UNIVERZA V LJUBLJANI
BIOTEHNIŠKA FAKULTETA

Tanja BOHINC

INTERAKCIJE KAPUSOVIH BOLHAČEV (*Phyllotreta* spp.) IN KAPUSOVIH STENIC (*Eurydema* spp.) Z ZELJEM IN IZBRANIMI PRIVABILNIMI POSEVKI

DOKTORSKA DISERTACIJA

Ljubljana, 2013

UNIVERZA V LJUBLJANI
BIOTEHNIŠKA FAKULTETA

Tanja BOHINC

INTERAKCIJE KAPUSOVIH BOLHAČEV (*Phyllotreta* spp.) IN KAPUSOVIH STENIC (*Eurydema* spp.) Z ZELJEM IN IZBRANIMI PRIVABILNIMI POSEVKI

DOKTORSKA DISERTACIJA

INTERACTIONS OF CABBAGE FLEA BEETLES (*Phyllotreta* spp.) AND CABBAGE STINK BUGS (*Eurydema* spp.) WITH CABBAGE AND SELECTED TRAP CROPS

DOCTORAL DISSERTATION

Ljubljana, 2013

Doktorska disertacija je zaključek podiplomskega študija bioloških in biotehniških znanosti ter se nanaša na znanstveno področje agronomije. Praktični del poskusa je bil izveden na dveh lokacijah, na Laboratorijskem polju Biotehniške fakultete v Ljubljani in na njivi v vasi Zgornja Lipnica v občini Radovljica. Laboratorijski del poskusa je bil izveden v Laboratoriju za fitomedicino Katedre za fitomedicino, kmetijsko tehniko, poljedelstvo, pašništvo in travništvo, na Oddelku za agronomijo Biotehniške fakulteti ter na Oddelku za agrokemijo in pivovarstvo Inštituta za hmeljarstvo in pivovarstvo Slovenije v Žalcu.

Na podlagi Statuta Univerze v Ljubljani ter po sklepu Senata Biotehniške fakultete in Sklepa Senata Univerze z dne 8.6.2011 je bilo potrjeno, da kandidatka izpolnjuje pogoje za neposredni prehod na doktorski Podiplomski študij bioloških in biotehniških znanosti ter opravljanje doktorata znanosti s področja agronomije. Za mentorja je bil imenovan prof. dr. Stanislav Trdan.

Komisija za oceno in zagovor:

Predsednik: doc. dr. Dragan Žnidarčič
Univerza v Ljubljani, Biotehniška fakulteta, Oddelek za agronomijo

Član: prof. dr. Stanislav Trdan
Univerza v Ljubljani, Biotehniška fakulteta, Oddelek za agronomijo

Član: doc. dr. Iztok Jože Košir
Inštitut za hmeljarstvo in pivovarstvo Slovenije, Oddelek za agrokemijo in pivovarstvo, Žalec

Datum zagovora:

Naloga je rezultat lastnega raziskovalnega dela. Izjavljam, da so vsa vključena znanstvena dela identična objavljenim verzijam. Podpisana se strinjam z objavo svojega dela v polnem tekstu na spletni strani Digitalne knjižnice Biotehniške fakultete. Izjavljam, da je delo, ki sem ga oddala v elektronski obliki, identično tiskani verziji.

Tanja BOHINC

KLJUČNA DOKUMENTACIJSKA INFORMACIJA

ŠD Dd

DK UDK 632.75/.76: 635.342: 632.936.23 (043.3)

KG interakcije/kapusovi bolhači/*Phyllotreta* spp./kapusove stenice/*Eurydema* spp./zelje/poljski poskus/poškodbe/privabilni posevki/krmna ogrščica/bela gorjušica/oljna redkev/glukozinolati/naravna odpornost/mešani posevki/okoljski dejavniki/

KK AGRIS H01/H10

AV BOHINC, Tanja, univ. dipl. inž. agr.

SA TRDAN, Stanislav (mentor)

KZ SI-1111 Ljubljana, Jamnikarjeva 101

ZA Univerza v Ljubljani, Biotehniška fakulteta, Podiplomski študij bioloških in biotehniških znanosti, področje agronomije

LI 2013

IN INTERAKCIJE KAPUSOVIH BOLHAČEV (*Phyllotreta* spp.) IN KAPUSOVIH STENIC (*Eurydema* spp.) Z ZELJEM IN IZBRANIMI PRIVABILNIMI POSEVKI

TD Doktorska disertacija

OP VII, 74 str., 18 pril., 1 sl., 80 vir.

IJ sl

JI sl/en

AI Med leti 2009 in 2010 je potekal poljski poskus, kjer smo preučevali učinkovitost metode privabilnih posevkov za zmanjševanje poškodb kapusovih bolhačev (*Phyllotreta* spp.) in kapusovih stenic (*Eurydema* spp.) na glavni rastlinski vrsti, na dveh hibridih zelja. Kot privabilne posevke smo uporabili krmno ogrščico (*Brassica napus* [L.] ssp. *oleifera* f. *biennis*), belo gorjušico (*Sinapis alba* [L.]) in oljno redkev (*Raphanus sativus* [L.] var. *oleiformis*). Poskus je potekal na dveh različnih lokacijah, na Laboratorijskem polju Biotehniške fakultete v Ljubljani in na njivi v vasi Zgornja Lipnica v občini Radovljica. Obseg poškodb *Phyllotreta* spp. je bil najvišji na oljni redkvi, medtem ko so se *Eurydema* spp. najbolj intenzivno prehranjevale na krmni ogrščici. Kapusove bolhače smo najprej opazili v začetku maja, medtem ko se kapusove stenice začele pojavljati v drugi polovici maja. Ugotovili smo, da se dovzetnost rastlin za poškodbe predstavnikov rodov *Phyllotreta* in *Eurydema* med rastno dobo spreminja, kar lahko pripišemo naravni odpornosti rastlin. Ugotovili smo, da se vsebnost glukozinolatov razlikuje med posameznimi rastlinskimi vrstami, med posameznimi organi iste rastlinske vrste rastlin. Vsebnost pa je pogojena tudi z okoljskimi vplivi. Glukobrasicin je bil prisoten v vseh rastlinskih vrstah, v skoraj vseh pa je zaviral prehranjevanje kapusovih bolhačev, razen v krmni ogrščici, kjer je v tej zvezi deloval stimulativno. Ugotavljamo, da se vpliv posameznega glukozinolata na vrste iz rodov *Phyllotreta* in *Eurydema* razlikuje med posameznimi rastlinskimi vrstami. Zaradi variabilnosti glukozinolatov, kot tudi različne preference preučevanih skupin škodljivcev do obravnavanih rastlinskih vrst, kot možnost izrabe glukozinolatov v varstvu rastlin izpostavljamo uporaba mešanih privabilnih posevkov križnic. S tem bi omogočili širšo dostopnost metode v različnih pridelovalnih sistemih in na geografsko različnih lokacijah.

KEY WORDS DOCUMENTATION

DN Dd

DC UDC 632.75/.76: 635.342: 632.936.23 (043.3)

CX interactions/cabbage flea beetles/*Phyllotreta* spp./cabbage stink bugs/*Eurydema* spp./field experiment/injuries/trap crops/oil rape/white mustard/oil radish/oil radish/glucosinolates/natural resistance/mixed cropping/environmental factors

CC AGRIS H01/H10

AU Bohinc, Tanja

AA Trdan, Stanislav (supervisor)

PP SI-1111 Ljubljana, Jamnikarjeva 101

PB University of Ljubljana, Biotechnical Faculty, Postgraduate study program of Biological and Biotechnical Sciences, Field: Agronomy

PY 2013

PI INTERACTIONS OF CABBAGE FLEA BEETLES (*Phyllotreta* spp.) and CABBAGE STINK BUGS (*Eurydema* spp.) WITH CABBAGE AND SELECTED TRAP CROPS

DT Doctoral dissertation

NO VII, 74 p., 18 ann., 1 fig., 80 ref.

LA sl

AL sl/en

AB Between 2009 and 2010 we carried out a field experiment studying the effectiveness of a trap crop method for reducing the damage done by cabbage flea beetles (*Phyllotreta* spp.) and cabbage stink bugs (*Eurydema* spp.) on the main crops, which were two cabbage hybrids. Oil rape (*Brassica napus* [L.] ssp. *oleifera* f. *biennis*), white mustard (*Sinapis alba* L.) and oil radish (*Raphanus sativus* [L.] var. *oleiformis*) were used as trap crops. The experiment was carried out at two different locations – at the Laboratory field of the Biotechnical Faculty in Ljubljana and at a field in the village Zgornja Lipnica in the municipality Radovljica. The extent of damage done by cabbage flea beetles was highest on oil radish, while cabbage stink bugs most frequently fed on oil rape. Cabbage flea beetles were in our study first noticed in the beginning of May, while cabbage stink bugs began appearing in the second half of May. Susceptibility of plants to damage done by *Phyllotreta* spp. and *Eurydema* spp. during the growth period varies. The reason that susceptibility of plants varied during the growth period can be explained by natural resistance of plants. Among the parameters of natural resistance on which our study focused are also glucosinolates. Our study found out that the content of glucosinolates differs between individual plant species, as well as between individual plant organs. The content of glucosinolates is conditioned also by environmental influences. Among the analysed glucosinolates, glucobrassicin was present in all plant species. In almost all plant species it inhibited the feeding of cabbage flea beetles, except in oil rape, where it had stimulative effects. We have established that the influence of individual glucosinolate on *Phyllotreta* spp. and *Eurydema* spp. is not identical as it differs between individual plant species. Because of the variability of glucosinolates, as well as different preferences of the studied groups of harmful pests in regard to the said plant species, one of the options is the application of mixed crops. Thus we would enable wider accessibility of the method in different systems of production, as well as at geographically different locations

KAZALO VSEBINE

		str.
	Ključna dokumentacijska informacija (KDI)	III
	Key words documentation (KWD)	IV
	Kazalo vsebine	V
	Kazalo prilog	VI
	Kazalo slik	VII
1	**UVOD**	1
2	**ZNANSTVENI ČLANKI**	7
2.1	POVEZAVA MED KONCENTRACIJO GLUKOZINOLATOV IN OBSEGOM POŠKODB KAPUSOVIH STENIC (*Eurydema* spp., Heteroptera: Pentatomidae) NA RAZLIČNIH KRIŽNICAH	7
2.2	VSEBNOST GLUKOZINOLATOV KOT OBRAMBNI MEHANIZEM KRIŽNIC PRED NAPADOM KAPUSOVIH BOLHAČEV	16
2.3	VPLIV OKOLJSKIH DEJAVNIKOV NA VSEBNOST GLUKOZINOLATOV (Brassicaceae)	23
2.4	UPORABA PRIVABILNIH POSEVKOV Z NAMENOM ZMANJŠANJA POŠKODB KAPUSOVIH STENIC (*Eurydema* spp.) in KAPUSOVIH BOLHAČEV (*Phyllotreta* spp.) NA BELEM ZELJU: DEJSTVO ALI FANTAZIJA?	28
2.5	GLUKOZINOLATI V VARSTVU RASTLIN - PREGLEDNI ČLANEK	35
2.6	ZA ZMANJŠEVANJE POŠKODB KAPUSOVIH BOLHAČEV (*Phyllotreta* spp.) NA ZELJU SE PRIPOROČA SETEV MEŠANIH PRIVABILNIH POSEVKOV KRIŽNIC	44
3	**RAZPRAVA IN SKLEPI**	53
3.1	RAZPRAVA	53
3.2	SKLEPI	59
4	**POVZETEK (SUMMARY)**	62
4.1	POVZETEK	62
4.2	SUMMARY	65
5	**VIRI**	68
	ZAHVALA	
	PRILOGE	

KAZALO PRILOG

Priloga A1: Parjenje kapusovih stenic (*Eurydema oleracea*) na cvetu bele gorjušice (foto: T. Bohinc)
Priloga A2: Poškodbe kapusovih bolhačev (*Phyllotreta* spp.) na oljni redkvi (foto: T. Bohinc)
Priloga A3: Ličinka (levo) in odrasel osebek (desno) pisane stenice (*Eurydema ventrale*) (foto: T. Bohinc)
Priloga A4: Poškodbe kapusovih stenic (*Eurydema* spp.) na krmni ogrščici v letu 2010 na njivi na Gorenjskem (foto: T. Bohinc)
Priloga A5: Poškodbe kapusovih bolhačev (*Phyllotreta* spp.) na listu oljne redkve (foto: T. Bohinc)
Priloga B1: Poljski poskus na njivi na Gorenjskem v letu 2010 (foto: T. Bohinc)
Priloga B2: Poljski poskus na Laboratorijskem polju Biotehniške fakultete v Ljubljani (foto: T. Bohinc)
Priloga C1: Vzorec cvetov bele gorjušice, namenjen liofiliziranju, in posledično analizi glukozinolatov (foto: T. Bohinc)
Priloga D1: Dovoljenje, da je članek »Association between glucosinolate concentration and injuries caused by cabbage stink bugs Eurydema spp. … on different Brassicas« lahko del doktorske disertacije, ki bo predstavljena v papirnati in elektronski obliki.
Priloga E1: Dovoljenje, da je lahko članek »Glucosinolates as arsenal for defending Brassicas against cabbage flea beetles attack« del doktorske disertacije, ki bo predstavljena v elektronski in papiranati oblikI

Priloga F1: Prošnja, naslovljena na WFL Publisher, ki se nanaša na distribucijo članka »Environmental factors affecting the glucosinolate content in Brassicaceae« v okviru doktorske naloge
Priloga F2: Prošnja, naslovljena na WFL Publisher, ki se nanaša na distribucijo članka »Trap crops for reducing damage caused by cabbage stink bugs (*Eurydema* spp.) and flea beetles (*Phyllotreta* spp.) on white cabbage: fact or fantasy?« v okviru doktorske naloge
Priloga F3: Dovoljenje, ki se navezuje na članka, omenjena v prošnjah v Prilogi G1 in Prilogi G2.
Priloga G1: Dovoljenje, da lahko članek »Glucosinolates in plant protection strategies« uporabimo v doktorski nalogi, ki bo dostopna v elektronski in papirnati verziji
Priloga H1: Prošnja naslovljena na založbo »Taylor & Francis«, v zvezi z uporabo članka »Sowing mixtures of Brassica trap crops is recommended to reduce *Phyllotreta* beetles injury to cabbage«, kot del doktorske naloge v elektronski in papirnati obliki
Priloga H2: Odgovor založbe »Taylor & Francis, v zvezi s prošnjo v Prilogi I1.
Priloga H3: Odgovor založbe »Taylor & Francis, v zvezi s prošnjo v Prilogi I1.
Priloga H4: Odgovor založbe »Taylor & Francis, v zvezi s prošnjo v Prilogi I1.

KAZALO SLIK

str.

Slika 1: Strukturne formule glukozinolatov, ki smo jih analizirali v naši raziskavi 6

1 UVOD

Skrb za okolje ima pri zmanjševanju številčnosti škodljivih žuželk, med katere uvrščamo tudi kapusove stenice (*Eurydema* spp.) in kapusove bolhače (*Phyllotreta* spp.), ki jih obravnavamo v pričujoči disertaciji, vedno večji pomen. V tej zvezi se v zadnjih desetih letih na različnih območjih sveta (Osakabe in Honda, 2002; Soroka in sod., 2005; Hummel in sod., 2009; Olson in sod., 2012; Wallingford in sod., 2013) izvajajo raziskave okoljsko sprejemljivih načinov njihovega zatiranja, z namenom implementacije takšnih načinov v pridelavo živeža. Nujnost razvoja okoljsko sprejemljivih načinov zatiranja kapusovih bolhačev in kapusovih stenic v Sloveniji pogojuje tudi dejstvo, da za zatiranje kapusovih stenic nimamo registriranega insekticida, medtem ko lahko kapusove bolhače zatiramo le z insekticidi na podlagi lambda-cihalotrina, alfa-cipermetrina in azadirahtina A (Seznam registriranih …, 2013).

Zaradi obrambnih mehanizmov rastlin se škodljivi organizmi velikokrat pojavljajo na samo določenih delih rastlin. Rastline se pred svojimi napadalci branijo na različne načine. Nekaterim rastlinam pri obrambi pred škodljivci pomaga odebeljena kutikula, druge rastline varuje epikutikularni vosek na povrhnjici (Stoner, 1992a), spet tretje rastline kot obrambni mehanizem uporabljajo trihome (Stoner, 1992b; Tian in sod., 2012), trne, bodice in druge dele rastlinskih organov (Harrewijn in sod., 1995). Lahko pa rastline v namen obrambe pred škodljivimi organizmi izkoriščajo tudi sekundarni metabolite (Hadacek, 2002).

Rastlinske vrste, ki smo jih vključili v naše poskuse, uvrščamo v družino križnic. Med križnice (Brassicaceae) uvrščamo 3200 rastlinskih vrst, ki so po svoji uporabnosti lahko krmne rastline, zelenjadnice, okrasne rastline ali pa le pleveli (Ahuja in sod., 2010). Med križnicami, ki jim pripisujemo zelo velik pomen v pridelavi živeža so zelje (*Brassica oleracea* L. var. *capitata* L.), cvetača (*Brassica oleracea* L. var. *botrytis* Alef.), brokoli (*Brassica oleracea* L. convar. *botrytis* L. var. *italica* Plenk), brstični ohrovt (*Brassica olearacea* L. var. *gemmifera* D.C.) in še nekatere druge (Osvald in Kogoj-Osvald, 2005; Ahuja in sod., 2010).

Med zelenjadnicami, ki jih v Sloveniji pridelujemo na 1498 ha, je za pridelavo kapusnic namenjenih 442 ha oziroma 29,50 % površin pod zelenjadnicami (Popis tržnega vrtnarstva, 2010). Zelje spada med najbolj razširjene kapusnice (Osvald in Kogoj-Osvald, 2005; Maiti in sod., 2012), pridelava zelja pa je v Evropi pomembna kmetijska panoga (Trdan in sod., 2005b). Vrsta, ki prvotno izvira iz obale Sredozemskega morja, spada med toplotno srednje zahtevne vrtnine (Osvald in Kogoj-Osvald, 2005; Maiti in sod., 2012).

Krmna ogrščico lahko uspešno vključimo v kolobar, je pa tudi pomemben vir krme za živino (Valantin-Morison in sod., 2007). Bela gorjušica predstavlja v nekaterih primerih uspešen varovalni posevek, kot tudi oljna redkev (Talgre in sod., 2011). Rastlinski deli oljne redkve, nadzemski in podzemni, pa so pomemben vir živalske krme (Dixon, 2007). Pridelovanje v različnih podnebnih razmerah (Björkman in sod., 2011) je križnicam omogočilo velik pomen v rastlinski pridelavi in marsikje pridelovalcem zagotavljajo soliden dohodek (Vaughn in Berhow, 2005).

Obe skupini preučevanih škodljivcev, kapusove stenice (*Eurydema* spp.) in kapusovi bolhači (*Phyllotreta* spp.), spadata med polifage, kar pomeni, da povzročajo škodo na različnih rastlinskih vrstah iz družine križnic. Kapusovi bolhači (Coleoptera, Chrysomelidae) (Priloga A2) predstavljajo eno od ključnih skupin škodljivcev na kapusnicah (Trdan in sod., 2005b). V Sloveniji se najpogosteje pojavljajo vrste ***Phyllotreta armoraciae*** (Koch), ***Phyllotreta undulata*** (Kutchera), ***Phyllotreta nemorum*** (L.), ***Phyllotreta striolata*** (Fabricius), ***Phyllotreta atra*** (Fabricius) in ***Phyllotreta vittula*** (Redtenbacher) (Brelih in sod., 2003). Kapusovi bolhači prezimijo kot odrasli osebki (Vrabl, 1992) in imajo v srednji Evropi navadno en rod na leto (Trdan in sod., 2005b). Pojav odpornosti na sintetične insekticide je pri njih precej pogost (Trdan in sod., 2005b). Kapusovi bolhači z načinom prehranjevanja povzročajo izjede na listih (Vrabl, 1992) in posledično lahko celo povzročijo propad rastlin (Palaniswamy in Lamb, 1992). Pojavljanje teh škodljivcev v poznejših razvojnih stadijih kot posledica neenakomerne rasti rastlin, zmanjšane količina semena in podobno, pa lahko vpliva na manjšo količino ali kakovost pridelka (Tansey in sod., 2009) (Priloga A5).

