – the

density was 8.2 plants per square meter. The extent of damage on cabbage leaves was in the years 2010 and 2011 assessed at 10-day intervals, while in the years 2006 and 2008 we carried out two or one damage assessment. The extent of damage on cabbage leaves due to the biting by cabbage flea beetles was assessed by the 5-grade visual scale EPPO (Guidelines …, 2002), while the extent of damage due the sucking by cabbage stink bugs was assessed by the 6-grade scale (Stoner in Shelton, 1988).

The results of our experiment showed that the studied groups of harmful pests have preferences for individual cabbage genotypes. Cabbage flea beetles began appearing already on the first assessment date, i.e. at the end of May. On the first assessment date in 2010 the highest extent of damage due to the biting by cabbage flea beetles was detected in early (2.17±0.05) and mid-early cabbage genotypes (2.17±0.05), the findings in the second year were similar. The economic threshold of damage done by cabbage flea beetles was for the first time exceeded in the second decade of June in the early cabbage genotypes, that was when the said genotypes began developing vegetative parts suitable for harvesting. The exceeded economic threshold of damage in the said interval was not attributed any great significance, as we know that greater extent of damage done by cabbage flea beetles on cabbage is from the economic point of view more acute in young plants.

Our study confirms the findings of some earlier studies – the number of adult specimens of the cabbage flea beetle (and consequently the extent of damage) is highest in the second decade of July. At that time the economic threshold of damage was exceeded in early, mid-early and mid-late cabbage genotypes. The said held true for the years 2010 and 2011. In the said interval in 2010 we thus in general established the extent of damage on cabbage 3.17±0.05. The hybrids in general had the extent of damage 3.13±0.10, the cultivars in general had the extent of damage 3.23±0.11, the early genotypes had the extent of damage 3.19±0.08, the mid-early genotypes had the extent of damage 3.27±0.11, and mid-late genotypes had the extent of damage 2.98±0.05.

In the group of early genotypes in the said interval in 2011 we established the extent of damage 3.18±0.08. The extent of damage in mid-late genotypes did not exceed the average index 3, which means that in this group of cabbage genotypes the economic threshold of damage was reached but not exceeded. If we neglect the division of genotypes into groups, we see that in the second decade of July in both years of the experiment two cabbage cultivars were most damaged, namely 'Varaždinsko' and 'Futoško'. The said cultivars proved to be most susceptible to attacks by cabbage flea beetles also at the first assessment in both years of the experiment. Among the genotypes which displayed the lowest extent of damage at the first assessment in both years of the experiment we can point out 'Erfurtsko', 'Holandsko pozno' and 'Red dinasty F1'.

Our study confirms the previously proved fact that individual groups of cabbage genotypes are differently susceptible to damage done by cabbage stink bugs. When stink bugs first appeared (the period from the last decade of May until the first decade of June), damage was first noticed on white cabbage genotypes. The extent of damage done by cabbage stink bugs on white genotypes was at the first assessment in 2010 1.42±0.05, in 2011 there was almost no damage done by cabbage stink bugs. In 2010 the extent of damage on the red genotypes at the first assessment was considerably lower (1.17±0.06). Our study shows that an important factor of susceptibility of cabbage to cabbage stink bugs may be also the length of growth period of this garden vegetable, although our research of antixenotic influence of epicuticular wax, which was carried out between the years 2006 and 2008, did not confirm this. We suppose that in the latter research other factors were more prominent. We can thus state that the extent of damage done by cabbage stink bugs varies both with the length of growth period of cabbage and the developmental stage of this garden vegetable.

With the method based on DPPH* radical we determined the value of antioxidative potential in outer leaves of cabbage at technological maturity. We found out that the values of antioxidative potential differ between individual genotypes, and that the content of antioxidative potential is determined by several factors. The cabbage cultivars included in our study have much higher value of antioxidative potential (0.59±0.03 mmol/100 g) than hybrids (0.47±0.01 mmol/100 g). The white cabbage genotypes had the average value of antioxidative potential 0.47±0.04 mmol/100 g, while the red genotypes had 0.67±0.03 mmol/100 g). The results of our experiment confirmed that antioxidative potential is an important factor of natural resistance of plants. We thus established a negative influence on the feeding by cabbage stink bugs in all studied genotypes. The content of antioxidative potential had the strongest negative influence on the feeding by cabbage stink bugs in the hybrid 'Red dinasty F1', in which we recorded the Spearman's correlation coefficient (r) -0.83.