Kapusove stenice (Heteroptera, Pentatomidae) uvrščamo med fitofagne stenice, ki sicer predstavljajo 60 % znanih vrst stenic (Schafer in Panizzi, 2000). V Sloveniji sta med kapusovimi stenicami najpogostejši kapusova stenica (*Eurydema oleracea* L.) (Priloga A1) in pisana stenica (*Eurydema ventrale* Kolenati) (Priloga A3) (Janežič, 1951; Vrabl, 1992). Z imenom kapusove stenice namreč poimenujemo tako vrsto, kot tudi skupino več vrst stenic, ki se hranijo na zelju in nekaterih drugih kapusnicah. Janežič (1951) opisuje stenice kot široke ploščate žuželke, ki imajo prednja krila debelo usnjata in segajo do 2/3 njihove dolžine. Stenice z svojim načinom prehranjevanja na listih povzročijo bele pege. Ob močnejšem napadu je list prosojen, preluknjan. Rastlinsko tkivo znotraj teh pik je nekrotizirano. Če so poškodbe obsežne, se list oziroma rastlina lahko posuši. Poškodb je sicer navadno največ na mladih rastlinah, po kalitvi ali po presajanju (Trdan in sod., 2006; Bohinc in Trdan, 2011a). Za ocenjevanje poškodb kapusovih bolhačev smo v naši raziskavi uporabili 5-stopenjsko lestvico EPPO (OEPP/EPPO, 2002), medtem ko smo ocenjevanje poškodb kapusovih stenic izvedli s 6-stopenjsko lestvico Stonerjeve in Sheltona (1998). Najvišja ocena na lestvici, ki je bila namenjena ocenjevanju poškodb kapusovih bolhačev, predstavlja več kot 25 % poškodovane listne površine, medtem ko je več kot 50 % poškodovane listne površine po lestvici Stonerjeve in Sheltonona ovrednoteno kot ocena 6.

Glede na podatke iz Seznama registriranih fitofarmacevtskih sredstev (2013) je v Sloveniji za zatiranje kapusovih bolhačev dovoljena uporaba insekticida Fastac 100 EC, medtem ko za zatiranje kapusovih stenic ni registriranega nobenega insekticida. Za zatiranje bolhačev lahko uporabljamo tudi pripravek Karate Zeon 5 CS (aktivna snov lambda cihalotrin) (Seznam registriranih…, 2013). Med insekticidi, ki so dovoljeni v integrirani pridelavi kapusnic za zatiranje kapusovih bolhačev, najdemo poleg pripravkov Karate Zeon 5 CS in Fastac 100 EC (aktivna snov alfa cipermetrin) tudi pripravek Neemazal – T/S (aktivna snov azadirahtin A) (Tehnološka navodila …, 2013). Številne insekticide, katerih uporaba je v Evropi in Sloveniji prepovedana, pa še vedno uporabljajo na nekaterih drugih območjih sveta; na Kitajskem, v Ameriki (Andersen in sod., 2006; Trdan in sod., 2006; Tansey in sod., 2009; Chen in sod., 2012).

V Tehnoloških navodilih za integrirano pridelavo zelenjave (2013) je kot pomemben alternativni ukrep za zmanjševanje poškodb na različnih gostiteljskih rastlinah navedena uporaba zaščitnih mrež oziroma prekrivk. Učinkovitost prekrivk iz 100 % propilena so potrdili tudi Andersen in sod. (2006). Poleg tega v tej zvezi priporočajo vse ukrepe, ki spodbujajo rast rastlin. Posebnih alternativnih metod za zmanjševanje škodljivosti kapusovih stenic v omenjenih navodilih ni navedenih. Ker smo želeli preučiti doslej še nepreučene alternativne metode v varstvu kapusnic za zatiranje kapusovih bolhačev in kapusovih stenic, smo v letih 2009 in 2010 preučevali delovanje privabilnih posevkov.

Pri metodi privabilnih posevkov izkoriščamo lastnosti za škodljivce dovzetnih rastlin. Te posadimo oziroma posejemo med rastline glavnega posevka ali v njegovo bližino, z namenom, da bi na dovzetne rastline privabili škodljivce in obenem zmanjšali njihovo številčnost na glavnem posevku (Gray in Koch, 2002; Wallingford in sod., 2013). Metoda privabilnih posevkov pride do izraza tudi takrat, ko glavni posevek nima odpornosti na škodljivega organizma oziroma za njegovo zatiranje ni registriranih kemičnih pripravkov.

Lastnosti gostiteljskih rastlin sicer lahko izkoriščamo tudi pri drugih okoljsko sprejemljivih ukrepih varstva rastlin; medsetvah (angl. intercropping), varovalnih posevkih (angl. cover crops) in drugih (Cook in sod., 2006). Tako lahko pri metodi privabilnih posevkov izkoriščamo rastlinske vrste, ki pripadajo istemu botaničnemu rodu kot glavni posevek oziroma za privabilni posevek uporabimo rastline, ki spadajo v druge botanične družine.

V primeru, ko rastlinske vrste privabilnih posevkov spadajo v enako botanično skupino kot rastline glavnega posevka, lahko predstavniki različnih vrst pri obrambi pred škodljivci izrabljajo iste parametre antiksenoze (Safraz in sod., 2007; Tansey in sod., 2010). Med takšne na primer spadajo sekundarni metaboliti. Pomen sekundarnih metabolitov rastlin je v varstvu rastlin v zadnjih letih predmet številnih raziskav (Ahuja in sod., 2010). Glukozinolatom, kot specifičnim sekundarnim metabolitom, ki se nahajajo v križnicah, se namreč v številnih raziskavah pripisuje pomen pri naravni odpornosti rastlin na škodljivce (Ahuja in sod., 2010). Glukozinolati se sicer pojavljajo v rastlinskih vrstah iz 13 različnih botaničnih družin (Newton in sod., 2009).

Vsebnost glukozinolatov je pogojena tudi z rastlinsko vrsto (Moyes in sod., 2007), različnimi organi iste rastlinske vrste (Fahey in sod., 2001), razvojnim stadijem rastline (Lambdon in sod., 2003), rezultati raziskav nekaterih avtorjev pa kažejo tudi na vpliv okoljskih dejavnikov na vsebnost glukozinolatov v rastlinah (Velasco in sod., 2007). Van Doorn in sod. (1999) navajajo, da na vsebnost glukozinolatov vplivajo tudi škodljivi organizmi, tako povzročitelji bolezni kot povzročitelji poškodb.

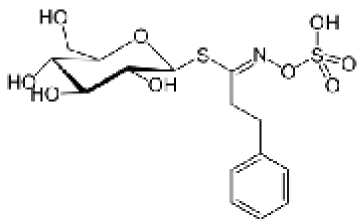
Vsebnost glukozinolatov v rastlinah naj bi imela različne vplive na škodljive žuželke. Nekatere monofagne škodljive vrste lahko glukozinolate uspešno razgradijo v manj škodljive produkte, druge vrste, tako monofagne kot polifagne, pa lahko glukozinolate uporabijo za svojo obrambo (Poelman in sod., 2008). Tako je bilo na primer ugotovljeno, da je vitalnost polonic *Adalia bipunctata* (L.) in *Coccinella semptempunctata* (L.), ki se hranijo z ličinkami mokaste kapusove uši (*Brevicoryne brassicae*), odvisna od vsebnosti sinigrina (Prat in sod., 2008). Sinigrin namreč negativno vpliva na vrsto *A. bipunctata*, na polonico *C. semptempunctata* pa niso ugotovili kakršnegakoli vpliva (Prat in sod., 2008).

Glukozinolati, predvsem njihovi razgradni produkti, lahko delujejo tudi na talne škodljive organizme (proces biofumigacije) (Gimsing in Kirkegard, 2009). Vpliv glukozinolatov na koristne organizme pa tudi ni zanemarljiv, saj posledično - udeleženi so namreč v škodljivih organizmih, s katerimi se naravni sovražniki hranijo (npr. mokaste kapusove uši) -, delujejo negativno na njene naravne sovražnike (Kos in sod., 2012).

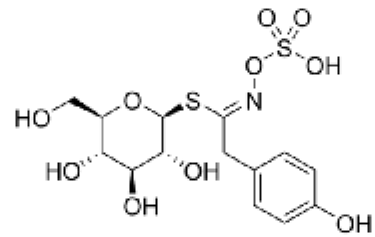
Omenjene sekundarne metabolite lahko razdelimo na tri kemične skupine, in sicer glede na aminokislino, na katero so vezani (Hopkins in sod., 1998). Alifatski glukozinolati vsebujejo metionin, indolni so vezani na triptofan, medtem ko aromatski vključujejo tirozin ali fenilalanin (Padilla in sod., 2006). V kapusnicah so najpomembnejši alifatski glukozinolati. Glukozinolati so kemične spojine, katerih vsebnost se v rastlini oziroma v njenih listih spreminja glede na razvojni stadij rastlin. Vsebnost le-teh naj bi bila višja v mladih listih, kar bi lahko pomenilo, da se dovzetnost rastlin za napade škodljivcev proti koncu rastne dobe povečuje (Lambdon in sod., 2003). Pri hidrolizi glukozinolatov nastane vrsta fiziološko aktivnih komponent, vključno z izotiocianati, nitrili, tiocianati in oksazolidintioni (Al-Gendy in sod., 2010).

Izmed aromatskih glukozinolatov smo v naši raziskavi ugotavljali prisotnost sinalbina in glukonasturtiina, med indolnimi nas je zanimala prisotnost glukobrasicina. V skupini alifatskih glukozinolatov smo se osredotočili na vsebnost glukoiberina, progoitrina, epiprogoitrina, sinigrina, glukonapina in glukorafenina.

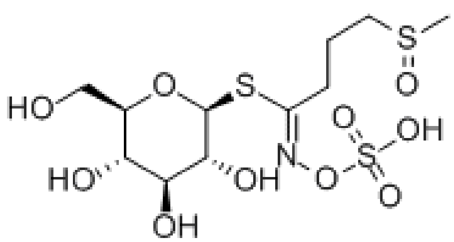
V nadaljevanju so predstavljene strukturne formule glukozinolatov, ki smo jih analizirali v naši raziskavi (Chemical Book, 2010). Ime glukozinolata je napisano v levem spodnjem kotu pod strukturno formulo.



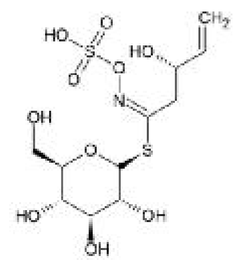
glukonasturtiin



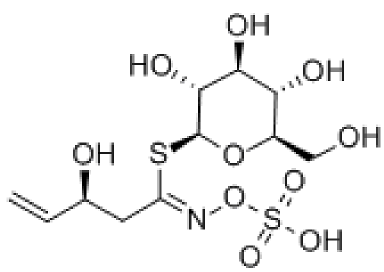
sinalbin



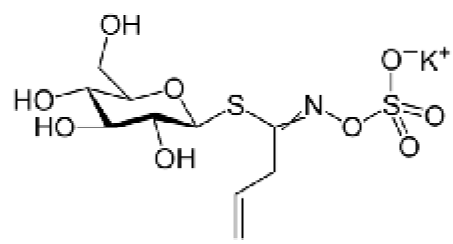
glukoiberin



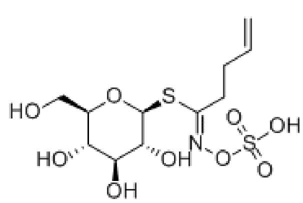
progoitrin



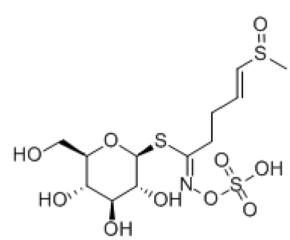
epiprogoitrin



sinigrin

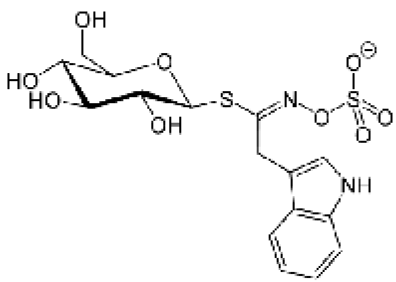


glukonapin



glukorafenin

Slika 1: se nadaljuje…



glukobrasicin

Slika 1: Strukturne formule glukozinolatov, ki smo jih analizirali v naši raziskavi

Figure 1: Glucosinolates, analysed in our survey, presented by structural classes

V raziskavi smo želeli preučiti sezonsko dinamiko predstavnikov dveh rodov škodljivcev, kapusovih stenic (*Eurydema* spp.) in kapusovih bolhačev (*Phyllotreta* spp.), na dveh lokacijah v Sloveniji. Analizirali smo sestavo glukozinolatov v dveh hibridih belega zelja in treh vrstah križnic (bele gorjušice [*Sinapis alba*], krmne ogrščice [*Brassica napus* spp. *oleifera* f. *biennis*] in oljne redkve [*Raphanus sativus* var. *oleiformis*]), z namenom, da bi preučili interakcije med vsebnostjo glukozinolatov v rastlinah in obsegom poškodb zaradi hranjenja preučevanih škodljivcev.

Postavili smo naslednje hipoteze:

- različne koncentracije in razmerja med glukozinolati v rastlinah privabilnih posevkov vplivajo na njihovo različno dovzetnost za kapusove stenice in kapusove bolhače, zato predvidevamo, da so različno ustrezne za privabljanje preučevanih škodljivcev.
- predvidevamo, da je vsebnost glukozinolatov različna med posameznimi organi iste rastlinske vrste, kar posledično na njih vodi do različnega obsega poškodb zaradi hranjenja preučevanih skupin škodljivcev.
- domnevamo, da na vsebnost sekundarnih metabolitov (glukozinolatov) v preučevanih križnicah vplivajo tudi okoljski dejavniki, predvsem temperatura zraka.
- predpostavljamo, da obstaja povezava med vrsto glukozinolatov in občutljivostjo/odpornostjo rastlinskih vrst na napad preučevanih škodljivcev.
- obseg poškodb, ki jih povzročajo preučevani škodljivci, je odvisen tudi od načel dobre kmetijske prakse in upoštevanja agrotehničnih ukrepov. V ta namen bomo izpostavili povezavo med povprečnim indeksom poškodb škodljivcev skozi rastno dobo in morfološkimi lastnostmi rastlin privabilnih posevkov in zelja kotglavne rastlinske vrste.
- z izbiro rastlinskih vrst, ki se med seboj razlikujejo tudi po dolžini rastne dobe, želimo vplivati tudi na bionomijo preučevanih skupin škodljivcev, *Phyllotreta* spp. in *Eurydema* spp. Predvidevamo, da obstajajo razlike v dovzetnosti za poškodbe tudi med dvema kultivarjema zelja, ki se med seboj razlikujeta v dolžini rastne dobe.

2 ZNANSTVENI ČLANKI

2.1 POVEZAVA MED KONCENTRACIJO GLUKOZINOLATOV IN OBSEGOM POŠKODB KAPUSOVIH STENIC (*Eurydema* spp., Heteroptera: Pentatomidae) NA RAZLIČNIH KRIŽNICAH

BOHINC, Tanja, HRASTAR, Robert, KOŠIR, Iztok Jože in TRDAN Stanislav
Association between glucosinolate concentration and injuries caused by cabbage stink bugs *Eurydema* spp. (Heteroptera: Pentatomidae) on different Brassicas.
Acta Scientiarum Agronomy, 2013, 35: 1-8.
Prejeto: 27. 12. 2011, sprejeto: 19.03.2012

V letu 2010 smo vsebnost glukozinolatov preučevali v različnih vrstah križnic, z namenom preučitve vpliva vsebnosti teh sekundarnih metabolitov na obseg poškodb kapusovih stenic (*Eurydema* spp.). Naša raziskava je potrdila, da vsebnost glukozinolatov variira med posameznimi rastlinskimi vrstami in različnimi organi iste rastlinske vrste. Ugotavljamo, da je vsebnost glukozinolatov pogojena tudi s terminom ocenjevanja. V naših vzorcih so prevladovali alifatski glukozinolati (glukoiberin, progoitrin, epiprogoitrin, sinigrin, glukonapin, glukoraphenin, sinalbin). Prisotnost glukobrasicina (pomembnega indolnega glukozinolata) smo potrdili v vseh križnicah v poskusu. Vsebnost glukobrasicina je bila signifikatno najvišja v vzorcih oljne redkve v prvem terminu ocenjevanja (30 dni po sajenju, 8,84±0,65 µmol/g mase suhega semena), medtem ko smo v vzorcih krmne ogrščice ugotovili signifikatno najnižjo vsebnost omenjenega glukozinolata v zadnjem terminu ocenjevanja (134 dni po sajenju, 4,30±0,80 µmol/g mase suhega semena). Stimulativno delovanje posameznega glukozinolata oziroma negativen vpliv na hranjenje kapusovih stenic v križnicah, uporabljenih v poskusu, ni bilo povsod enako izraženo. Na podlagi rezultatov dvoletnega poljskega poskusa v letih 2009 in 2010 lahko trdimo, da je krmna ogrščica najbolj ustrezen privabilni posevek za kapusove stenice. V prihodnosti bi lahko vsebnost glukozinolatov v križnicah predstavljalaa pomeben dejavnik pri našem odločanju za pridelavo omenjenih rastlin v razmerah, kjer se pojavljajo in povzročajo škodo kapusove stenice.

Acta Scientiarum
http://www.uem.br/acta
ISSN printed: 1679-9275
ISSN on-line: 1807-8621
Doi: 10.4025/actasciagron.v35i1.15622

Association between glucosinolate concentration and injuries caused by cabbage stink bugs *Eurydema* spp. (Heteroptera: Pentatomidae) on different Brassicas

Tanja Bohinc[1*], Robert Hrastar[2], Iztok Jože Košir[2] and Stanislav Trdan[1]

*[1]University of Ljubljana, Biotechnical Faculty, Department of Agronomy, Jamnikarjeva 101, SI-1111 Ljubljana, Ljubljana, Slovenia. [2]Department of Agrochemistry and Brewing, Slovenian Institute of Hop Research and Brewing, SI-3310 Žalec, Žalec, Slovenia. *Author for correspondence. E-mail: tanja.bohinc@gmail.com*

ABSTRACT. In 2010, we were determining the contents of glucosinolates in different Brassicas in order to study their influence on feeding of cabbage stink bugs (*Eurydema* spp.) and the consequent extent of damage. We confirmed that glucosinolates content depends on plant species, plant organs and the time of sampling. In the samples aliphatic glucosinolates (glucoiberin, progoitrin, epiprogoitrin, epiprogoitrin, sinigrin, gluconapin, glucoraphenin, sinalbin) prevailed. Glucobrassicin, an important indolic glucosinolate compound, was detected in all tested Brassicas. Its concentration in the oil radish samples was highest during the first assessment (30 DAS), 8.84 ± 0.65 μmol g^{-1} ds, while the oilseed rape samples displayed lowest concentration during the last assessment (134 DAS), 4.30 ± 0.80 μmol g^{-1} ds. The stimulative activity of individual glucosinolates or their negative influence on feeding of cabbage stink bugs in the Brassicas used in our experiment was not uniformly manifested. Based on a two-year field experiment we concluded that oil rape was the most adequate trap crop used to allure cabbage stink bugs. In future, glucosinolates should be employed to a greater extent in environmentally acceptable ways of food production, one of which is also the use of trap crops in order to reduce harmful effects of cabbage stink bugs.

Keywords: Phytochemicals, cabbage stink bugs, cabbage, white mustard.

Associação entre a concentração glucosinolada e danos causados pelo percevejo *Eurydema* spp. (Heteroptera: Pentatomidae) em diferentes gramíneas

RESUMO. Em 2010 o conteúdo de glucosinolatos em diferentes plantas brássicas foi determinado para estudar a influência sobre a alimentação de percevejos de repolho (*Eurydema* spp.) e a extensão do dano causado. Através do método do cultivo armadilha, durante o período de crescimento foram amostradas as partes aéreas de óleo de colza, mostarda branca, nabo forrageiro e dois híbridos de repolho branco. O conteúdo de glucosinolatos depende das espécies de plantas, órgãos de plantas e período da amostragem. Predominaram nas amostras os glucosinolatos alifáticos (glucoiberina, progoitrina, epiprogoitrina, sinigrina, gluconapina, glucoraphenina, sinalbina). A glucobrassicina foi detectada em todas as brássicas testadas. Sua concentração no nabo forrageiro foi mais alta na primeira avaliação (30 DAS), 8.84 ± 0.65 μmol g^{-1} ds, enquanto houve a menor concentração no óleo de colza durante a última avaliação (134 DAS), 4.30 ± 0.80 μmol g^{-1} ds. Considerando o experimento de dois anos, conclui-se que o óleo de colza foi o mais adequado como cultivo armadilha para atrair o percevejo do repolho. Glucosinolatos podem ser usados no futuro mais extensivamente em várias modalidades de produção de alimentos, entre as quais o emprego de cultivo armadilha para diminuir os danos do percevejo do repolho.

Palavras-chave: fitoquímicos, percevejo do repolho, repolho, nabo forrageiro, mostarda branca.

Introduction

Secondary metabolites (KLIEBENSTEIN et al., 2001) are often said to be important factors of plants' resistance to biotic (WINDE; WITTSTOCK, 2011) and abiotic stress (Björkman et al., 2011). Glucosinolates are classified as the secondary metabolites (KLIEBENSTEIN et al., 2005; SILVA CARVALHO et al., 2010), characteristic for the Caparales order (AL-GENDY et al., 2010; BJÖRKMAN et al., 2011), present particularly in the Brassicaceae family (GRIFFITHS et al. 2001; JOHNSON, 2002; DE VILLENA et al., 2007; CARTEA et al., 2008 BLAŽEVIĆ; MASTELIĆ, 2009; AL-GENDY et al., 2010; MÜLLER, et al., 2010; YANG et al., 2010; BJÖRKMAN et al., 2011; WINDE; WITTSTOCK, 2011).

Different Brassicaceae species are important from the agronomical (FONT et al., 2005; VAUGHN; BERHOW, 2005; CARTEA et al., 2008; BLAŽEVIĆ; MASTELIĆ, 2009; HASAN; ANSARI, 2011) and the economic point of view (RAYBOULD; MOYES, 2001; VAUGHN; BERHOW, 2005). Glucosinolates are composed of the β-D-glucan group, the functional group of sulphated oxime and a variable side-chain (BEEKWILDER et al., 2008; VIG et al., 2009; AL-GENDY et al., 2010). Glucosinolates are according to their side-chain categorised as aliphatic, indole and aromatic (CARTEA; VELASCO, 2008; VAN EYLEN et al., 2009) and the concentration of these varies between individual plant organs (FAHEY et al., 2001; WINDE; WITTSTOCK, 2011), plant species (MOYES et al., 2000; CHAPLIN-KRAMER et al., 2011), developmental stages of the same plant species (DE VILLENA et al., 2007; CARTEA et al., 2008; SARIKAMIŞ; YANMAZ, 2011), and it depends also on weather conditions (VELASCO et al., 2007; WINDE; WITTSTOCK, 2011).

The hydrolysis of glucosinolates creates several physiologically active components, including isothyocianates, nitriles, thyocianates and oxazolidinones (BROWN; MORRA, 1996; BEEKWILDER et al., 2008; VIG et al., 2009; BLAŽEVIĆ; MASTELIĆ, 2009). These components and also glucosinolates as a whole can protect plants from infections by pathogens (HOPKINS et al., 1998; BLAŽEVIĆ; MASTELIĆ, 2009) and attacks of generalists (HOPKINS et al., 1998; MOYES et al., 2000; HOOKS; JOHNSON, 2003), while their influence on specialists is often stimulative (HOPKINS et al., 1998; MOYES, et al., 2000; HOOKS; JOHNSON, 2003; MÜLLER, 2009); MÜLLER et al., 2010; CHAPLIN- KRAMER et al., 2011).

Among the pests that can infest Brassicas, cabbage stink bugs *Eurydema* spp. (Heteroptera: Pentatomidae) feed on outer leaves of older plants developing bronze discolorations, and when they suck on young plants, bright specks are the symptoms caused by their feeding (TRDAN et al., 2006; DEMIREL, 2009; ELTEZ; KARSAVURAN, 2010; BARIĆ; PAJAĆ, 2011). Trdan et al. (2006) in one of the previous studies confirmed their detrimental effects on cabbage crops. Our study was spurred by the fact that cabbage stink bugs in the South-East Europe (BARIĆ; PAJAĆ, 2011) and Asia Minor (DEMIREL, 2009) significantly harm the family Brassicaceae, and it seems that their population density and thus harmfulness are increasing, and also by the fact that some countries either lack registered insecticides for their suppression or they have been drastically reduced. The study deals with the trap crop method which takes advantage of characteristics of those plants which are susceptible to harmful pests. This method belongs to the group of environmentally acceptable methods for reducing the economic impact of harmful pests on plants (TRDAN et al., 2005; COOK et al., 2006).

The purpose of our study was to establish glucosinolate content in different species of Brassicas so as to determine practical applications in protection of cabbage against cabbage stink bugs. The connection between the extent of damage caused by these pests on cabbage and the concentration of glucosinolates in this Brassica has so far not been studied, the same holds true for the significance of glucosinolate concentration in oilseed rape, white mustard and oil radish, which have been used in this study to trap cabbage stink bugs and thus deter them from feeding on cabbage.

Plant material

The two-year field experiment (2009-2010) was carried out in the village Zgornja Lipnica (46°19' N latitude, 14°10' E longitude, 511 m above the sea level) in Slovenia, during the growth period in the second year of the experiment we were collecting samples of selected above-the-ground parts of oilseed rape (*Brassica napus* [L.] ssp. *oleifera* f. *biennis*), cv. Daniela (supplier: Semenarna Ljubljana, d. d., Ljubljana, Slovenia), white mustard (*Sinapis alba* [L.]), cv. Zlata (supplier: Semenarna Ljubljana, d. d., Ljubljana, Slovenia), oil radish (*Raphanus sativus* [L.] var. *oleiformis*), cv. Apoli (supplier: Semenarna Ljubljana, d. d., Ljubljana, Slovenia), the early hybrid of white cabbage (*Brassica oleracea* [L.] var. *capitata* f. alba), cv. 'Tucana' (supplier: Semenarna Ljubljana, d. d., Ljubljana, Slovenia) and the medium-late cabbage hybrid (*Brassica oleracea* [L.] var. *capitata* f. alba), cv. 'Hinova' (producer: Bejo Zaden, Warmenhuizen, The Netherlands; supplier: Agroprogress, d. o. o., Ljubljana, Slovenia).

Field evaluation

The field of 528 m^2 was divided into four blocks, where the three species of trap crops were sowed within the plots in separate treatments, and the fourth treatment was the control, where no trap crop was sowed (bare surface). In each treatment we also sowed both cabbage hybrids in separate sub-plots. The treatments within blocks were arranged randomly. The trap crops which were used for chemical analysis were sowed on the April 19th 2010, while the cabbage was sowed outdoors on the April 20th 2010. The seedlings of cabbage were cultivated in the Department of Agronomy's greenhouse at the Biotechnical Faculty according to the protocol as described in Trdan et al., 2009. The injuries caused by the stink bugs on Brassicas were assessed by the 6-grade visual scale (STONER; SHELTON, 1988), which was developed for assessing the extent of damage done on cabbage by onion thrips (*Thrips tabaci* Lindeman), while Trdan et al. (2006) had successfully used a slightly modified scale to evaluate damage done on cabbage by cabbage stink bugs.

Glucosinolate analysis

Plant material for the analysis of glucosinolates was sampled at different intervals. The material was collected by scissors. Sampling of cabbage leaves was carried out at five different intervals (30 DAT (days after transplanting), 48 DAT, 66 DAT, 92 DAT and 111 DAT). Samplings of above-the-ground parts of oilseed rape was carried out at five different intervals (30 DAS (days after sowing), 50 DAS, 78 DAS, 103 DAS, 134 DAS), while the sampling of oil radish and white mustard was performed at four intervals (the former at 30 DAS, 50 DAS, 65 DAS and 103 DAS of July; the latter at 30 DAS, 50 DAS, 65 DAS and 78 DAS).

Four samples of individual plant species were collected at individual intervals of assessment. An individual sample (the specified part of a plant species) was the representative plant sample of one block. When analysing glucosinolates, the analysis of individual samples was repeated twice. The material was then freeze-dried (tip: LIO-10P; producer: Kambič Laboratorijska oprema, Semič, Slovenia) and homogenized before extraction of glucosinolates. The lyophilised samples were stored in 50 ml bottles in a freezer (type: U3286S; producer: Sanyo) at -80°C.

The glucosinolate extraction and analysis were performed according to ISO 9167:1-1992. As internal standards sinigrin or glucotropaeolin (C_2 Bioengineering ApS, Denmark) were added. The extracted glucosinolates were purified on a 1.5 cm DEAE Sephadex A-25 anion exchange column. The column was washed twice with 1 mL distilled water loaded with 2ml of the glucosinolates extract and then washed twice with 1 mL 20 mM NaAc-solution and treated with sulphatase (75 μL and 25 mg mL^{-1}). After an overnight reaction at room temperature the desulfoglucosinolates were eluted with distilled water (2*1mL). The eluate was filtered over 0.45 μm filter and then sample was ready for HPLC analysis.

For GLS quantification, twenty microlitres of desulfoglucosinolates solution were run on an Agilent 1200 Series HPLC system (Palo Alto, CA, USA) at 2 Ml min^{-1}. The column was a Discovery C18, 25 cm x 4.6 mm, 5 μm (Supelco). The mobile phases were water and methanol, running time: 28 min. The gradient changed as follows: 100% A for 1 min., then in 20 min. to 20% B, followed by 100% B for 5 min. Afterwards the column was equilibrated at 100 % A for 3 min. The desulfoglucosinolates were detected with DAD detector at 229 nm. The desulfoglucosinolates were identified with external standards (C2 Bioengineering ApS, Denmark). The certified reference material used was BCR-367R. The content of each GLS was back calculated and expressed in micromoles per gram (μmol g^{-1}) of dry seed.

Data analysis

The experiment's results were statistically evaluated by the program Statgraphics Centurion XVI (STATGRAPHICS CENTURION XVI, 2009). The differences between values of glucosinolates during the growth period and between individual plant species were calculated by the analysis of variance (ANOVA) and Duncan's test of multiple comparisons ($p < 0.05$). We calculated correlations between the concentration of an individual glucosinolate and the average injury index of the plant species.

Results and discussion

Glucosinolate content in trap crops

The samples of oil radish had six different glucosinolates, which can be divided into three different groups (Table 1). The time of assessment of the developmental stage of the studied plants had significant influence on the concentration of glucobrassicin in the samples ($p = 0.0005$; $F = 8.68$; $Df = 3$). The average value of glucobrassicin content was significantly highest during the first assessment (8.84 ± 0.65 μmol g^{-1} of dry seed (ds)), while during the last assessment the concentration was 0.84 ± 0.18 μmol g^{-1} ds. On average the concentration of glucoraphenin in the oil radish samples was 8.66 ± 1.81 μmol g^{-1} ds.

The concentration of the said glucosinolate is not conditioned by the time of assessment ($p = 0.3755$; $F = 1.10$; $Df = 3$). The established average value of sinalbin in the samples was 0.36 ± 0.12 μmol g^{-1} ds, while the said concentration was not significantly conditioned by the time of sampling ($p = 0.7193$; $F = 0.37$; $Df = 2$). Glucoiberin and progoitrin were present in traces only.

The analysis of oilseed rape samples found out eight different glucosinolates (Table 1). The most frequent among aromatic glucosinolates was gluconasturtiin (0.13 ± 0.04 μmol g^{-1} ds), which was not significantly influenced by different times of assessment ($p = 0.0646$; $F = 96.33$; $Df = 1$). We also found out that the concentration of glucobrassicin was significantly influenced by the times of assessment or developmental stages ($p = 0.0044$; $F = 6.19$; $Df = 4$).

Table 1. *Average values of glucosinolates in individual plant species (in μmol g⁻¹ ds). Zgornja Lipnica, Slovenia, 2009/2010.*

Systematic name	Trivial name	Oil radish	Oil rape	White mustard	'Hanova'	'Tucana'
Aliphatic						
3-Methlysufinylpropyl	Glucoiberin	< 0.1	< 0.1	< 0.1	0.33 ± 0.08	0.38 ± 0.09
2 (R)-2-Hydroxy-3-butenyl	Progoitrin	< 0.1	1.55 ± 0.51	< 0.1	1.13 ± 0.02	0.27 ± 0.03
2 (S)-2-Hydroxy-3-butenyl	Epiprogoitrin	x	0.19 ± 0.06	2.45 ± 0.38	y	Y
2-Propenyl	Sinigrin	x	X	x	0.30 ± 0.03	0.35 ± 0.06
3-Butenyl	Gluconapin	x	0.38 ± 0.05	x	0.17 ± 0.03	0.24 ± 0.10
4-Methylsulfinyl-3-butenyl	Glucoraphenin	8.66 ± 1.81	0.99 ± 0.87	0.51 ± 0.19	< 0.1	< 0.1
Indole						
3-Indolmethyl	Glucobrassicin	3.24 ± 0.86	1.39 ± 0.49	1.71 ± 0.88	0.40 ± 0.15	0.79 ± 0.27
Aromatic						
2-Phenylethyl	Gluconasturtiin	< 0.1	0.13 ± 0.04	x	y	Y
4-Hydroxybenzyl	Sinalbin	0.36 ± 0.12	11.16 ± 6.50	30.12 ± 5.52	y	Y

x - type of glucosinolate not present in the plant species; y – detection was made, but there was no glucosinolate.

While the concentration of glucobrassicin in the oil radish samples was significantly highest during the first assessment, the concentration of glucobrassicin in the oilseed rape samples was significantly lowest during the last assessment (4.30 ± 0.80 μmol g⁻¹ ds). We found out that the concentration of gluconapin in the samples was conditioned by the times of assessment ($p = 0.0365$; $F = 3.76$; $Df = 4$).

The concentration of gluconapin was significantly lowest in the second (0.20 ± 0.04 μmol g⁻¹ ds) and the first assessment (0.22 ± 0.01 μmol g⁻¹ ds), the highest was during the last assessment (0.60 ± 0.07 μmol g⁻¹ ds). The concentration of sinalbin in the oilseed rape samples was not conditioned with the times of assessment ($p = 0.2035$; $F = 2.63$; $Df = 1$). No significant influence of the times of sampling on the concentration of glucoraphenin in the oilseed rape samples was found ($p = 0.5729$; $F = 0.76$; $Df = 4$). We found out that the concentration of epiprogoitrin in the plant samples varied with the times of sampling ($p = 0.0013$; $F = 782.29$; $Df = 1$). The significantly highest concentration of epiprogoitrin was found in the samples which were collected at 30 DAS or during the first assessment (0.37 ± 0.01 μmol g⁻¹ ds), while the value of the said glucosinolate during the last assessment was the significantly lowest (0.13 ± 0.03 μmol g⁻¹ ds). The analysis of the oilseed rape sample revealed significant influence of the time of sampling on the concentration of progoitrin in the plant tissue of oilseed rape ($p < 0.001$; $F = 219.78$; $Df = 4$). Despite the fact that the concentration of the said glucosinolate in the plant varies, it was significantly the highest during the last assessment (3.64 ± 0.12 μmol g⁻¹ ds). Glucoiberin was present in traces only (< 0.1 μmol g⁻¹ ds).

In the white mustard samples we found the presence of six glucosinolates. The most distinctive among aliphatic glucosinolates was the presence of sinalbin and it was conditioned by the times of assessment ($p = 0.0168$, $F = 4.43$; $Df = 3$). The times of sampling also significantly influenced the concentration of glucobrassicin ($p = 0.0096$; $F = 5.23$; $Df = 3$;) and epiprogoitrin ($p = 0.0008$; $F = 13.30$. $Df = 3$). Its concentration was significantly the highest during the first assessment (6.59 ± 2.91 μmol g⁻¹ ds), while the concentration of epiprogoitrin was the significantly lowest in the first sampling (0.25 ± 0.12 μmol g⁻¹ ds).

Glucosinolate content in cabbage hybrids

In the samples of the early cabbage hybrid six different glucosinolates were discovered. The concentration of glucobrassicin varies with the times of assessment ($p = 0.0056$; $F = 5.82$; $Df = 4$). The average value was the significantly highest in the samples which were collected in the first sampling (2.34 ± 0.37 μmol g⁻¹ ds). The concentration of the said glucosinolate varies throughout the growth period, at the end of it the average value was 0.15 ± 0.02 μmol g⁻¹ ds. The average value during the entire growth period was 0.79 ± 0.27 μmol g⁻¹ ds). The times of assessment also did not significantly influence the concentration of sinigrin ($p = 0.4628$; $F = 0.61$; $Df = 1$) and glucoiberin ($p = 0.6575$; $F = 0.45$; $Df = 2$) in the cabbage samples. The concentration of sinigrin varied from 0.41±0.06 μmol g⁻¹ ds during the first assessment to 0.30 ± 0.12 μmol g⁻¹ ds during the assessment in the second decade of August (0.30 ± 0.12 μmol g⁻¹ ds). The concentration of glucoiberin, on the other hand, varied from 0.35 ± 0.09 μmol g⁻¹ ds during the first assessment to 0.33 ± 0.11 μmol g⁻¹ ds during the last but one assessment. The limit of detection for glucoraphenin is < 0.1 μmol g⁻¹ ds.

The samples of the medium-late hybrid were tested on nine glucosinolates. We found out that the concentration of glucoiberin depended on the times of assessment – the concentration varies during the growth period ($p = 0.0016$; $F = 12.13$; $Df = 3$). Sinigrin, whose concentration was not conditioned by the times of sampling ($p = 0.1054$; $F = 3.63$; $Df = 1$), was present in the hybrid from 0.25 ± 0.03

during the first sampling to 0.36 ± 0.05 μmol g^{-1} dt during the assessment in mid-August. No significant influence of the times of sampling on the concentration of gluconapin was found (p = 0.1648; F = 2.88; Df = 1). The concentration of glucobrassicin in the samples of the hybrid 'Hinova' was also conditioned by time (p = 0.0000; F = 28.83; Df = 4) and was significantly the highest during the first assessment.

The results of our study show that glucosinolates content in individual parts of Brassicas varies, what was already confirmed by Fahey et al., (2001) and Winde and Wittstock (2011). Data obtained from our survey can confirm that glucosinolate content also differs between individual species of Brassicas (MOYES et al., 2000; CHAPLIN-KRAMER et al., 2011); and that their concentration varies during the growth period (DE VILENA et al., 2007; CARTEA et al., 2008; SARIKAMIŞ; YANMAZ, 2011).

High concentrations of aliphatic glucosinolates (BEEKWILDER et al., 2008) (in our case progoitrin,epiprogoitrin,gluconapin, glucoraphenin) are evaluated as an important factor determining resistance of these plants. The presence of progoitrin in the plants of oil radish and white mustard was below the limit of detection, which enables a wider spectrum of their use since the said aliphatic glucosinolate can cause negative effects in feeding of animals (VAN DOORN et al., 1998; PADILLA et al., 2007). Data obtained from our survey confirm the highest concentration of progoitrin in oil rape during the last assessment. Although stimulative effects of progoitrin is present.

The harmful pests' distinct preference for oil rape, in our case this holds true for both years of the field experiment (BOHINC; TRDAN, 2012), often deters farmers from planting the said species (VALANTIN-MORISON et al., 2007). We can relate the extent of damage to the concentration of gluconapin.

From the results of our study we can conclude that the concentration of progoitrin in cabbage hybrids is an important factor of selecting cabbage hybrids to be cultivated, since the concentration of the said glucosinolate in the two cabbage hybrids differed and caused different extent of injuries done by the studied harmful pests. The data obtained by our study show that higher values of glucobrassicin in above-the-ground parts of white mustard, oil radish, the early and the mid-late cabbage hybrid reduce the extent of injuries done by cabbage stink bugs. Stimulative effects of glucosinolates on these insects were found only in oilseed rape. We suggest oil rape as one of the plant species that can be used as a trap crop to allure cabbage stink bugs.

Correlations between glucosinolate contents in different Brassicas and damage caused by cabbage stink bugs

Stimulative influence of epiprogoitrin on feeding of cabbage stink bugs was found in the white mustard samples (r = 0.69), while the concentration of epiprogoitrin in oilseed rape plants negatively influenced feeding of cabbage stink bugs (r = -0.99) (Table 2). The concentration of glucobrassicin negatively influenced feeding of cabbage stink bugs on the plants of white mustard (r = -0.54), oil radish (r =-0.30), the early cabbage hybrid (r = -0.29), the medium-late cabbage (r = -0.59), while they stimulated feeding of bugs on oilseed rape (r = 0.24). Stimulative influence of progoitrin on feeding of cabbage stink bugs was found in the medium-late cabbage samples (r = 0.76) and in the oilseed rape samples (r = 0.51), while its concentration negatively influenced feeding on the early cabbage hybrid (r = -1.0). We established positive correlation between the concentration of gluconapin in the samples of the early hybrid (r = 0.31), the samples of oilseed rape (r = 0.64), and the extent of injuries, while negative influence of the said glucosinolate was established in the samples of the medium-late hybrid (r= -0.62).

The results of our study show the potential of trap crops to be used in ecological or integrated production of cabbage, which could be particularly important due to the increasing limitations in the use of synthetic insecticides (BJÖRKMAN et al., 2011), which are ever more frequently reflected even by complete absence of registered preparations (THE LIST OF REGISTERED…, 2011). Among such insects in the country where our study took place are cabbage gall midges *Contarinia nasturtii* (Kieffer), cabbage flies *Delia radicum* (L.) and also cabbage stink bugs *Eurydema* spp. Since the concentration of chemical substances in plant tissue of Brassicas is one of the main factors of susceptibility to injuries by herbivores (HOOKS; JOHNSON, 2003), which differs also between plant species.