In the years 2006 and 2008 we determined the mass of epicuticular wax in eight cabbage genotypes at technological maturity and its influence on the feeding of the selected harmful pests. On the basis of the results of our experiment we ascertain that the content of epicuticular wax is significantly highest in red genotypes, in which we in 2006 on average established 49.91±3.20 µg of wax per cm2, while the white genotypes on average contained 33.53±2.35 µg of wax per cm2. In 2008 we measured 29.33±2.62 µg of wax per cm2 on the white genotypes, the red genotypes had 38.30±2.02 µg of wax per cm2. In the first year of the experiment (26.50±2.64 µg/cm2) and in 2008 (16.24±0.00 µg/cm2) we established the lowest mass of epicuticular wax in the genotype 'Varaždinsko'. In the majority of the selected genotypes we also detected a pronounced negative influence of epicuticular wax's surface on the feeding by cabbage flea beetles and cabbage stink bugs. The results of our research prove that antioxidative potential and epicuticular wax are important factors of natural resistance of cabbage to attacks by cabbage flea beetles and cabbage stink bugs, and that they, in combination with other alternative methods for protection of cabbage from harmful insects, represent factors which should be in future (when selecting naturally more resistant genotypes) taken into account in environmentally acceptable systems of cabbage production.

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PRILOGA A

Slikovno gradivo – lokacija in zasnova poskusa

Priloga A1: Poljski poskus na Laboratorijskem poskusu Biotehniške fakultete Univerze v Ljubljani leta 2010 (foto: J. Rupnik)

Priloga A2: Zgled zasnove poskusa v letih 2010 in 2011, ko smo v vsakem od obravnavanj v blokih posadili po 9 sadik istega genotipa zelja (foto: T. Bohinc)

Priloga A3: Hibrid Sunta F1 v poljskem poskusu na Laboratorijskem polju v Ljubljani v letu 2010 (foto: J. Rupnik)

Priloga A4: Sorta Varaždinsko v poljskem poskusu na Laboratorijskem polju v Ljubljani v letu 2010 (foto: J. Rupnik)

PRILOGA B

Slikovno gradivo – preučevane škodljive vrste in tipi poškodb

Priloga B1: Odrasel osebek kapusovega bolhača (*Phyllotreta* spp.) (foto: J. Rupnik)

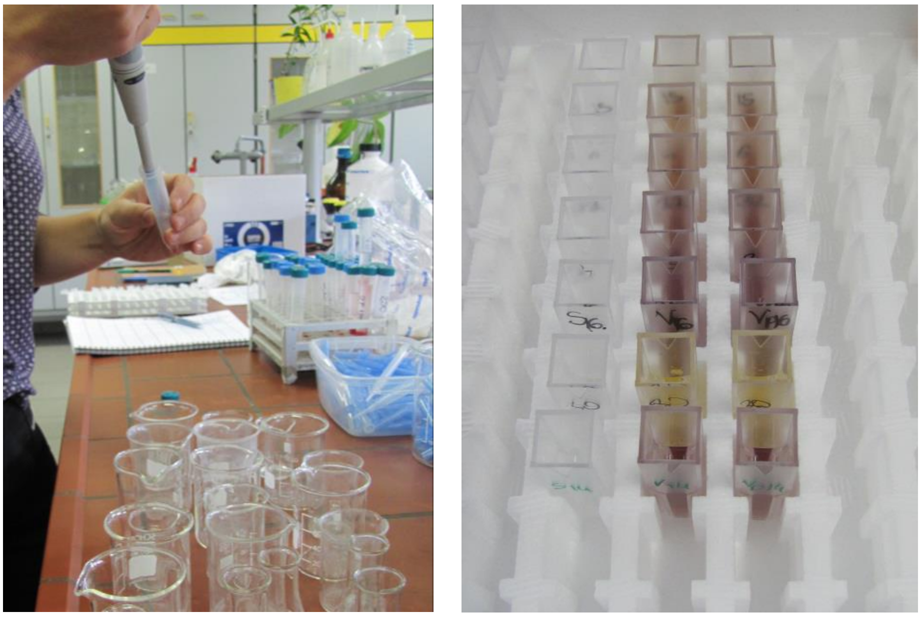
Priloga B2: Odrasli osebki kapusovih bolhačev (*Phyllotreta* spp.) pri hranjenju na listu zelja (foto: T. Bohinc)

Priloga B3: Samec (zgoraj) in samica pisane stenice (*Eurydema ventrale*) pri parjenju (foto: S. Trdan)