Among the other cabbage pests, which occur on the area, where the present research was carried out, cabbage aphid (*Brevicoryne brassicae* [L.]) is known for its explotation of glucosinolates for the purpose of defending against natural enemies (BROEKGAARDEN et al., 2008), while stimulative effect of glucosinolates on oviposition was detected for cabbage butterfly (*Pieris brassicae* [L.]) (SMALLEGANGE et al., 2007). In one study the presence of sinigrin in Brassicas was confirmed to induce defence mechanisms against diamondback moth (*Plutella xylostella* [L.]) (SILVA CARVALHO et al., 2010).

Table 2. Correlations between concentrations of different glucosinolates in individual plant species and the extent of injuries done by cabbage stink bugs ($p < 0.005$ Duncan's multiple range test). Zgornja Lipnica, Slovenia. 2009/2010.

	r	P	Y
			White mustard
Epiprogoitrin	0.69	0.0037*	1.74265 – 0.433471*epiprogoitrin
Glucobrassicin	-0.54	0.0119*	2.45713 - 0.121135*glucobrassicin
Glucoraphenin	0.26	0.4902	2.40176 - 0.462470*glucoraphenin
Sinalbin	0.36	0.0996	1.55608 + 0.0113671*sinalbin
			Oil rape
Gluconasturtiin	-0.96	0.1333	7.65868 - 30.7078*gluconasturtiin
Glucobrassicin	0.24	0.3232	3.21056 + 0.171768*glucobrassicin
Gluconapin	0.64	0.009	2.09333 + 1.3665*gluconapin
Sinalbin	-0.68	0.2035	2.37452 - 0.0514846*sinalbin
Glucoraphenin	0.09	0.7148	2.373715 + 0.0252182*glucoraphenin
Epiprogoitrin	-0.93	0.0919*	7.32541 – 16.8443*epiprogoitrin
Progoitrin	0.51	0.1123	3.26631 + 0.451754*progoitrin
Glucoiberin	-1	-	4.8250-0.208333*glucoiberin
			Oil radish
Glucobrassicin	-0.33	0.1272	2.97621 - 0.2798455*glucobrassicin
Glucoraphenin	0.15	0.5100	2.23785 + 0.0213208*glucoraphenin
Sinalbin	0.40	0.3670	1.76274 + 1.28602*sinalbin
			'Tucana'
Glucobrassicin	-0.25	0.2318	1.77559 - 0.153006*glucobrassicin
Sinigrin	-0.32	0.4611	2.25673 - 1.36632*sinigrin
Gluconapin	0.31	0.3258	1.58063 + 0.88506*gluconapin
Glucoiberin	0.03	0.9915	1.83206 + 0.0105065*glucoiberin
Progoitrin	-1.0	-	2.54313 - 1.67504*progoitrin
			'Hinova'
Glucobrassicin	-0.59	0.0095*	2.30235 - 0.764395*glucobrassicin
Sinigrin	0.50	0.2124	0.297512 + 5.43967*sinigrin
Gluconapin	-0.62	0.1813	3.22748 - 5.78140*gluconapin
Glucoiberin	0.37	0.2078	1.66917 + 1.06091*glucoiberin
Progoitrin	0.76	0.2408	2.41404 + 2.218105*progoitrin

r = correlation coefficient; * = $p < 0.05$; Y = linear regression model which shows dependence of injuries done by cabbage stink bugs on contents of individual glucosinolates in a plant species.

In future we will have to perform even more studies on the influence of different Brassica species on bionomics of economically important insect pests (AL-ZYOUD et al., 2005; DE ALBUQUERQUE et al., 2006). Globally, Brassicas have a very wide spectrum of use (BJÖRKMAN, et al., 2011), and they are also very important in human diet. If we suppose that negative influence of glucosinolates on human diet is ruled out (BRANCA et al., 2002; PADILLA et al., 2007), the role of safely produced food is even more important.

Conclusion

Oil rape is the most adequate trap crop used to allure cabbage stink bugs;

In future glucosinolates should be employed to a greater extent in environmentally acceptable ways of food production.

Acknowledgements

The results presented in this paper were obtained by research work within the national project V4-1067, which is cofinanced by the Slovenian Research Agency, and the Ministry of Agriculture and the Environment of the Republic of Slovenia. We thank Dr. Dragan Žnidarčič for helping us to cultivate seedlings, Dr. Matej Vidrih for helping us to select seeds for trap crops and Jaka Rupnik for all the technical assistance.

References

AL-GENDY, A. A.; EL-GINDI, O. D.; HAFEZ, A. S.; ATEYA, A. M. Glucosinolates, volatile constituents and biological activities of *Erysimum corinthium* Boiss. (Brassicaceae). **Food Chemistry**, v. 118, n. 3, p. 519-524, 2010.

AL-ZYOUD, F.; TORT, N.; SENGONCA, N. Influence of leaf portion and plant species on the egg-laying behaviour of the predatory ladybird *Serangium parcesetosum* Sicard (Col., Coccinellidae) in the presence of a natural enemy. **Journal of Pest Science**, v. 78, n. 3, p. 167-174, 2005.

BARIĆ, B.; PAJAČ, I. Harmful stink bugs on cabbage and their control. **Glasilo Biljne Zaštite**, v. 11, n. 3, p. 214-217, 2011.

BEEKWILDER, J.; VAN LEEUWEN, W.; VAN DAM, N. M.; BETROSSI, M.; GRANDI, V.; MIZZI, L.; SOLOWIEV, M.; SZABADOS, L.; MOLTHOFF, J. W.; SCHIPPER, B.; VERBOCHT, H.; DE VOS, R. C. H.; MORANDINI, P.; AARTS, M. G. M.; BOVY, A. The impact of the absence of aliphatic glucosinolates on insect herbivory in *Arabidopsis*. **Plos One**, v. 3, p. 1-12, 2008.

BLAŽEVIĆ, I.; MASTELIĆ, J. Glucosinolate degradation products and other bound and free volatiles in the leaves and roots of radish (*Raphanus sativus* L.). **Food Chemistry**, v. 113, n. 1, p. 96-102, 2009.

BJÖRKMAN, M.; KLINGEN, I.; BIRCH, A. N. E.; BONES, A. M.; BRUCE T. J. A.; JOHANSEN, T. J.; MEADOW, R.; MØLMANN, J.; SELJÅSEN, R.;

SMART, L. E.; STEWART, D. Phytochemicals of Brassicaceae in plant protection and human health – Influences of climate, environment and agronomic practice. **Phytochemistry**, v. 72, n. 7, p. 538-556, 2011.

BOHINC, T.; TRDAN, S. Trap crops for reducing damage caused by cabbage stink bugs (*Eurydema* spp.) and flea beetles (*Phyllotreta* spp.) on white cabbage: fact or fantasy? **Journal of Food, Agriculture and Environment**, v. 10, n. 2, p. 1365-1370, 2012.

BROEKGAARDEN, C.; POELMAN, E. H.; STEENHUIS, G.; VOORRIPS, R. E.; DICKE, M.; VOSMAN, B. Responses of *Brassica oleracea* cultivars to infestation by the aphid *Brevicoryne brassicae*: an ecological and molecul aproach. **Plant, Cell and Environment**, v. 31, n. 11, p. 1592-1605, 2008.

BRANCA, F.; LI, G.; GOYAL, S.; QUIROS, C. Survey of aliphatic glucosinolates in Sicilian wild and cultivated Brassicaceae. **Phytochemistry**, v. 59, n. 7, p. 717-724, 2002.

BROWN, P. D.; MORRA, M. J. Hydrolysis products of glucosinolates in Brassica napus tissues as inhibitors of seed germination. **Plant and Soil**, v. 181, n. 2, p. 307-316, 1996.

CARTEA, M. E.; VELASCO, P.; OBRÉGON, S.; PADILLA, G.; DE HARO, A. Seasonal variation in glucosinolate content in *Brassica oleracea* crops grown in northwestern Spain. **Phytochemistry**, v. 69, n. 2, p. 403-416, 2008.

CARTEA, M. E.; VELASCO, P. Glucosinolates in Brassica foods: bioavability in food and significance for human health. **Phytochemistry Review**, v. 7, n. 2, p. 213-229, 2008.

CHAPLIN-KRAMER, R.; KLIEBENSTEIN, D. J.; CHIEM, A.; MORILL, E.; MMILLS, N. J., KREMEN, C. Chemically mediated tritrophic interactions: opposing effects of glucosinolates on a specialist herbivore and its predator. **Journal of Applied Ecolology**, v. 48, n. 4, p. 880-887, 2011.

COOK, S. M.; SMART, L. E.; MARTIN, J. L.; MURRAY, D. A.; WATTS, N. P.; WILLIAMS, I. H. Explotation of host plant preferences in pest management strategies for oilseed rape (*Brassica napus*). **Entomologia Experimentalis et Applicata**, v. 119, n. 3, p. 221-229, 2006.

DE ALBUQUERQUE, F. A.; CROCOMO, W. B.; SCAPIM, C. A. Influencia de sistemas de plantio e armadilha adesiva na incidencia de *Frankliniella williamsi* Hood na cultura do milho. **Acta Scientiarum. Agronomy**, v. 28, n. 3, p. 393-397, 2006.

DE VILLENA, F. A.; FRITZ, V. A.; COHEN, J. D.; HUTCHISON, V. D. Changes in gluconasturtiin concentrations in chinese cabbage with increasing cabbage looper density. **HortScience**, v. 42, n. 6, p. 1337-1340, 2007.

DEMIREL, N. Determination of heteroptera species on canola plants in Hatay province of Turkey. **African Journal of Agricultural Research**, v. 4, n. 11, p. 1226-1233, 2009.

ELTEZ, S.; KARSAVURAN, Y. Food preference in the cabbage bug *Eurydema ornatum* (L.) (Heteroptera: Pentatomidae). **Pakistan Journal of Zoology**, v. 42, n. 4, p. 407-412, 2010.

FAHEY, J. W.; ZALCMANN, A. T.; TALALAY, P. The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. **Phytochemistry**, v. 56, n. 1, p. 5-51, 2001.

FONT, R.; DEL RIO-CELESTINO, M.; CARTEA, E.; DE HARO-BAILÓN, A. Quantification of glucosinolates in leaves of leaf rape (*Brassica napus* ssp. *pabularia*) by near-infrared spectroscopy. **Phytochemistry**, v. 66, n. 2, p. 175-185, 2005.

GRIFFITHS, D. W.; DEIGHTON, N.; BIRCH, A. N. E.; PATRIAN, B.; BAUR, R.; STÄDLER, E. Identification of glucosinolates on the leaf surface of plants from the cruciferae and other closely related species. **Phytochemistry**, v. 57, n. 5, p. 693-700, 2001.

HASAN, F.; ANSARI, M. S. Population growth of *Pieris brassicae* (L.) (Lepidoptera: Pieridae) on different cole crops under laboratory conditions. **Journal of Pest Science**, v. 84, n. 2, p. 179-186, 2011.

HOPKINS, R. J.; EKBOM, B.; HENKOW, L. Glucosinolate content and suscebility for insect attack of three populations of *Sinapis alba*. **Journal of Chemical Ecolology**, v. 24, n. 7, p. 1203-1215, 1998.

HOOKS, C. R. R.; JOHNSON, M. W. Impact of agricultural diversification on the insect community of cruciferous crops. **Crop Protection**, v. 22, n. 2, p. 223-238, 2003.

JOHNSON, I. T. Glucosinolates in the human diet. Bioavailability and implications for health. **Phytochemistry Review**, v. 1, n. 2, p. 183-188, 2002.

KLIEBENSTEIN, D. J.; GERSHENZON, J.; MITSCHELL-OLDS, T. Comparative quantative trait loci mapping of aliphatic, indolic and benzylic glucosinolate production in *Arabidopsis thaliana* leaves and seeds. **Genetics**, v. 159, n. 1, p. 359-370, 2001.

KLIEBENSTEIN, D. J.; KROYMAN, J.; MITSCHELL-OLDS, T. The glucosinolate myrosinaze system in an ecological and evolutionary context. **Current Opinion in Plant Biolology**, v. 8, n. 3, p. 264-271, 2005.

MOYES, C. L.; COLLIN, H. A.; BRITTON, G.; RAYBOULD, A. F. Glucosinolates and differential herbivory in wild populations of *Brassica oleracea*. **Journal of Chemical Ecology**, v 26, n. 11, p. 2625-2641, 2000.

MÜLLER, C. Role of glucosinolates in plant invasiveness. **Phytochemistry Review**, v. 8, n. 1, p. 227-242, 2009.

MÜLLER, R.; DE VOS, M.; SUN, J. Y.; SØNERBY, I. E.; HALKIER, B. A.; WITTSTOCK, U.; JANDER, G. Differential effects of indole and aliphatic glucosinolates on Lepidopteran herbivores. **Journal of Chemical Ecolology**, v. 36, n. 8, p. 905-913, 2010.

PADILLA, G.; CARTEA, M. E.; VELASCO, P.; DE HARO, A.; ORDÁS, A. Variation of glucosinolates in vegetable crops of *Brassica rapa*. **Phytochemistry**, v. 68, n. 4, p. 536-545, 2007.

RAYBOULD, A. F.; MOYES, C. L. The ecological genetics of aliphatic glucosinolates. **Heredity**, v. 87, n. 4, p. 383-391, 2001.

SARIKAMIŞ, C.; YANMAZ, R. Effects of cultivar and developmental stage on glucosinolates in garden cress (*Lepidium sativum* L.). **Journal of Medicinal Plants Research**, v. 5, n. 17, p. 4388–4392, 2011.

SILVA CARVALHO, J.; DE BORTOLI, S. A.; THULER, R. T.; MARCHI GOULART, R.; LINHARES VOLPE, H. X. Efeito de sinigrina aplicada em folhas de brássicas sobre características biológicos de *Plutella xylostella* (L.) (Lepidoptera: Plutellidae). **Acta Scientiarum. Agronomy**, v. 32, n. 1, p. 15-20, 2010.

SMALLEGANGE, R. C.; VAN LOON, J. J. A.; BLATT, S. E.; HARVEY, J. A.; AGERBIRK, N.; DICKE, M. Flower vs. leaf feeding by *Pieris brassicae*: glucosinolate-rich flower tissues are preferred and sustain higher growth rate. **Journal of Chemical Ecology**, v. 33, n. 10, p. 1831-1844, 2007.

STONER, K. A.; SHELTON, A. M. Effects of planting date and timing of growth stage on damage to cabbage by onion thrips (*Thrips tabaci*). **Journal of Econonomic Entomology**, v. 81, n. 4, p. 329-333, 1988.

STATGRAPHICS CENTURION XVI. Warrenton: Statpoint technologies Inc., 2009.

THE LIST OF REGISTERED PLANT PROTECTION PRODUCT. **Ministry of Agriculture, Forestry and Food.** Phytosanitary Administration of the Republic of Slovenia. Available from: <http://www.spletni2.furs.gov.si/FFS/REGSR/index.htm>. Access on: Oct. 10, 2011.

TRDAN, S.; VALIČ, N.; ŽNIDARČIČ, D.; VIDRIH, M.; BERGANT, K.; ZLATIČ, E.; MILEVOJ, L. The role of Chinese Cabbage as a trap crop for flea beetles (Coleoptera: Chrysomelidae) in production of white cabbage. **Scientia Horticulturae**, v. 106, n. 1, p. 12-24, 2005.

TRDAN, S.; ŽNIDARČIČ, D.; VALIČ, N. Field efficacy of three insecticides against cabbage stink bugs (Heteroptera: Pentatomidae) on two cultivars of white cabbage. **International Journal of Pest Management**, v. 52, n. 2, p. 79-87, 2006.

TRDAN, S.; VALIČ, N.; VOVK, I.; MARTELANC, M.; SIMONOVSKA, B., VIDRIH, R.; VIDRIH, M.; ŽNIDARČIČ, D. Natural resistance of cabbage against three insect pests. In: COLLIER, R. (Ed.). **IOBC/wprs Bulletin**, v. 51, p. 93-106, 2009.

VALANTIN-MORISON, M.; MEYNARD, J. M.; DORÉ, T. Effects of crop management and surrounding field environment on insect incidence in organic winter oilseed rape (*Brassica napus* L.). **Crop Protection**, v. 26, n. 8, p. 1108-1120, 2007.

VAN DOORN, H. E.; VAN DEN KRUK, G. C.; VAN HOLST, G. J.; RAAIJMAKERS-RUIJS, N. C. M. E.; POSTMA, E.; GROENEWEG, B.; JONGEN, W. H. F. The glucosinolates sinigrin and progoitrin are important determinants for taste preference and bitterness of brussels sprout. **Journal of the Science of Food and Agriculture**, v. 78, n. 1, p. 30-38, 1998.

VAN EYLEN, D.; BELLOSTAS, N.; STROBEL, B. W.; OEY, I.; HENDRICKS, M.; VAN LOEY, A.; SØRENSEN, H.; SØRENSEN, J. C. Influence of pressure/temperature treatments on glucosinolate conversion in broccoli (*Brassica oleracea* L. cv. *italica*). **Food Chemistry**, v.112, n. 3, p. 646–653, 2009.

VAUGHN, S. F.; BERHOW, M. A. Glucosinolate hydrolisis products from various plant sources: Ph effects, isolation, and purification. **Industrial Crop Production**, v. 21, n. 2, p. 693-202, 2005.

VELASCO, P.; CARTEA, M. E.; GONZALEZ, C.; VILAR, M.; ORDÁS, A. Factors affecting the glucosinolate content of kale (*Brassica oleracea acephala* group). **Journal of Agricultural and Food Chemistry**, v. 55, n. 2, p. 955-962, 2007.

VIG, A. P.; RAMPAL, G.; THIND, T. S.; ARORA, S. Bio-protective effects of glucosinolates – a review. **LWT – Food Science and Technology**, v. 42, n. 10, p. 1561-1572, 2009.

YANG, B.; QIU, D.; QUIROS, C. F. Variation of five major glucosinolate genes in *Brassica rapa* in relation to *Brassica oleracea* and *Arabidopsis thaliana*. **Spanish Journal of Agricultural Research**, v. 8, n. 3, p. 662–671, 2010.

WINDE, I.; WITTSTOCK, U. Z. Insect herbivore counteradaptions to the plant glucosinolate-myrosinase system. **Phytochemistry**, v. 72, n. 13, p. 1566-1575, 2011

Received on December 27, 2011.
Accepted on March 19, 2012.

2.2 VSEBNOST GLUKOZINOLATOV KOT OBRAMBNI MEHANIZEM KRIŽNIC PRED NAPADOM KAPUSOVIH BOLHAČEV

BOHINC, Tanja, KOŠIR, Iztok, Jože in TRDAN Stanislav
Glucosinolates as arsenal for defending *Brassicas* against cabbage flea beetles attack.
Zemdirbyste-Agriculture, 2013, 100: 199-204.
Prejeto: 19.10.2012; sprejeto: 12.04.2013

Prehranjevanje kapusovih bolhačev (*Phyllotreta* spp.) na različnih vrstah križnic lahko vpliva na manjšo produktivnost rastlin. V želji po zmanjševanju rabe sintetičnih insekticidov za zatiranje kapusovih bolhačev je bila naša raziskava usmerjena v preučevanje naravne odpornosti krmne ogrščice, bele gorjušice, oljne redkve in dveh kultivarjev zelja na napad kapusovih bolhačev. Rezultati raziskave, ki je potekala v letu 2010, so pokazali, da se vsebnost glukozinolatov razlikuje med posameznimi rastlinskimi vrstami in tudi različnimi rastlinskimi organi. Med indolnimi glukozinolati smo prisotnost glucobrasicina ugotovili v vseh rastlinskih vrstah, vendar pa se njegov vpliv na prehranjevanje kapusovih bolhačev po posameznih rastlinskih vrstah razlikuje. Ugotovili smo stimulativen vpliv progoitrina (r=0,51), sinalbina (r=0,61), glukonapina (r=0,67) na prehranjevanje kapusovih bolhačev na krmni ogrščici, medtem ko je glukonasturtiin (r=-0,99) negativno vplival na prehranjevanje kapusovih bolhačev. Poškodbe kapusovih bolhačev so bile na krmni ogrščici zelo izrazite, vendar pa pri tej rastlinski vrsti vpliva glukonasturtiina in glukoiberina na škodljivost teh vrst žuželk nismo potrdili. Uporaba oljne redkve kot privabilnega posevka se je izkazala za najbolj ustrezno iz vidika privabljanja kapusovih bolhačev. Lahko trdimo, da glukozinolati predstavljajo pomemben vir odpornosti rastlin pred napadom kapusovih bolhačev, vendar bodo potrebne še nadaljnje raziskave, da bodo obravnavani sekundarni metaboliti dobili večji pomen v varstvu rastlin.

ISSN 1392-3196 Zemdirbyste-Agriculture Vol. 100, No. 2 (2013) 199

ISSN 1392-3196 / e-ISSN 2335-8947
Zemdirbyste-Agriculture, vol. 100, No. 2 (2013), p. 199–204
DOI 10.13080/z-a.2013.100.026

Glucosinolates as arsenal for defending *Brassicas* against cabbage flea beetle (*Phyllotreta* spp.) attack

Tanja BOHINC[1], Iztok Jože KOŠIR[2], Stanislav TRDAN[1]

[1]University of Ljubljana
Jamnikarjeva 101, SI-1111 Ljubljana, Slovenia
E-mail: tanja.bohinc@bf.uni-lj.si

[2]Slovenian Institute of Hop Research and Brewing
SI-3310 Žalec, Slovenia

Abstract

Feeding of cabbage flea beetles on various *Brassica* species can reduce crop productivity. While progressing towards the goal of reducing the use of synthetic pesticides and promotion of environmental protection, we wish to exploit plants' natural resilience. The results of our study carried out in 2009–2010 show that glucosinolate contents vary with plant species and plant organs. Among the indole glucosinolates, all *Brassica* species (cabbage, oil radish, oil seed rape and white mustard) displayed the presence of glucobrassicin, whose influence on cabbage flea beetles varied according to the plant species. We established that gluconasturtiin content in oil seed rape negatively ($r = -0.99$) influenced the feeding of flea beetles, while the progoitrin ($r = 0.51$), sinalbin ($r = 0.61$) and gluconapin ($r = 0.67$) stimulated the feeding of flea beetles in this crop. No significant influence of glucobrassicin on flea beetles was detected in oilseed rape, while this glucosinolate negatively influenced the intensity of flea beetles feeding in oil radish and white mustard ($r = -0.32$, $r = -0.64$). Oil radish thus proved to be the most suitable species as a trap crop for flea beetles. We conclude that protection of the *Brassicas* against flea beetles can depend on glucosinolate content, but additional tests to confirm practical meaning of this study in environmentally acceptable cabbage production are required.

Key words: cabbage, glucosinolates, oil radish, oil seed rape, *Phyllotreta* spp., white mustard.

Introduction

Plants can react to herbivores in two ways – by defending and by developing tolerance (Breuker et al., 2007). Besides morphological defensive factors, e.g., waxy epidermis (Trdan et al., 2009), trichomes (Carmona et al., 2011) etc., plants can defend against harmful pests also with secondary metabolites (Breuker et al., 2007; Jõgar et al., 2008).

The typical secondary metabolites, characteristic of the *Brassicaceae*, and consequently affecting interactions between herbivores and the target plants, are glucosinolates (Bidart-Bouzat, Kliebenstein, 2008). Glucosinolates are present primarily in the *Brassicas* (Kim, Jander, 2007), but their influence on the resistance of plants to bacteria and fungi has also been proved (Griffiths et al., 2001). According to the origin (the amino acid they are derived from), glucosinolates are classified into three groups (classes) – aliphatic, indole and aromatic (Padilla et al., 2007). Cabbage flea beetles (*Phyllotreta* spp.) are among major harmful pests causing damage to the *Brassicas* (Trdan et al., 2005). Feeding on *Brassica* seedlings can cause plant death, while feeding at later developmental stages can reduce final yield due to uneven growth of plants (Trdan et al., 2005; Tansey et al., 2009). It has been established that during feeding cabbage flea beetles can transmit pathogens from infected to healthy plants (Toshova et al., 2009). Among the proven successful alternative methods of suppressing cabbage flea beetles are trap cropping (Trdan et al., 2005; Bohinc, Trdan, 2012), different times of sowing, different densities of plants per plot and crop rotation (Toshova et al., 2009).

Given that growing different plant species on a certain plot reduces the density of a harmful pest population (Bohinc, Trdan, 2012), and the secondary metabolites content (glucosinolates) varies among plant species (Moyes et al., 2000), we wished to study susceptibility of various *Brassicas* to cabbage flea beetles in view of using the trap cropping method in an environmentally friendly food production strategy.

The aim of our research was to determine glucosinolate content in different *Brassica* species during the growing season, to acquire the information about the suitability of different trap crops to flee beetle attack. In this way we would gain the results, which would be useful for growing cabbage without the use of synthetic insecticides.

Materials and methods

Plant material. A two-year (2009–2010) field experiment was carried out in the village Zgornja Lipnica (46°19′ N latitude, 14°10′ E longitude, 511 m a.s.l.) in Slovenia. During the growth period of cash crop (two hybrids of cabbage) in 2010, we were collecting samples of the selected above-ground parts of oil seed rape (*Brassica napus* L. ssp. *oleifera* f. *biennis*), cv. 'Daniela' (supplier: Semenarna Ljubljana Ltd., Ljubljana, Slovenia), white mustard (*Sinapis alba* L.), cv. 'Zlata' (supplier: Semenarna Ljubljana Ltd., Ljubljana, Slovenia), oil radish (*Raphanus sativus* L. var. *oleiformis*), cv. 'Apoll' (supplier: Semenarna Ljubljana

Ltd., Ljubljana, Slovenia), the early hybrid of white cabbage (*Brassica oleracea* L. var. *capitata* f. alba), cv. 'Tucana' (supplier: Semenarna Ljubljana Ltd., Ljubljana, Slovenia) and the medium-late cabbage hybrid (*Brassica oleracea* L. var. *capitata* f. alba), cv. 'Hinova' (producer: Bejo Zaden, the Netherlands; supplier: Agroprogress Ltd., Ljubljana, Slovenia).

Field evaluation. The field of 528 m² was divided into four blocks. The three species of trap crops (oil seed rape, oil radish, white mustard) were sown within the blocks in separate treatments. The fourth treatment was the control, where no trap crop was sowed (bare surface). The arrangement of different *Brassicas* in our experiment is presented in Figure. In each treatment we also sowed both cabbage hybrids in separate sub-plots. Cabbage hybrids were the main crops in our experiment. The treatments within blocks were arranged randomly. The trap crops which were used for chemical analysis were sowed on 19 April 2010, while the cabbage seedlings were transplanted outdoors on 26 April 2010. The damage done on *Brassicas* by flea beetles was assessed by the 5-grade visual scale. The plants were evaluated on a scale from 1 (no damage) to 5 (more than 25% leaf area eaten), as follows: 2) up to 2% leaf area eaten, 3) between 3% and 10% leaf area eaten and 4) 11–25% leaf area eaten (OEPP/EPPO, 2002). The seedlings of cabbage were grown in the Department of Agronomy's glasshouse at the Biotechnical Faculty, University of Ljubljana, Slovenia according to the protocol as described in Trdan et al. (2009).

		cabbage		
4th block	white mustard	Tucana Hinova	White mustard	4th block
	oil seed rape	Tucana Hinova	oil seed rape	
	control	Tucana Hinova	control	
	oil radish	Tucana Hinova	oil radish	
3rd block	oil seed rape	Tucana Hinova	oil seed rape	3rd block
	control	Tucana Hinova	control	
	white mustard	Tucana Hinova	white mustard	
	oil radish	Tucana Hinova	oil radish	
2nd block	control	Tucana Hinova	control	2nd block
	oil seed rape	Tucana Hinova	oil seed rape	
	oil radish	Tucana Hinova	oil radish	
	white mustard	Tucana Hinova	white mustard	
1st block	control	Tucana Hinova	control	1st block
	oil seed rape	Tucana Hinova	oil seed rape	
	white mustard	Tucana Hinova	white mustard	
	oil radish	Tucana Hinova	oil radish	

Figure. Arrangement of different *Brassicas* in the field experiment

Zgornja Lipnica, Slovenia

Determination of glucosinolates. Plant material for the analysis of glucosinolates was sampled at different intervals. The plant material was cut down with scissors. Four samples of leaves or blossoms (flowers) of the species were collected on each assessment date. Individual samples (a part of the species) represented a representative sample of the plants in one plot. On certain assessment dates we collected, besides the samples of white mustard leaves (8 June, 3 July) and oil radish leaves (8 and 25 June, 31 July), also samples of blossoms of the trap crop species tested. Sampling of cabbage leaves was carried out at five different intervals, on 26 May, 16 June, 4 and 29 July, and 17 August 2010. Sampling of leaves of oil seed rape was carried out at five different intervals (19 May, 8 June, 3 and 31 July, and 31 August), while the sampling of oil radish and white mustard was performed at four intervals (the former: 19 May, 8 and 25 June, and 31 July; the latter 19 May, 8 and 25 June, and 3 July). Four samples of individual plant species were collected at individual intervals of assessment. An individual sample (leaves or blossoms or flowers) was the representative plant sample of one block. When analysing glucosinolates, the analysis of individual samples was repeated twice. The material was then freeze-dried (type: LIO-10P, producer: Kambič laboratorijska oprema, Slovenia) and homogenized before extraction of glucosinolates. The lyophilised samples were stored in 50 ml bottles in a freezer (type: U3286S, producer: Sanyo) at −80°C. The glucosinolate extraction and analysis were performed according to ISO 9167:1-1992. As internal standards sinigrin or glucotropaeolin (C, Bioengineering ApS, Denmark) were added. The extracted glucosinolates were purified on a 1.5 cm DEAE Sephadex A-25 anion exchange column. The column was washed twice with 1 ml distilled water loaded with 2 ml of the glucosinolates extract and then washed twice with 1 ml 20 mM NaAc-solution and treated with sulphatase (75 μL and 25 mg ml^{-1}). After an overnight reaction at room temperature the desulfoglucosinolates were eluted with distilled water (2 × 1 ml). The eluate was filtered over 0.45 μm filter and then sample was ready for HPLC (high performance liquid chromatography) analysis.

For glucosinolate (GLS) quantification, twenty microlitres of desulfoglucosinolates solution were run on an Agilent 1200 Series HPLC system (Palo Alto, USA) at 2 ml min^{-1}. The column was a Discovery C18, 25 cm × 4.6 mm, 5 μm (Supelco). The mobile phases were water and methanol, running time: 28 min. The gradient changed as follows: 100% A for 1 min, then in 20 min to 20% B, followed by 100% B for 5 min. Afterwards the column was equilibrated at 100% A for 3 min. The desulfoglucosinolates were detected with diode array detector at 229 nm. The desulfoglucosinolates were identified with external standards (C, Bioengineering ApS, Denmark). The certified reference material used was BCR-367R. The content of each GLS was back calculated and expressed in micromoles per gram (μmol g^{-1}) of dry seed.

Data analysis. Prior to statistical analysis, the experimental results were statistically evaluated by the program *Statgraphics Centurion XVI* (2009). The differences between values of glucosinolates during the growth period and between individual plant species were calculated by analysing the variance (*ANOVA*) and Duncan's test of multiple comparisons ($P < 0.05$). We calculated correlations between the concentration of an individual glucosinolate and level of injury caused by flea beetles on the plant species.

Results and discussion

Content of glucosinolates in different Brassicas. The results of the statistical analysis show that the glucoiberin content is significantly influenced by plant species ($P = 0.0233$, $F = 4.39$, Df = 2), while the content between leaves and blossoms (flowers) does not vary ($P = 0.3544$, $F = 0.3544$, Df = 3). The glucoiberin content in the samples of white mustard, oil seed rape, oil radish was below detection limit (in all cases <0.1 μmol g^{-1} of dry seed (ds), while the samples of the cabbage hybrid 'Hinova' had the average content 0.33 ± 0.08 μmol g^{-1} ds, and the samples of the hybrid 'Tucana'

ISSN 1392-3196 Zemdirbyste-Agriculture Vol. 100, No. 2 (2013) 201

0.38 ± 0.09 µmol g⁻¹ ds (Table 1). The *Brassicaceae* species used in our experiment displayed no significant differences in the glucoiberin content ($P = 0.0656$, $F = 3.55$, $Df = 1$), while the glucoraphenin content was significantly influenced by individual *Brassicaceae* species ($P = 0.0003$, $F = 9.89$, $Df = 2$). The average glucoraphenin content was significantly higher in oil radish (8.66 ± 1.81 µmol g⁻¹ ds), while it was precisely above the detection limit in the samples of both cabbage hybrids (<0.1 µmol g⁻¹ ds). The epiprogoitrin content was influenced by individual species ($P = 0.0055$, $F = 10.29$, $Df = 2$), significant differences were detected also between leaves and blossoms (flowers) of the plants ($P < 0.0001$, $F = 32.15$, $Df = 1$).

Table 1. Average values of glucosinolates occurring in different *Brassica* species (in µmol g⁻¹ ds)

Systematic name	Trivial name	Oil radish	Oil seed rape	White mustard	Cabbage	
					'Hinova'	'Tucana'
Aliphatic						
3-Methylsulfinylpropyl	Glucoiberin	<0.1	<0.1	<0.1	0.33 ± 0.08	0.38 ± 0.09
2 (R)-2-Hydroxy-3-butenyl	Progoitrin	<0.1	1.55 ± 0.51	<0.1	1.13 ± 0.02	0.27 ± 0.03
2 (S)-2-Hydroxy-3-butenyl	Epiprogoitrin	–	0.19 ± 0.06	2.45 ± 0.38	<0.1	<0.1
2-Propenyl	Sinigrin			–	0.30 ± 0.03	0.35 ± 0.06
3-Butenyl	Gluconapin	–	0.38 ± 0.05	–	0.17 ± 0.03	0.24 ± 0.10
4-Methylsulfinyl-3-butenyl	Glucoraphenin	8.66 ± 1.81	0.99 ± 0.87	0.51 ± 0.19	<0.1	<0.1
Indole						
3-Indolmethyl	Glucobrassicin	3.24 ± 0.86	1.39 ± 0.49	1.71 ± 0.88	0.40 ± 0.15	0.79 ± 0.27
Aromatic						
2-Phenylethyl	Gluconasturtiin	<0.1	0.13 ± 0.04	–	<0.1	<0.1
4-Hydroxybenzyl	Sinalbin	0.36 ± 0.12	11.16 ± 6.50	30.12 ± 5.52	<0.1	<0.1

Note. <0.1 – glucosinolate present below detection level.

The content of the aliphatic glucosinolate was in comparison to leaves (0.21 ± 0.03 µmol g⁻¹ ds) significantly higher in the blossom samples (2.81 ± 0.01 µmol g⁻¹ ds) (Table 2). Plant species did not significantly affect the progoitrin content ($P = 0.5088$, $F = 0.80$, $Df = 3$), the content also did not differ between leaves and blossoms (flowers) ($P = 0.5551$, $F = 0.72$, $Df = 3$).

Table 2. Average values of glucosinolates occurring in different *Brassica* species (µmol g⁻¹ ds) through growth period, related to different plant parts

Date	Plant species	Plant part	GIB	PRO	SIN	E-PRO	GRE	GNA	SLB	GBS	GST
19.05	oil radish	leaves	<0.1	<0.1			0.22 ± 0.13	<0.1	0.12 ± 0.09	3.84 ± 0.07	<0.1
08.06	oil radish	leaves	<0.1	<0.1			0.11 ± 0.10	<0.1	<0.1	2.15 ± 0.01	<0.1
08.06	oil radish	flowers	<0.1	<0.1	–	–	13.52 ± 0.10	<0.1	0.53 ± 0.04	2.22 ± 0.06	<0.1
25.06	oil radish	leaves	<0.1	<0.1	–	–	0.29 ± 0.10	<0.1	0.52 ± 0.05	2.24 ± 0.04	<0.1
25.06	oil radish	flowers	<0.1	<0.1	–	–	16.42 ± 0.05	<0.1	0.23 ± 0.11	10.87 ± 0.17	<0.1
31.07	oil radish	leaves	<0.1	<0.1	–		2.86 ± 0.05	<0.1	<0.1	1.14 ± 0.05	<0.1
31.07	oil radish	flowers	<0.1	<0.1	–		16.71 ± 0.84	<0.1	<0.1	0.53 ± 0.11	<0.1
19.05	white mustard	leaves	<0.1	<0.1	–	0.24 ± 0.11	0.22 ± 0.13	–	34.77 ± 0.09	6.39 ± 0.34	–
08.06	white mustard	leaves	<0.1	<0.1	–	<0.1	0.18 ± 0.01		5.51 ± 0.11	<0.1	–
08.06	white mustard	flowers	<0.1	<0.1	–	2.14 ± 0.10	<0.1		25.85 ± 0.32	0.08 ± 0.02	–
25.06	white mustard	flowers	<0.1	<0.1	–	1.39 ± 0.04	0.09 ± 0.01		71.77 ± 0.19	0.18 ± 0.02	–
03.07	white mustard	leaves	<0.1	<0.1	–	<0.1	0.82 ± 0.04		6.73 ± 1.45	0.21 ± 0.09	
03.07	white mustard	flowers	<0.1	<0.1		2.30 ± 0.05	<0.1	–	56.53 ± 0.07	0.15 ± 0.06	
19.05	oil seed rape	leaves	<0.1	1.03 ± 0.04	–	0.375 ± 0.04	0.13 ± 0.05	0.22 ± 0.04	18.41 ± 0.15	2.16 ± 0.04	0.21 ± 0.15
08.06	oil seed rape	leaves	<0.1	0.15 ± 0.02		<0.1	3.82 ± 0.15	0.20 ± 0.05	<0.1	0.17 ± 0.05	<0.1
03.07	oil seed rape	leaves	<0.1	0.23 ± 0.03		<0.1	0.08 ± 0.06	0.29 ± 0.04	<0.1	0.33 ± 0.09	<0.1
31.07	oil seed rape	leaves	<0.1	0.27 ± 0.04	–	<0.1	0.12 ± 0.04	0.40 ± 0.17	<0.1	0.45 ± 0.12	<0.1
31.08	oil seed rape	leaves	<0.1	2.64 ± 0.26	–	0.13 ± 0.01	0.13 ± 0.05	0.60 ± 0.12	<0.1	4.20 ± 0.17	0.08 ± 0.05
	Cabbage:										
26.05	'Hinova'	leaves	0.16 ± 0.02	<0.1	0.25 ± 0.21	<0.1	<0.1	<0.1	<0.1	1.46 ± 0.02	<0.1
16.06	'Hinova'	leaves	<0.1	<0.1	0.25 ± 0.22	<0.1	<0.1	<0.1	<0.1	0.03 ± 0.02	<0.1
04.07	'Hinova'	leaves	0.11 ± 0.02	<0.1	<0.1	<0.1	<0.1	0.25 ± 0.03	<0.1	0.04 ± 0.01	<0.1
29.07	'Hinova'	leaves	0.82 ± 0.16	1.13 ± 0.07	<0.1	<0.1	<0.1	0.15 ± 0.02	<0.1	0.04 ± 0.04	<0.1
17.08	'Hinova'	leaves	0.22 ± 0.13	<0.1	0.35 ± 0.16	<0.1	<0.1	<0.1	<0.1	0.36 ± 0.01	<0.1
26.05	'Tucana'	leaves	0.35 ± 0.04	<0.1	0.41 ± 0.09	<0.1	<0.1	0.14 ± 0.02	<0.1	2.34 ± 0.05	<0.1
16.06	'Tucana'	leaves	<0.1	<0.1	<0.1	<0.1	<0.1	0.14 ± 0.02	<0.1	0.35 ± 0.05	<0.1
04.07	'Tucana'	leaves	<0.1	<0.1	<0.1	<0.1	<0.1	0.06 ± 0.02	<0.1	0.04 ± 0.04	<0.1
29.07	'Tucana'	leaves	0.56 ± 0.13	0.27 ± 0.12	<0.1	<0.1	<0.1	0.49 ± 0.15	<0.1	1.23 ± 0.05	<0.1
17.08	'Tucana'	leaves	0.35 ± 0.12	<0.1	0.31 ± 0.04	<0.1	<0.1	0.14 ± 0.02	<0.1	0.15 ± 0.12	<0.1

Note. GIB – glucoiberin, PRO – progoitrin, SIN – sinigrin, E-PRO – epiprogoitrin, GRE – glucoraphenin, GNA – gluconapin, SLB – sinalbin, GBS – glucobrassicin, GST – gluconasturtiin; <0.1 – glucosinolate present below detection level.

Among indole glucosinolates glucobrassicin was detected in all plant species, its content, however, varied significantly among the plant species ($P = 0.0282$, $F = 3.15$, $Df = 3$), from 0.40 ± 0.15 µmol g⁻¹ ds in the samples of the cabbage hybrid 'Hinova' to 3.24 ± 0.86 µmol g⁻¹ ds in the samples of oil radish (Table 1). No significant differences in glucobrassicin content of individual above-ground plant organs were detected ($P = 0.1273$, $F = 1.94$, $Df = 3$). In detecting aromatic glucosinolates we established that the gluconasturtiin content was influenced by the *Brassicaceae* species ($P = 0.04643$, $F = 0.12$, $Df = 1$). There were no differences detected in the gluconasturtiin content of various plant organs ($P = 0.7643$, $F = 0.49$, $Df = 1$). Gluconasturtiin was in oil radish samples present in traces (<0.1 µmol g⁻¹ ds), meanwhile its presence in cabbage samples was below detection level. The sinalbin content varied with different plant species ($P = 0.0090$, $F = 5.50$, $Df = 1$), as well as with individual plant organs ($P = 0.0001$, $F = 20.84$, $Df = 1$). The sinalbin content was significantly higher in the samples of white mustard (30.12 ± 5.52 µmol g⁻¹ ds); in the samples of oil seed rape it was 11.16 ± 6.50 µmol g⁻¹ ds, while in the samples of cabbage, early and mid-late hybrid, no sinalbin was detected.