PRILOGA C

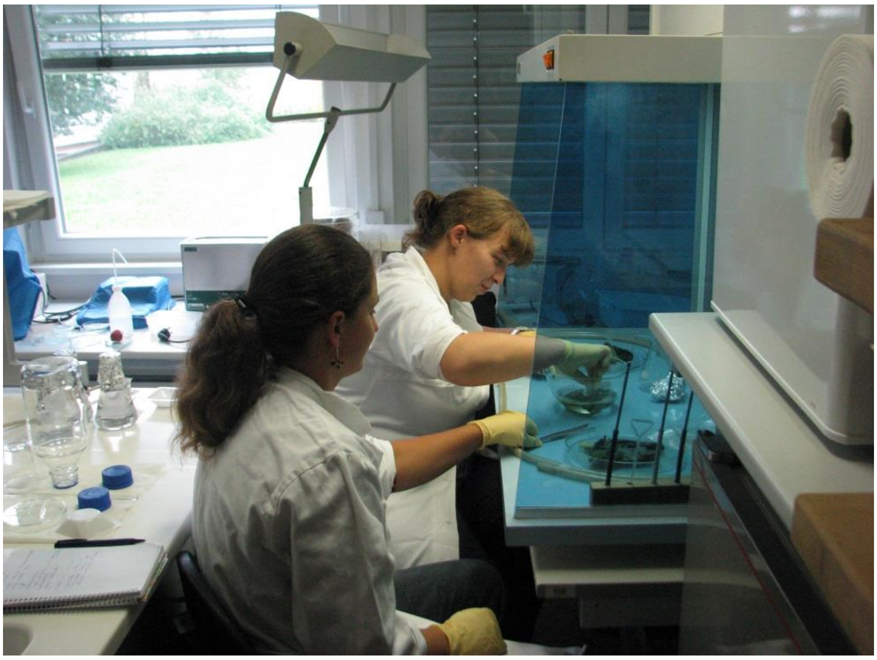
Slikovno gradivo - laboratorijsko delo

Priloga C1: Vzorci zelja namenjeni za kemične analize. 10 g svežih listov zelja smo zmešali s 4% metafosforno kislino, in vzorce shranili na -20°C (foto: T. Bohinc).



Priloga C2: Priprava vzorcev za analizo antioksidacijskega potenciala (levo) in vzorci zelja (desno), ki čakajo na merjenje absorbance pri 517 nm (foto: T. Bohinc).

Priloga C3: Merjenje absorbance vzorca pri 517 nm. Vzorec je sestavljen iz slepega vzorca, vzorca 1 in njegove ponovitve (foto: T. Bohinc).



Priloga C4: Ekstrakcija epikutikularnega voska z listov zelja v Laboratoriju za fitopatologijo Oddelka za agronomijo leta 2010 (foto: J. Rupnik).

Priloga C5: Izparevanje topila pri ekstrakciji epikutikularnega voska v rotavaporju v Laboratoriju za entomologijo Oddelka za agronomijo leta 2010 (foto: J. Rupnik).

PRILOGA D

Potrdilo, da lahko članek »Susceptibility of 20 cabbage genotypes to flea beetles attack under field conditions« uporabimo kot del doktorske disertacije, ki bo dostopna v elektronski in tiskani verziji

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LETTER OF ACCEPTANCE & PERMISSION

Dear Mr. Damir Marković,

Herewith I avail myself of the opportunity of informing you that the paper under the title:

Association between antioxidative potential and level of injury caused by *Eurydema* spp. feeding on red and white cabbage genotypes

authored by Damir Marković, Tanja Bohinc, and Stanislav Trdan has been accepted on the session (January 2014) of the Editorial Committee of the Archives of Biological Sciences, Belgrade, and will therefore be published in vol. 66, 4 (2014).

I agree with the intent that paper mentioned above will present a part of the PhD Thesis of Mr. Marković which will be published in paper and electronic version

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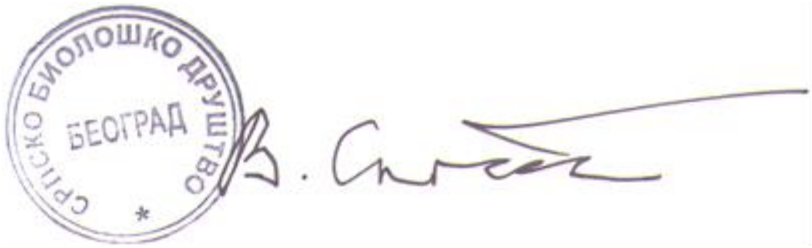

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PRILOGA F

Potrdilo, da lahko članek "Leaf epicuticular wax as a factor of antixenotic resistance of cabbage to cabbage flea beetles and cabbage stink bugs attack" uporabimo kot del doktorske disertacije, ki bo dostopna v elektronski in tiskani verziji

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