Correlation between level of injury caused by cabbage flea beetles on plants belonging to trap crop and concentration of glucosinolate. We discovered a very high negative correlation ($r = -0.99$) between the gluconasturtiin content and level of injury done by flea beetles (Table 3). A low negative correlation ($r = -0.22$) was established between the glucoraphenin content in the oil seed rape samples and level of injury. A stimulating influence of epiprogoitrin on feeding of cabbage flea beetles was detected in the white mustard samples ($r = 0.56$), while the epiprogoitrin content negatively influenced feeding habits on oil seed rape ($r = -0.80$). The correlation between level of injury and the glucoiberin content in the samples of oil seed rape is explained with the model Ŷ = 4.15 − 0.416667*. The results of the statistical analysis show stimulating influence of glucobrassicin on feeding of the species from the genus *Phyllotreta* on oil seed rape ($r = 0.39$), while higher glucobrassicin content in the oil radish ($r = -0.32$) and white mustard ($r = -0.64$) negatively influenced the intensity of the flea beetles feeding (Table 3). A moderate positive correlation ($r = 0.67$) was established between level of injury done by flea beetles and gluconapin content in the oil seed rape samples. In the oil seed rape samples we also detected stimulating influence of sinalbin ($r = 0.61$) and progoitrin ($r = 0.51$) on feeding intensity of flea beetles.

Table 3. Correlation between level of injury caused by *Phyllotreta* spp. and glucosinolate (GLS) concentration ($P < 0.05$ Duncan's multiple range test) in three trap crop species

GLS	Oil seed rape				Oil radish				White mustard			
	r	*p*	a	b	*r*	*p*	a	b	*r*	*p*	a	b
GST	−0.99	0.0646	4.5913	−6.7922			x				–	
GRE	0.22	0.3863	3.4482	−0.0293	0.11	0.6239	3.3539	0.0099	0.09	0.8127	2.7598	−0.0739
E-PRO	−0.80	0.1950	4.1831	−2.7732			–		0.56	0.0364*	2.5833	0.13010
GIB	−1.00	–	4.1500	−0.4166			x				x	
GBS	0.39	0.1062	3.3010	0.0812	−0.32	0.0998	3.5159	−0.0478	−0.64	0.0019*	2.9538	−0.0635
GNA	0.67	0.0045*	2.8510	1.5742			x					
SLB	0.61	0.2724	3.0531	0.0109	0.13	0.7780	2.8938	0.1441	0.04	0.8526	2.9151	0.0005
PRO	0.51	0.1035	3.5192	0.1461			x				x	
SIN											–	

Notes. GST – gluconasturtiin, GRE – glucoraphenin, E-PRO – epiprogoitrin, GIB – glucoiberin, GBS – glucobrassicin, GNA – gluconapin, SLB – sinalbin, PRO – progoitrin, SIN – sinigrin. x – because glucosinolate was present at trace level (<0.1 µmol g^{-1} ds) we were not able to calculate correlation, *r* – correlation coefficient, * – $p < 0.05$, a – intercept, b – slope.

Correlation between level of injury caused by flea beetles on cash crops and glucosinolate concentration. A moderate positive correlation was established between the glucoiberin content in the mid-late hybrid samples 'Hinova' and level of injury done by flea beetles ($r = 0.52$), while the correlation between the glucosinolate content and the level of injury on the early hybrid 'Tucana' plants is explained by the model Ŷ = 2.39784 − 0.0448347* glucoiberin (Table 4). The analysis of results shows stimulating influence of progoitrin on the intensity of feeding by flea beetles on the early hybrid plants ($r = 1$), while the correlation between the level of injury on the mid-late hybrid and the glucosinolate content is explained by the model Ŷ = 2.25781 − 0.079386* progoitrin. The analysis of results shows low (positive) correlation between the level of injury done by flea beetles and the sinigrin content in the mid-late hybrid samples ($r = 0.31$). Generally speaking, oil radish proved to be the most efficient trap crop, which was also confirmed in our previous study (Bohinc, Trdan, 2012). Significant preference towards feeding on white mustard was not shown in our study, nor in a similar experiment (Hiiesaar et al., 2006). We confirmed the findings of some researchers who found out that the glucosinolate content varied between different *Brassicaceae* species (Moyes et al., 2000), between plant organs (Bellostas et al., 2007), and the concentrations of these secondary metabolites varied also during the growth period (De Villena et al., 2007). Differences in the glucosinolate content were detected also in a single species – between two cabbage hybrids (Bellostas et al., 2007). In the studied *Brassicaceae* we detected aliphatic glucosinolates which are characteristic also of other *Brassicaceae* species (Cartea et al., 2008); their presence is genetically predetermined (Agerbirk et al., 2009).

Among indole glucosinolates we established the presence of glucobrassicin whose content was to some extent probably influenced also by temperatures (Kim, Jander, 2007). While the research results show negative influence of glucosinolates on generalists (Pontoppidan et al., 2005), stimulating influence is most frequently established with the specialists who feed exclusively on *Brassicaceae* (Nielsen et al., 2001). There is, however, not much known about the significance of individual glucosinolates in plant protection (Kim, Jander, 2007). In view of the fact that some plants (Bidart-Bouzat, Kliebenstein, 2008), including oil seed rape, are very susceptible to level of injury by various

Table 4. Correlation between level of injury caused by *Phyllotreta* spp. and glucosinolate (GLS) concentration ($P < 0.05$ Duncan's multiple range test) in two different cabbage hybrids as main crops

GLS	'Tucana'				'Hinova'			
	r	*p*	a	b	*r*	*p*	a	b
GST			–				–	
GRE			x				x	
E-PRO			–					
GIB	−0.06	0.8649	2.3978	0.0448	0.52	0.0650	2.2123	0.3646
GBS	−0.08	0.7289	2.4449	−0.0134	−0.26	0.3010	2.3214	−0.0853
GNA	−0.08	0.8001	2.4226	0.0416	−0.03	0.9602	2.3601	−0.0773
SLB							–	
PRO	1.00	–	2.3323	1.1166	0.55	0.4555	2.2578	0.0793
SIN	−0.07	0.8737	2.3999	−0.0797	0.31	0.4584	2.0568	0.7657

Explanations under Table 3

ISSN 1392-3196 Zemdirbyste-Agriculture Vol. 100, No. 2 (2013) 203

phytophagous insects (Valantin-Morison et al., 2007). These characteristic can be explained by the content of various chemical substances, also glucosinolates (Bidart-Bouzat, Kliebenstein, 2008). The effect of glucosinolates on feeding of various herbivorous species can vary (Agerbirk et al., 2008). Besides stimulating effect of progoitrin on feeding of cabbage flea beetles with oil seed rape and the main crops, its presence can also have negative effects if plants with excessive glucosinolate content are used as fodder (Padilla et al., 2007; Sun et al., 2011). Consequently, the emphasis is on plant breeding, though no negative effects on human nutrition have been detected (Sun et al., 2011). The potential negative influence of glucobrassicin on feeding of cabbage flea beetles, which we proved by our research, can positively influence human nutrition, since the decomposition products of indole glucosinolates have positive (anticarcinogenic) effects on human health (Sun et al., 2011). Beneficial to human health are also some other glucosinolates including sinigrin, glucoiberin, progoitrin and gluconasturtiin (Sun et al., 2011).

In the past (Bodnaryk, 1991), one of the major "chemical" factors of defending white mustard against attacks by cabbage flea beetles was considered the presence of sinalbin. Abundance of sinalbin was also evaluated as an important defensive factor against *Mamestra configurata* (Ulmer et al., 2001). However, its presence can also have a stimulating effect on *Pieris rapae* feeding (Agerbirk et al., 2009). Oil seed rape can be used for fodder (Valantin-Morison et al., 2007), white mustard as a means to prevent nitrate leaching from agricultural soil during winter (Dorsainvil et al., 2005), and as a living mulch (Romaneckas et al., 2012), oil radish as a plant species affects the balance of carbon in soil (Mutegi et al., 2011). Results of our research indicate a potentially wider application of the studied *Brassicaceae* – also to protect other cruciferous plants against harmful pests. Their applicability for protecting plants against the pests which are numerous and thus harmful in Europe is manifested by the fact that registered synthetic pesticides are on the decrease (The list…, 2012) as they negatively influence our environment (Bommarco et al., 2011).

Conclusions

1. The tested alternative method of cabbage protection against the flea beetle adults proved to be efficient. The level of injury caused by flea beetles was significantly the highest on oil radish, thus oil radish proved to be the most suitable as trap crop.

2. Glucosinolate content differed between plant species and even between plant organs of the same *Brassica* species. Sinigrin was only present in cabbage cultivars; gluconapin was present in oil seed rape and cabbage cultivars. Glucoraphenin and sinalbin contents were only confirmed in *Brassica* trap crops. In comparison to different plant parts, glucoraphenin was more abundant in oil radish flowers than in leaves. Epiprogoitrin was only abundant in oil seed rape and white mustard, but below detection level in cabbage cultivars. Progoitrin, as the most abundant in oil seed rape and cabbage cultivars, was also present in oil radish and white mustard, but below detection level (<0.1 μmol g^{-1} dry seed). The level of glucoiberin was significantly the highest in cabbage cultivars, while gluconasturtiin was the most abundant in oil seed rape.

3. Glucobrassicin was the most abundant glucosinolate detected in our study. It was the most abundant in oil radish.

4. Based on the results of our study, we conclude that the glucosinolates content in different *Brassicas* can play a significant role in selection of mentioned field crops in the areas, where the cabbage is exposed to numerous pests. Additional tests are undoubtedly needed to confirm the applicability of these results in environmentally acceptable cabbage production.

Acknowledgements

The results presented in this paper were obtained by research work within the national project V4-1067, which is cofinanced by the Slovenian Research Agency, The Ministry of Agriculture and the Environment of the Republic of Slovenia. Jaka Rupnik is acknowledged for technical assistance.

Received 19 10 2012
Accepted 12 04 2013

References

Agerbirk N., De Vos M., Kim J. H., Jander G. 2009. Indole glucosinolate breakdown and its biological effects. Phytochem Review, 8 (1): 101–120 http://dx.doi.org/10.1007/s11101-008-9098-0

Bellostas N., Kachlicki P., Sørensen J. C., Sørensen H. 2007. Glucosinolate profiling of seeds and sprouts of *B. oleracea* varieties used for food. Scientia Horticulturae, 114 (4): 234–242 http://dx.doi.org/10.1016/j.scienta.2007.06.015

Bidart-Bouzat M. G., Kliebenstein D. J. 2008. Differential levels of insect herbivory in the field associated with genotypic variation in glucosinolates in *Arabidopsis thaliana*. Journal of Chemical Ecology, 34 (8): 1026–1037 http://dx.doi.org/10.1007/s10886-008-9498-z

Bodnaryk R. P. 1991. Developmental profile of sinalbin (p-hydroxybenzyl glucosinolate) in mustard seedlings, *Sinapis alba* L. and its relationship to insect resistance. Journal of Chemical Ecology, 17 (8): 1543–1556 http://dx.doi.org/10.1007/BF00984687

Bohinc T., Trdan S. 2012. Trap crops for reducing damage caused by cabbage stink bugs (*Eurydema* spp.) and flea beetles (*Phyllotreta* spp.) on white cabbage: fact or fantasy. Journal of Food Agriculture and Environment, 10 (2): 1365–1370

Bommarco R., Miranda F., Bylund H., Björkman C. 2011. Insecticides suppress natural enemies and increase pest damage in cabbage. Journal of Economic Entomology, 104 (3): 782–791 http://dx.doi.org/10.1603/EC10444

Breuker C. J., De Jong P. W., Victorir K., Vrieling K., Brakefield P. M. 2007. Pleiotropic effects associated with an allele enabling the flea beetle *Phyllotreta nemorum* to use *Barbarea vulgaris* as host plant. Evolutionary Ecology, 21 (1): 13–26 http://dx.doi.org/10.1007/s10682-006-9121-0

Carmona D., Lajeunesse M. J., Johnson M. T. J. 2011. Plant traits that predict resistance to herbivores. Functional Ecology, 25 (2): 358–367 http://dx.doi.org/10.1111/j.1365-2435.2010.01794.x

Cartea M. E., Rodríguez V.M., de Haro A., Velasco P., Ordás A. 2008. Variation of glucosinolates and nutritional value in nabicol (*Brassica napus pabularia* group). Euphytica, 159 (1–2): 111–122

De Villena F. A., Fritz V. A., Cohen J. D., Hutchison V. D. 2007. Changes in gluconasturtiin concentrations in chinese cabbage with increasing cabbage looper density. HortScience, 42 (6): 1337–1340

Dorsainvil F., Dürr C., Justes E., Carrera A. 2005. Characterisation and modelling of white mustard (*Sinapis alba* L.) emergence under several sowing conditions. European Journal of Agronomy, 23 (2): 146–158 http://dx.doi.org/10.1016/j.eja.2004.11.002

Griffiths D. W., Deighton N., Birch A. N. E., Patrian B., Baur R., Städler E. 2001. Identification of glucosinolates on the leaf surface of plants from the *Cruciferae* and other closely related species. Phytochemistry, 57 (2): 693–700 http://dx.doi.org/10.1016/S0031-9422(01)00138-8

Hiiesaar K., Metspalu L., Jõgar K. 2006. Attractiveness and susceptibility of *Brassica rapa*, *B. napus* and *Sinapis alba* to the flea beetles (*Coleoptera: Chrysomelidae*). Agronomy Research, 4: 191–196

Jõgar K., Metspalu L., Hiiesaar K., Ploomi A., Kuusik A., Men'shykova A. L. 2008. Abundance of the small white (*Pieris rapae* L.) on different cabbage cultivars. Zemdirbyste-Agriculture, 95 (3): 88–93

Kim J. K., Jander G. 2007. *Myzus persicae* (green peach aphid) feeding on Arabidopsis induces the formation of a deterrent indole glucosinolate. Plant Journal, 49 (6): 1008–1019 http://dx.doi.org/10.1111/j.1365-313X.2006.03019.x

Moyes C. L., Collin H. A., Britton G., Raybould A. F. 2000. Glucosinolates and differential herbivory in wild populations of *Brassica oleracea*. Journal of Chemical Ecology, 26 (11): 2625–2641 http://dx.doi.org/10.1023/A:1005549115751

Mutegi J. K., Petersen B. J., Munkholm L. J., Hansen E. M. 2011. Belowground carbon input and translocation potential of fodder radish cover-crop. Plant Soil, 344 (1–2): 159–175 http://dx.doi.org/10.1007/s11104-011-0737-7

Nielsen J. K., Hansen M. L., Agerbirk N., Petersen B. L., Halkier B. A. 2001. Responses of the flea beetles *Phyllotreta nemorum* and *P. cruciferae* to metabolically engineered *Arabidopsis thaliana* with an altered glucosinolate profile. Chemoecology, 11 (2): 75–83 http://dx.doi.org/10.1007/PL00001835

OEPP/EPPO. 2002. Guidelines for the efficancy evaluation of insecticides. *Phyllotreta* spp. on rape. OEPP/EPPO Bulletin, 32: 361–365

Padilla G., Cartea M. E., Velasco P., de Haro A., Ordás A. 2007. Variation of glucosinolates in vegetable crops of *Brassica rapa*. Phytochemistry, 68 (4): 536–545 http://dx.doi.org/10.1016/j.phytochem.2006.11.017

Pontoppidan B., Hopkins R., Rask L., Meijer J. 2005. Differential wound induction of the myrosinase system in oilseed rape (*Brassica napus*): contrasting insect damage with mechanical damage. Plant Science, 168 (3): 715–722 http://dx.doi.org/10.1016/j.plantsci.2004.10.003

Romaneckas K., Adamavičienė A., Pilipavičius V., Šarauskis E., Avižienytė D., Buragienė S. 2012. Interaction of maize and living mulch. Crop weediness and productivity. Zemdirbyste-Agriculture, 99 (1): 23–30

Statgraphics Centurion XVI. 2009. Statpoint Technologies Inc., Warrenton, USA

Sun B., Liu N., Zhao Y., Yan, H., Wang Q. 2011. Variation of glucosinolates in three edible parts of Chinese kale (*Brassica alboglabra* Bailey) varieties. Food Chemistry, 124 (3): 941–947 http://dx.doi.org/10.1016/j.foodchem.2010.07.031

Tansey J. A., Dosdall L. M., Keddie B. A. 2009. *Phyllotreta cruciferae* and *Phyllotreta striolata* responses to insecticidal seed treatments with different models of action. Journal of Applied Entomology, 133 (3): 201–209 http://dx.doi.org/10.1111/j.1439-0418.2008.01321.x

The list of registrated plant protection product on 16.02.2012. The Ministry of Agriculture and the Environment of the Republic of Slovenia. Phytosanitary Administration of the Republic of Slovenia. <http://spletni2.furs.gov.si/FFS/REGSR/index.htm> [accessed 16 02 2012] (in Slovenian)

Toshova T. B., Csonka E., Subschev M. A., Tóth M. 2009. The seasonal activity of flea beetles in Bulgaria. Journal of Pest Science, 82 (3): 295–303 http://dx.doi.org/10.1007/s10340-009-0254-0

Trdan S., Valič N., Žnidarčič D., Vidrih M., Bergant K., Zlatič E., Milevoj L. 2005. The role of Chinese cabbage as a trap crop for flea beetles (*Coleoptera*: *Chrysomelidae*) in production of white cabbage. Scientia Horticulturae, 106 (1): 12–24 http://dx.doi.org/10.1016/j.scienta.2005.03.005

Trdan S., Valič N., Vovk I., Martelanc M., Simonovska B., Vidrih R., Vidrih M., Žnidarčič D. 2009. Natural resistance of cabbage against three insect pests. Integrated Protection of Field Vegetables. Collier R. (ed.). IOBC/wprs Bulletin, 51: 93–106

Ulmer B., Gillot C., Erlandson M. 2001. Feeding preferences, growth, and development of *Mamestra configurata* (*Lepidoptera*: *Noctuidae*) on *Brassicaceae*. Canadian Entomologist, 133 (4): 509–519 http://dx.doi.org/10.4039/Ent133509-4

Valantin-Morison M., Meynard J. M., Doré T. 2007. Effects of crop management and surrounding field environment on insect incidence in organic winter oilseed rape (*Brassica napus* L.). Crop Protection, 26 (8): 1108–1120 http://dx.doi.org/10.1016/j.cropro.2006.10.005

ISSN 1392-3196 / e-ISSN 2335-8947
Zemdirbyste-Agriculture, vol. 100, No. 2 (2013), p. 199–204
DOI 10.13080/z-a.2013.100.026

Gliukozinolatai kaip natūrali bastutinių augalų apsauga nuo kryžmažiedinės spragės (*Phyllotreta* spp.) pažeidimų

T. Bohinc[1], I. J. Košir[2], S. Trdan[1]

[1]Liublijanos universitetas, Slovėnija
[2]Slovėnijos apynių ir alaus tyrimų institutas

Santrauka

Kryžmažiedinės spragės, mintančios įvairiais bastutiniais augalais, gali sumažinti jų produktyvumą. Siekiant mažinti sintetinių pesticidų naudojimą ir skatinti aplinkos apsaugą, turėtų būti panaudota natūrali augalų geba apsisaugoti nuo kenkėjų pažeidimų. 2009–2010 m. atlikto tyrimo rezultatai parodė, kad gliukozinolatų kiekis įvairuoja priklausomai nuo augalo rūšies ir organo. Visų rūšių bastutiniuose augaluose (kopūstuose, aliejiniuose ridikuose, rapsuose ir baltosiose garstyčiose) tarp indolinių gliukozinolatų buvo nustatytas gliukobrasicinas, kurio įtaka kryžmažiedinėms spragėms skyrėsi priklausomai nuo augalo rūšies. Tyrimo metu nustatytas stiprus neigiamas koreliacinis ryšys ($r = -0{,}99$) tarp gliukonasturtino kiekio rapsų augaluose ir kryžmažiedinių spragių pažeidimo intensyvumo. Tarp gliukonapino, sinalbino, progoitrino kiekių rapsuose ir kryžmažiedinių spragių pažeidimų intensyvumo nustatyti vidutinio stiprumo koreliaciniai ryšiai (atitinkamai $r = 0{,}67$, $r = 0{,}61$ ir $r = 0{,}51$). Esminės gliukobrasicino įtakos kryžmažiedinių spragių pažeidimams rapsuose nebuvo nustatyta, tačiau šis gliukozinolatas turėjo neigiamos įtakos spragių pažeidimams aliejiniuose ridikuose ($r = -0{,}32$) ir baltosiose garstyčiose ($r = -0{,}64$). Nustatyta, kad aliejinis ridikas yra tinkamiausia rūšis privilioti kryžmažiedines spragės ir gali būti naudojamas kaip spąstai šiam kenkėjui. Padaryta išvada, kad bastutinių augalų apsaugos nuo kryžmažiedinių spragių intensyvumas gali priklausyti nuo gliukozinolatų kiekio augaluose, tačiau siekiant šio tyrimo rezultatus pritaikyti ekologiškai auginamiems kopūstams, reikia papildomų tyrimų.

Reikšminiai žodžiai: aliejinis ridikas, baltoji garstyčia, gliukozinolatai, gūžinis kopūstas, *Phyllotreta* spp., rapsas.

2.3 VPLIV OKOLJSKIH DEJAVNIKOV NA VSEBNOST GLUKOZINOLATOV V KRIŽNICAH (Brassicaceae)

BOHINC, Tanja, TRDAN, Stanislav

Environmental factors affecting the glucosinolate content in Brassicaceae.
International Journal of Food, Agriculture & Environment, 2012, 10: 357-380.
Prejeto: 18. 01. 2012; sprejeto: 02.05.2012

V omenjeni raziskavi smo preučevali vpliv okoljskih dejavnikov (povprečne in najvišje dnevne temperature, povprečne dnevne relativne zračne vlage, trajanje sončnega obsevanja) na vsebnost glukozinolatov v križnicah (Brassicaceae). Rezultati naše raziskave kažejo, da je vsebnost glukobrasicina (indolnega glukozinolata) pogojena z povprečno dnevno in najvišjo temperaturo. Okoljski dejavniki so signifikatno najbolj vplivali na vsebnost indolnih glukozinolatov, manj pa na vsebnost alifatskih oziroma aromatskih glukozinolatov. Kljub temu, da je bil vpliv okolja na alifatske in aromatske glukozinolate v našem poskusu spremenljiv, pa lahko potrdimo njegov signifikanten vpliv na posamezne glukozinolate, alifatske oziroma aromatske skupine. Ugotavljamo, da so okoljski dejavniki pomembni v načrtovanju pridelave hrane tudi v kontekstu pričujoče raziskave, saj naši rezultati kažejo na signifikanten vpliv okolja na vsebnost glukozinolatov v križnicah.

WFL Publisher
Science and Technology

Meri-Rastilantie 3 B, FI-00980
Helsinki, Finland
e-mail: info@world-food.net

Journal of Food, Agriculture & Environment Vol.10 (2): 357-366. 2012 www.world-food.net

Environmental factors affecting the glucosinolate content in Brassicaceae

Tanja Bohinc and Stanislav Trdan

Chair of Phytomedicine, Agricultural Engineering, Crop Production, Pasture and Grassland Management, Department of Agronomy, Biotechnical Faculty, University of Ljubljana, Jamnikarjeva 101, SI-1111 Ljubljana, Slovenia.
e-mail: tanja.bohinc@gmail.com, stanislav.trdan@bf.uni-lj.si

Received 15 January 2012, accepted 2 May 2012.

Abstract

This study describes the effects of environmental factors: the average and highest daily temperature, average relative air humidity, and the duration of the daily solar radiation, on the glucosinolate content in Brassicaceae. The results of our study indicate that the content of indole glucosinolate, glucobrassicin, is influenced by the average daily and the highest air temperature. Indole glucosinolates were much more susceptible to environmental factors than aliphatic or aromatic glucosinolates. Although the impact of the environment on the groups of aliphatic and aromatic glucosinolates was variable, there was a significant impact of the environment on specific aliphatic or aromatic glucosinolates. We conclude that climatic conditions cannot be neglected in the future planning of cropping systems, as the results of our investigation showed the important effect of environmental factors on the glucosinolate content in Brassicaceae.

Key words: Temperature, relative humidity, solar radiation, glucosinolates, Brassicaceae

Introduction

Plants are able to defend themselves [7, 12] against attack by insects and other herbivores. Increases in resistance to herbivory are thought to have generated the vast diversity of proteins and secondary metabolites with defence functions [2, 4]. Glucosinolates are secondary metabolites that are well known for their role in plant resistance to insects and pathogens [10, 19, 24] and are largely found in the order Brassicales, which includes the economically and nutritionally important Brassica crops [24].

The changing temperature regime in northern and central Europe results in the prolongation of the cultivation period for vegetables originating from the Mediterranean climate region [4]. Literature sources indicate that the amount of phytochemicals is dependent on many pre- and postharvest stages of the fruit and vegetable production chain [15], such as their storage conditions after harvest and processing parameters. During the thermal processing of Brassica vegetables, the glucosinolate contents can be reduced due to several mechanisms, i.e. enzymatic hydrolysis and thermal degradation [9, 11].

Environmental factors, such as solar radiation, temperature variation and climatic conditions, within a geographical location are also key in determining the glucosinolate content [5]. To our knowledge, only a few studies on the thermal degradation of individual glucosinolates exist [1].

The aim of the present work was to investigate the influence of air temperature, relative humidity and the duration of solar radiation on the content of specific glucosinolates in Brassicaceae. Our goal was a better understanding of the suitability of Brassicaceae cultivation in environmentally different regions, taking into account the role of glucosinolates as pest defence mechanisms.

Materials and Methods

Plant material: A field experiment was conducted in 2010 in the village Zgornja Lipnica (46°19' N latitude, 14°10' E longitude, 511 m above sea level) in Slovenia. The seedlings of white cabbage (*Brassica oleracea* [L.] var. *capitata* f. *alba*) were cultivated at the Department of Agronomy's (Biotechnical Faculty in Ljubljana, Slovenia) greenhouse according to the protocol described by Trdan *et al.* [25]. Oil rape (*Brassica napus* [L.] ssp. *oleifera* f. *biennis*), cv. Daniela, white mustard (*Sinapis alba* [L.]) cv. Zlata, and oil radish (*Raphanus sativus* [L.] var. *oleiformis*), cv. Apoll, were sown on the 19th April 2010, whereas white cabbage seedlings, cv. 'Tucana' (mid-early hybrid) and cv. 'Hinova' (medium-late hybrid), were transplanted in an open field on the 26th April 2010. A detailed description of the field experiment is described by Bohinc and Trdan [2].

Glucosinolate determination: Plant material for the analysis of glucosinolates was sampled at different intervals. The material was harvested using scissors. Four samples of individual plant parts were collected on each assessment date. Individual samples (from each species) were representative of the plants in one plot. On certain assessment dates, in addition to the samples of white mustard leaves (8th June 2010 and 3rd July 2010) and oil radish leaves (8th June 2010, 25th June 2010 and 31st July 2010), we also collected representative samples of blossoms of each species.

Sampling of the cabbage leaves was performed at five different intervals: on 26 May, 16 June, 4 July, 29 July and 17 August 2010. Sampling of the above ground parts of oil rape was performed at five different intervals (19 May, 8 June, 3 July, 31 July and 31 August 2010), whereas the sampling of oil radish and white mustard was performed at four intervals: the former, 19 May, 8 June, 25 June and

31 July 2010, and the latter, 19 May, 8 June, 25 June and 3 July 2010.

Four samples of individual plant species were collected at individual intervals, and each individual sample (the specified part of a plant species) was the representative plant sample of one block. When analysing the glucosinolates, the analysis of individual samples was repeated twice. The material was freeze-dried (LIO-10P; Kambič Laboratorijska oprema, Semič, Slovenia) and homogenised prior to the extraction of glucosinolates. The lyophilised samples were stored in 50 ml bottles in a freezer (U3286S; Sanyo) at -80°C. The glucosinolate extraction and analysis were performed according to ISO 9167-1-1992.

Weather conditions: The average air relative humidity, average and maximum air temperatures, and the duration of solar radiation in the location of the nearest agrometeorological station (Lesce, 46°21'36''N latitude, 14°9'23'' E longitude, 494 m above sea level) during the experimental period in 2010 are presented in Table 1.

Data analysis: The results were statistically evaluated using Statgraphics Centurion XVI [17]. We calculated the correlations between the concentration of an individual glucosinolate in all four Brassicaceae species and weather parameters based on the analysis of the pooled results of glucosinolate content and the weather parameters on specific days (days 5, 2 and 1 before sampling and at the time of sampling).

Results

Influence of average daily temperature on a specific glucosinolate: A positive low correlation (r = 0.30) was established at 5 days before sampling between the average daily temperature and epiprogoitrin content (Fig. 1). We found a moderate correlation (r = 0.70) between the average daily temperature and the epiprogoitrin value at 2 days before sampling (Fig. 2). The results of the statistical analysis showed a suppression effect (r = -0.25) of the average daily temperature on the gluconapin content at 5 days before sampling, whereas the average daily temperature had a moderate negative effect (r = -0.67) on the gluconapin content 1 day before sampling.

With regard to the glucobrassicin content, we established a moderate negative impact (r = -0.60) of the average daily temperature 5 days before sampling, a moderate negative impact (r = -0.44) of the average daily temperature 2 days before sampling, and a negative effect (r = -0.33) of the average daily temperature 1 day before sampling.

Influence of the highest daily temperature on a specific glucosinolate: Analyses of the results demonstrated a low impact of the highest temperature on the content of glucobrassicin at 5 days before sampling (r = -0.22) (Fig. 3), whereas an influence of the highest temperature was very weak at 2 days before sampling (r = -0.10) (Fig. 4). The impact of the highest daily temperature on the content of specific aliphatic or aromatic glucosinolates varied.

The content of gluconapin was influenced (r = 0.84) by the highest daily temperature at 2 days before sampling, whereas there was no impact (r = 0.08) detected for the highest daily temperature at 5 days before sampling.

Influence of the average daily relative humidity on a specific glucosinolate: The content of gluconasturtiin was influenced by the mean relative humidity two and one days before sampling (r = 1), whereas we detected no impact (r = 0.07) at five days before sampling. The results showed a very weak negative impact of the mean relative humidity on the content of gluconapin at 5 days (r = -0.18) and two days (r = -0.01) before sampling, whereas the average relative humidity one day before sampling had a strong positive impact (r = 0.77) on the content of gluconapin.

Impact of the average duration of solar radiation on a specific glucosinolate: Interestingly, the results showed a very weak negative impact (r = -0.24) of the mean radiation at 5 days before sampling on the content of glucobrassicin. Similarly, strong positive impacts of the mean radiation duration on the gluconasturtiin content at 5 (r = 1) and 2 (r = 1) days before sampling were found. At one day before sampling, the influence of the mean radiation on the content of gluconasturtiin was very strong (r = -1). Similarly, a strong negative influence of the radiation duration on the content of epiprogoitrin at 2 days before sampling was detected (r = -0.81), whereas the impact of the average radiation duration on the sinalbin content at 2 days before sampling was positive and moderate (r = 0.56).

Impact of relative humidity and temperature at the time of sampling (at 7 a.m.): At the time of sampling, we detected a strong negative impact of the relative humidity on the content of glucobrassicin (r = -0.79), and a negative impact on epiprogoitrin was also detected (r = -0.32); there was also strong impact of the temperature at 7 a.m. on the content of epiprogoitrin (r = -0.85). We detected a strong negative impact of the temperature on the content of progoitrin (r = -1), whereas the impact of the relative humidity was strongly positive (r = 1).

Discussion

The results of our study indicate that the content of glucosinolates is influenced by environmental factors, which was previously reported by Rask *et al.* [15]. However, the findings reported in the literature mostly refer to the impact of drought [6-8]. In contrast, we focussed on the impact of the average daily temperature, the highest daily temperature, the average daily relative humidity, and the average duration of solar radiation and found that different groups of glucosinolates (aliphatic, indole, and aromatic) differ with regard to environmental influence. As Oerlemans *et al.* [11] we also found that glucobrassicin was the most important indole glucosinolates in Brassicaceae. We believe that the expression of glucobrassicin is related to the fact that indole glucosinolates are considered to be much more sensitive to heat treatment than aliphatic glucosinolates [2].

The indole glucosinolate, glucobrassicin, exhibited a stable degree of thermal degradation during the evaluation period [1] and, in our study, was mostly affected by the highest and average daily temperatures and

Table 1. Average weather parameters in Lesce in 2010.

	Rh (%)	$T_{average}$ (°C)	T_{max} (°C)	Solar radiation (hours)
April (last 10 days)	65.62±0.15	14.32±0.68	21.72±0.83	10.18±1.49
May	71.61±0.22	13.62±0.44	18.25±0.69	4.00±0.86
June	66.77±0.31	18.49±0.57	24.28±0.80	7.92±0.86
July	68.62±0.23	21.13±0.61	27.47±0.68	8.77±0.70
August	81.05±0.09	17.93±0.53	23.50±0.63	6.81±0.67
September	82.50±0.13	13.25±0.33	18.02±0.54	4.86±0.79

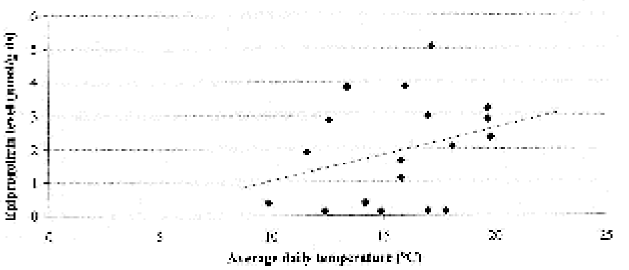


Figure 1. Relationship between the average daily highest temperature (°C) and the content of epiprogoitrin at 5 days before sampling.
$y = 0.1598x + 0.5675$ [illegible], $R^2 = 0.0608$ [illegible]

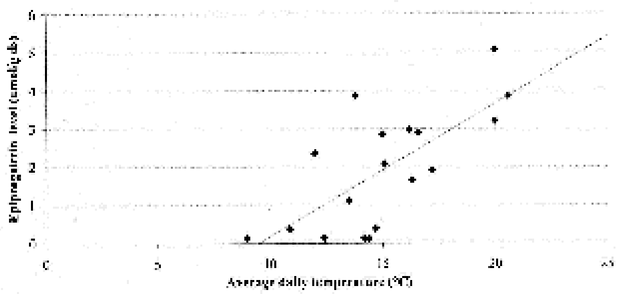


Figure 2. Relationship between the average daily highest temperature (°C) and the content of epiprogoitrin at 2 days before sampling.
$y = 0.3478x - 3.2957$ [illegible], $R^2 = 0.4567$ [illegible]

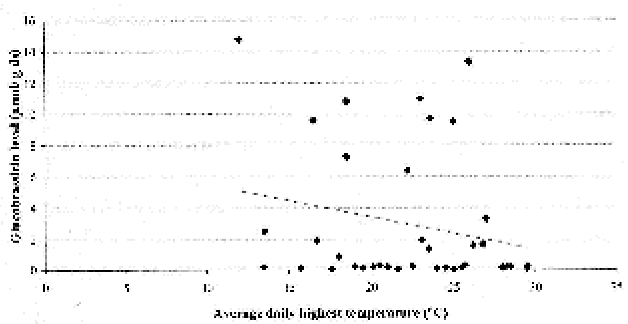


Figure 3. Relationship between the average daily highest temperature (°C) and the content of glucobrassicin at 5 days before sampling.
$y = -0.20890x + 7.6189$ [illegible], $R^2 = 0.0795$ [illegible].

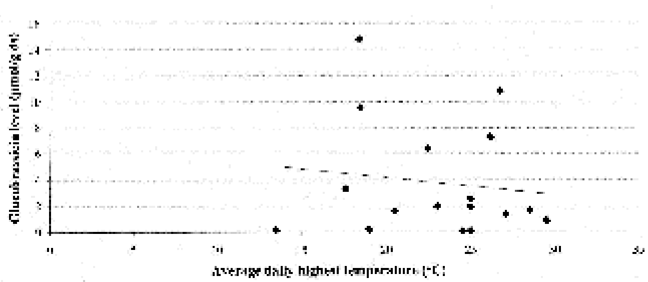


Figure 4. Relationship between the average daily highest temperature (°C) and the content of glucobrassicin at 2 days before sampling.
$y = -0.1331x + 6.3529$ [illegible], $R^2 = 0.0100$ [illegible].

relative humidity. However, indole glucosinolates, such as glucobrassicin, are precursors of cruciferous phytoalexins; thus, from a plant defence perspective, lowering the glucosinolate content could present an ecological risk [12].

Indole glucosinolates demonstrated more degradation than aliphatic glucosinolates at lower temperatures [11]. The results of our survey indicate that the influences of temperature, relative humidity, and duration of solar radiation differ with respect to specific glucosinolates. The different responses among the glucosinolate groups could be determined by the various enzymes involved in their syntheses [4].

Little is known about the impact of weather on the content of aromatic glucosinolates. The positive correlation between the content of two aromatic glucosinolates and the average daily temperature detected in our experiment is promising due to the beneficial impact of their degradation products on human health [15]. In addition to the weather parameters, there are also many other factors that can influence the contents of phytochemicals in fruit and vegetables [16,17].

Conclusions

Considering that the glucosinolate content varies among different plant varieties [5] and the hydrolysis products of sinigrin, glucobrassicin, glucoiberin, progoitrin and gluconasturtiin have been identified as cancer-suppressing agents [15], climatic conditions should not be neglected [7] when planning cropping systems (field experiments). The most serious cause for concern lies in the variability of the factors observed because poor control of the processes involved may impair our capacity to design reliable cropping techniques.

Acknowledgements

The results presented in this paper were obtained through research within national project V4-1067, which is cofinanced by the Slovenian Research Agency, the Ministry of Agriculture, Forestry and Food of the Republic of Slovenia and the Ministry of the Environment and Spatial Planning of the Republic of Slovenia. The authors are grateful to Dr. Robert Hrastar, Dr. Iztok Jože Košir, Assist. Prof. Matej Vidrih, Assist. Prof. Dragan Žnidarčič and Jaka Rupnik for their contributions to this work.

References

[1]Al-Gendy, A. A., El-gindi, O. D., Hafez, A. S. and Ateya, A. M. 2010. Glucosinolates, volatile constituents and biological activities of *Erysimum corinthium* Boiss. (Brassicaceae). Food Chem. 118:519-524.

[2]Bohinc, T. and Trdan, S. 2012. Trap crops for reducing damage caused by cabbage stink bugs (*Eurydema* spp.) and flea beetles (*Phyllotreta* spp.) on white cabbage: fact or fantasy? J. Food Agric. Environ. 10(2):1365-1370.

[3]Bones, A. M. and Rossiter, J. T. 2006. The enzymic and chemically induced decomposition of glucosinolates. Phytochem. 67:1053-1067.

[4]Björkman, M., Klingen, I., Birch, A. N. E., Bones, A. M., Bruce, T. J. A., Johansen, T. J., Meadow, R., Mølmann, J., Seljåsen, R., Smart, L. E. and Stewart, D. 2011. Phytochemicals of Brassicaceae in plant protection and human health. Influences of climate, environment and agronomic practice. Phytochem. 72:538-556.

[5]Cipollini, D., Busch, J. W., Stowe, K. A., Simms, E. L. and Bergelson, J. 2003. Genetic variation and relationships of constitutive and herbivore-induced glucosinolates, trypsin inhibitors and herbivore resistance in *Brassica rapa*. J. Chem. Ecol. 29:285-302.

[6]Chaplin-Kramer, R., Kliebenstein, D. J., Chiem, A., Morrill, E., Mills, N. J. and Kremen, C. 2011. Chemically mediated tritrophic interactions: Opposing effects of glucosinolates on a specialist herbivore and its predator. J. Appl. Ecol. 48:880-887.

[7]Charron, C. S., Saxton, A. M. and Sams, C. E. 2005. Relationship of climate and genotype to seasonal variation in the glucosinolate-myrosinase system. I. Glucosinolate content in ten cultivars of *Brassica oleracea* grown in fall and spring season. J. Sci. Food Agric. 85:671-681.

[8]Hanley, M. E., Lamont, B. B., Fairbanks, M. E. and Rafferty, C. M. 2007. Plant structural traits and their role in anti-herbivore defence. Perspect. Plant Ecol. Evol. Syst. 8:157-178.

[9]Hanschen, F. S., Rohn, S., Mewis, I., Schreiner, M. and Kroh, L. W. 2012. Influence of the chemical structure on the thermal degradation of the glucosinolates in broccoli sprouts. Food Chem. 130:1-8.

[10]Hopkins, R. J., van Dam, N. M. and van Loon, J. J. A. 2009. Role of glucosinolates in insect-plant relationships and multitrophic interactions. Ann. Rev. Entomol. 54:57-83.

[11]Oerlemans, K., Barrett, D.M., Bosch Suades, C., Verkerk, R. and Dekker, M. 2006. Thermal degradation of glucosinolates in red cabbage. Food Chem. 95:19-29.

[12]Pedras, M. S. C., Nycholat, C. M., Montaut, S., Xu, Y. and Khan, A. Q. 2002. Chemical defences of crucifers: Elicitation and metabolism of phytoalexins and indole-3-acetonitrile in brown mustard and turnip. Phytoch. 59:611-625.

[13]Rask, L., Andréasson, E., Ekbom, B., Eriksson, S., Pontoppidan, B. and Meijer, J. 2000. Myrosinase: gene family evolution and herbivore defense in Brassicaceae. Plant Mol. Biol. 42:93-113.

[14]Schonhof, I., Kläring, H. P., Krumbein, A., Claußen, W. and Schreiner, M. 2007. Effect of temperature increase under low radiation conditions on phytochemicals and ascorbic acid in greenhouse grown broccoli. Agric. Ecol. Environ. 119:103-111.

[15]Sun, B., Liu, N., Zhao, Y., Yan, H. and Wang, Q. 2011. Variation of glucosinolates in three edible parts of Chinese kale (*Brassica alboglabra* Bailey) varieties. Food Chem. 124:941-947.

[16]Sultana, T., Savage, G. P., McNeil, D. L., Porter, N. G. and Clark, B. 2003. Comparison of flavour compounds in wasabi and horseradish. J. Food Agric. Environ. 1(2):117-121.

[17]Statgraphics Centurion XVI. Statpoint technologies, Inc. Warrenton, Virginia.

[18]Tiwari, U. and Cummins, E. 2011. Factors influencing contents of phytochemicals in selected fruit and vegetables during pre- and post-harvest food processing operations. Food Research International. doi:10.1016/j.foodres.2011.09.007 Article (in press).

[19]Trdan, S., Žnidarčič, D., Kač, M. and Vidrih, M. 2008. Yield of early white cabbage grown under mulch and non-mulch conditions with low populations of onion thrips (*Thrips tabaci* Lindeman). Int. J. Pest Manage. 54:309-318.

[20]Winde, I. and Wittstock, U. 2011. Insect herbivore counteradaptations to the plant glucosinolate-myrosinase system. Phytochem. 72:1566-1575.

[21]Žnidarčič, D., Ban, D., Oplanić, M., Karić, L. and Požrl, T. 2010. Influence of postharvest temperatures on physicochemical quality of tomatoes (*Lycopersicon esculentum* Mill.). J. Food Agric. Environ. 8(1):21-25.

2.4 UPORABA PRIVABILNIH POSEVKOV Z NAMENOM ZMANJŠEVANJA POŠKODB KAPUSOVIH STENIC (*Eurydema* spp.) in KAPUSOVIH BOLHAČEV (*Phyllotreta* spp.) NA BELEM ZELJU: DEJSTVO ALI FANTAZIJA?

BOHINC, Tanja, TRDAN, Stanislav
Trap crops for reducing damage caused by stink bugs (*Eurydema* spp.) and flea beetles (*Phyllotreta* spp.) on white cabbage: fact of fantasy?
International Journal of Food, Agriculture & Environment, 2012, 10: 1365-1370.
Prejeto: 20.11.2011; sprejeto: 23.04.2012

V letih 2009 in 2010 smo izvedli poljski poskus, v katerem smo preučevali učinkovitost treh vrst privabilnih posevkov (oljne redkve, krmne ogrščice in bele gorjušice) kot metode zmanjševanja poškodb kapusovih stenic (*Eurydema* spp.) in kapusovih bolhačev (*Phyllotreta* spp.) na dveh hibridih belega zelja. Poskus je potekal v štirih blokih z naključno razporeditvijo. Obseg poškodb kapusovih stenic in kapusovih bolhačev na zelju in privabilnih posevkih smo ocenjevali v 10-dnevnih intervalih. V letu 2010 je bila krmna ogrščica najbolj dovzetna za poškodbe kapusovih stenic, medtem ko je bil obseg poškodb kapusovih bolhačev najvišji na oljni redkvi. V letu 2009 nismo ugotovili izrazite preference kapusovih bolhačev do posamezne vrste privabilnega posevka. Obseg poškodb kapusovih stenic je na glavnem posevku začel naraščati v začetku julija, medtem ko je obseg poškodb kapusovih bolhačev začel signifikatno naraščati ob koncu maja. Daljša rastna doba je lahko razlog, da je bilo zelje, cv. Hinova, signifikatno bolj dovzetno za poškodbe preučevanih skupin škodljivcev. Indeks poškodb je bil na zelju in rastlinah privabilnih posevkov višji v drugem letu poskusa, predvsem zaradi dejstva, da pred začetkom našega poskusa na omenjenem zemljišču in njegovi okolici niso pridelovali kapusnic. Izbira privabilnih posevkov ni imela signifikatnega vpliva na pridelek zelja.

WFL Publisher
Science and Technology
Meri-Rastilantie 3 B, FI-00980
Helsinki, Finland
e-mail: info@world-food.net

Journal of Food, Agriculture & Environment Vol.10 (2):1365-1379. 2012 www.world-food.net

Trap crops for reducing damage caused by cabbage stink bugs (*Eurydema* spp.) and flea beetles (*Phyllotreta* spp.) on white cabbage: fact or fantasy?

Tanja Bohinc [1] and Stanislav Trdan [2*]

*[1] Zgornja Lipnica 9a, SI-4246 Kamna Gorica, Slovenia. [2] Department of Agronomy, Biotechnical Faculty, Chair of Phytomedicine, Agricultural Engineering, Crop Production, Pasture and Grassland Management, Jamnikarjeva 101, SI-1111 Ljubljana, Slovenia. *e-mail: stanislav.trdan@bf.uni-lj.si*

Received 20 November 2011, accepted 23 April 2012.

Abstract

In 2009 and 2010, a field experiment was carried out to determine the effect of three trap crops (oil radish, oil rape and white mustard) as a protection method against cabbage stink bugs (*Eurydema* spp.) and flea beetles (*Phyllotreta* spp.) attack on two hybrids of white cabbage. The experiment was designed as randomized complete block with four treatments. The damage caused by cabbage stink bugs and flea beetles on the main crop and the trap crops was estimated in 10-day intervals. Oil rape was the most attractive trap crop for cabbage stink bugs, while flea beetles displayed specific preference to oil radish as a trap crop in 2010. Flea beetles displayed specific preference for oil radish as a trap crop in 2010, while they did not display specific preference to any of trap crops tested in 2009. The damage caused by cabbage stink bugs on cabbage started increasing in the beginning of July. The damage caused by flea beetles, on the other hand, started increasing at the end of May. Cabbage hybrid 'Hinova' was more heavily attacked by both groups of the pests, mostly due to its longer growth period. The extent of damage on cabbage and trap crops was higher in the second year, since before 2009 no Brassica crops were grown in the near vicinity of the experimental plot. The trap crop species has no important effect on the yield of cabbage, since the pests on trap crop plants were not control with insecticides.

Key words: Cabbage stink bugs, field experiment, flea beetles, trap crops, white cabbage.

Introduction

In European countries cabbage is known as a field-grown vegetable which is attacked by a large number of harmful insect pests [7, 15, 33]. Cabbage stink bugs (*Eurydema* spp., Heteroptera, Pentatomidae) and flea beetles (*Phyllotreta* spp., Coleoptera, Chrysomelidae) are important pests of cabbage leaves and their control is economically justified [30-32]. In Slovenia only deltamethrin is registered against flea beetles (FB), while no insecticide at all is registered for controlling cabbage stink bugs (CSB) [29].

The method of trap cropping is used frequently when no insecticides are registered against the pests, when the insecticide is expensive or when the main crop has no resistance to attack by the pest [8]. Trap crops are planted or sown among the main crop plants or in their vicinity in order to attract harmful pests and simultaneously reduce their number on the main crop [11]. Plants which serve as trap crops are also locations for oviposition. They can be treated with plant protection products and thus prevent further development of harmful organisms [27]. Trap crops can be an efficient alternative to insecticides [1, 19, 32], which were proven to reduce numbers of natural enemies in cabbage fields, consequently increasing economic significance of plant pests [3]. Trap crops as well as intercrops and intercrops also represent an important habitat for useful organisms [25, 26, 37].

In our study we wanted to determine the effect of oil radish, oil rape and white mustard as the trap crops against CSB and FB in production of white cabbage as the main crop.

Materials and Methods

Study site: The two-year field experiment (2009-2010) was carried out on the field of 528 m² in the village Zgornja Lipnica in Slovenia (46°19' N latitude, 14°10' E longitude, 511 m above the sea level). One year prior to our experiment no large field with plants of the family Brassicaceae was cultivated near the said location.

Field experiment: The field was divided into four blocks (Fig. 1). The three species of trap crops were sown within the plots in separate treatments. The fourth treatment was the control, where no trap crop was sowed (bare surface). The treatments within blocks were arranged randomly. In both years of the experiment

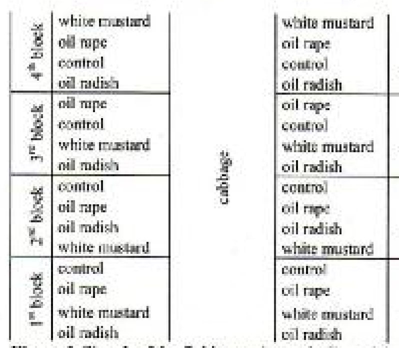


Figure 1. Sketch of the field experiment in Gorenjska in 2009 and 2010.

we sowed oil radish (*Raphanus sativus* var. *oleiformis*) of the sort 'Apoll', oil rape (*Brassica napus* ssp. *oleifera f. biennis*) of the sort 'Daniela' and white mustard (*Sinapis alba*) of the sort 'Zlata'. These were sown in two rows, one row on each outer side of all four blocks. On the inner side of both rows two different hybrids of cabbage were sown – 'Tucana' (growth period 60-65 days) and 'Hinova' (growth period 120-140 days). The cabbage was planted at the planting distance of 30 cm ×40 cm.

The trap crops were in the first year of the experiment sown on the 9th of April, in the second year of the experiment on the 19th of April. The planting of cabbage seedlings was in the first year carried out in two days (on the 25th and 26th of April), in the second year of the experiment we planted the seedlings of cabbage on the 26th of April. The seedlings of cabbage were planted on 240 m^2, the trap crops were sown on 288 m^2. The size of individual plots with trap crops within individual blocks was 18 m^2. In the second year of the experiment the said surface was treated with mineral fertilisers, namely with 2.5 kg of P_2O_5, 11.5 kg of K_2O, 1.5 kg of MgO and 4 kg of CaO.

Field observations and evaluation: The damage done by harmful pests to the cabbage and the trap crops were in both years evaluated thirteen times – in approximately 10-day intervals. In the first year of the experiment we began evaluating the damage on the trap crops on the 12th of May, and continued on the 25th of May. The evaluation of damage extent was completed on the 17th of September. In the second year of the experiment we began evaluating the damage on the trap crops on the 3rd of May, and continued on the 15th of May. The evaluation was completed on the 15th of September. In the first year of the experiment we began monitoring damage on the hybrid 'Tucana' caused by the species *Eurydema* spp. and *Phyllotreta* spp. on the 15th of May and continued on the 25th of May. The evaluation of damage extent was completed on the 5th of July. In the second year of the experiment we began evaluating the damage on the hybrid 'Tucana' on the 7th of May, and continued on the 15th of May. During the growth period we evaluated the damage extent on eleven different days, up to the 15th of August. The evaluation of the damage extent on the hybrid 'Hinova' was in 2009 carried out on twelve different days, from the 15th of May to the 17th of September. In 2010 began evaluating the damage on the 7th of May, and continued on the 15th of May. During the growth period we evaluated the damage extent on fourteen different days, up to the 15th of September.

The damage by cabbage stink bugs was evaluated by the 6-grade visual scale [28], which was originally developed for evaluating damage done by onion thrips (*Thrips tabaci* Lindeman) on outer cabbage leaves, while in 2006 successfully used it to evaluate damage done on cabbage leaves by cabbage stink bugs [13]. The damage done by flea beetles was evaluated by the 5-grade EPPO-scale [22]. Individual cabbage heads were weighed when technologically ripe. In 2009 the hybrid 'Tucana' reached technological ripeness on the 10th of July, in 2010 the heads of the same hybrid were collected on the 16th and 17th of August. In the first year of the experiment the heads of the hybrid 'Hinova' were collected on the 20th and 21st of September, in the second year of the experiment they were collected from the 17th to 20th of September. The extent of damage done to cabbage and cabbage crops by harmful pests was evaluated in regard to the distance between the cabbage and the trap crops. Besides recording the damage extent caused by the studied harmful pests during growth period, we evaluated the growth stages of both hybrids and all trap crops. The growth stages of the trap crops were identified with BBCH-scale for the growth stages of rape seed [7], while the growth stages of cabbage were identified with the BBCH-scale for leafy vegetables which form heads [17].

Data analysis: The experimental results were statistically evaluated with the program Statgraphics plus 4.0. The differences between averages were evaluated by variance analysis (MANOVA) and Student-Newman-Keuls multiple range test ($P \leq 0.05$). We calculated the correlation between the average index of damage and growth stages of trap crops and cabbage. We also calculated the correlation between the extent of damage on cabbage and the average mass of cabbage heads.

Results and Discussion

The extent of damage on trap crops caused by CSB (2009: $F = 8.15$; $df = 50$; $P<0.0001$; 2010: $F = 105.70$; $df = 44$; $P<0.0001$) and FB (2009: $F = 8.78$; $df = 50$; $P<0.0001$; 2010: $F = 24.25$; $df = 44$; $P<0.0001$) was significantly influenced by the growth stages of trap crops. This fact was already confirmed earlier for turnip rape as a trap crop for attracting two Coleopteran pests [5]. In 2009, weak correlation was established between the extent of damage done by FB ($r = 0.40$) and CSB ($r = 0.32$) and the growth stage of trap crops. In 2010, a moderate correlation ($r = 0.55$) between the growth stage of trap crops and the extent of damage done by CSB, and a weak correlation ($r = 0.40$) between the extent of damage done by FB and the growth stage of trap crops was established. When establishing correlation between individual growth stages of trap crops and the extent of damage done by the studied pests, we reached the conclusion that the connection between the extent of damage and the growth stage of plants varies throughout the growth period. The only highly significant ($P<0.01$) – positive and moderate - correlations (Table 1) in 2009 were established 11 decades (with white mustard as trap crop and damage caused by FB) and 14 decades (with oil rape as trap crop and damage caused by CSB) after cabbage transplanting (DACT). At that time oil rape and white mustard were between 73 and 86 BBCH growth stage. In 2010 highly significant – negative and moderate - correlation was established only 14 DACT with oil rape as trap crop and damage caused by FB. In the second year, significant ($P<0.05$) – negative and moderate – correlation was also detected 2 DACT with oil rape and white mustard as trap crops and damage caused by FB, and 4 DACT with oil rape as a trap crop and damage caused by CSB.

In 2009 the mean indexes of damage done by FB on the hybrid 'Tucana' reached significantly the highest value 4 DACT, while in 2010 the same was stated between 7 and 11 DACT (Table 2). In the most part of the growing seasons FB caused higher extent of damage compared to CSB. The latter started to feed on cabbage 4 DACT and caused significantly the highest mean index of damage at last evaluations in both years. FBs started to feed earlier than CBSs also on the hybrid 'Hinova' but at the end of the growing seasons the mean indexes of damage caused by CSB was higher (2009) or at least about the same (2010) compared to FB. Both groups of the pests caused significantly highest extent of damage at the end of the growing seasons; for FB this was true after 11

Table 1. Correlation coefficients between mean damage ratings caused by flea beetles and cabbage stink bugs feeding on trap crops, and phenological growth stages of trap crops in nine days of evaluation in 2009 and 2010.

	Oil rape			Oil radish			White mustard		
DACT	*Phyllotreta* spp.	*Eurydema* spp.	BBCH	*Phyllotreta* spp.	*Eurydema* spp.	BBCH	*Phyllotreta* spp.	*Eurydema* spp.	BBCH
2-09	-0.27	-	12.0-14.0	-0.34	-0.2	11.0-13.0	0.00	-0.57	12.0-15.0
3-09	0.09	0.07	12.0-15.0	0.01	-0.19	36.0-53.0	-0.35	0.16	53.0-63.0
4-09	-0.02	0.01	12.0-17.0	-0.15	0.02	39.0-64.0	0.14	0.16	63.0-65.0
6-09	-0.37	-0.28	28.0-64.0	0.18	-0.13	65.0-67.0	-0.11	0.25	65.0-69.0
7-09	0.29	-0.03	50.0-64.0	0.03	0.2	65.0-72.0	-0.11	-0.15	67.0-73.0
9-09	0.16	-0.05	57.0-65.0	0.03	-0.01	72.0-76.0	-0.43	-0.33*	79.0-84.0
11-09	-0.04	0.20	61.0-84.0	0.05	0.15	73.0-78.0	-	0.43***	83.0-86.0
14-09	0.48***	-0.01	73.0-86.0	0.04	-0.24	80.0-89.0	-		97.0-97.0
15-09	0.04	0.16	81.0-86.0	0.04	-0.24	80.0-89.0	-	-	97.0-97.0
3-10	-0.43**	-	11.0-13.0	0.28	0.01	12.0-14.0	-0.38**	-	13.0-16.0
2-10	0.06	0.19	13.0-14.0	-0.12	0.01	19.0-30.0	0.22	-0.06	24.0-30.0
4-10	-0.26	-0.41**	14.0-25.0		-	21.0-33.0	0.01	0.14	58.0-62.0
6-10	-	-	35.0-36.0	-	-	62.0-65.0		-	65.0-65.0
7-10	-	-0.07	35.0-37.0	-	-	65.0-65.0	-	-	65.0-67.0
9-10	-0.09	0.02	39.0-51.0	-0.06	0.18	67.0-69.0	-	-	71.0-73.0
11-10	0.24	0.44**	39.0-52.0	0.24	0.32*	67.0-69.1	-	0.04	72.0-73.0
14-10	-0.45***	0.11	50.0-52.0	0.14	0.11	67.0-74.0	-	-0.00	73.0-76.0
15-10	0.23	-0.23	50.0-53.0	0.17	-0.43	67.0-75.0		0.02	74.0-76.0

*p≤0.1; **p≤0.05; ***p≤0.01; DACT=days after cabbage transplanting; BBCH=growth stage of trap crop; 09 and 10 = year of experiment

Table 2. Mean damage ratings (±SE) for flea beetles and cabbage stink bugs on the leaves of white cabbage hybrids 'Tucana' and 'Hinova' in 2009 and 2010. The mean values followed by the same letter do not differ (P≤0.05) according to Student-Newman-Keuls multiple range test.

	'Tucana'				'Hinova'			
DACT	*Phyllotreta* spp.2009	*Phyllotreta* spp.2010	*Eurydema* spp.2009	*Eurydema* spp.2010	*Phyllotreta* spp.2009	*Phyllotreta* spp.2010	*Eurydema* spp.2009	*Eurydema* spp.2010
1	-	1.05±0.01 a	-	1.00±0.0 a	-	1.05±0.02 a	-	1.00±0.0 a
2	1.19±0.03 a	1.88±0.03 b	1.00±0.0 a	1.00±0.0 a	1.12±0.03 a	1.87±0.03 b	1.00±0.0 a	1.00±0.0 a
3	2.27±0.07 b	2.24±0.03 c	1.00±0.0 a	1.00±0.0 a	2.08±0.06 b	2.16±0.04 cd	1.00±0.0 a	1.00±0.0 a
4	2.35±0.07 b	2.35±0.04 d	1.07±0.02 a	1.07±0.03 a	2.28±0.06 d	2.24±0.04 cd	1.06±0.02 b	1.06±0.03 a
5	2.12±0.03 b	2.35±0.04 e	1.24±0.04 a	1.27±0.05 a	2.08±0.04 b	2.24±0.04 cd	1.12±0.03 bc	1.33±0.07 b
6	2.22±0.04 ce	2.37±0.04 de	1.25±0.04 b	1.38±0.06 b	2.24±0.05 cd	2.24±0.04 cd	1.19±0.03 bc	1.71±0.07 c
7	2.12±0.04 a	2.46±0.04 ef	1.56±0.05 c	1.26±0.06 a	2.36±0.04 bc	2.28±0.04 ce	1.29±0.04 b	1.79±0.01 c
8	-	2.46±0.04 ef	-	1.35±0.08 b	2.30±0.04 d	2.28±0.04 ce	1.40±0.04 c	1.87±0.01 c
9	-	2.51±0.04 f	-	2.15±0.08 d	2.14±0.05 bc	2.35±0.04 ce	1.65±0.06 d	2.66±0.07 d
10		2.51±0.04 f	-	2.37±0.08 e	2.31±0.04 d	2.4±0.04 e	2.40±0.06 e	2.80±0.09 de
11	-	2.51±0.04 f	-	2.54±0.07 f	2.50±0.04 e	2.4±0.05 e	2.80±0.06 f	2.88±0.08 e
12	-	-	-	-	2.50±0.04 e	2.68±0.05 f	2.91±0.06 f	3.35±0.06 f
13	-		-	-	2.50±0.04 e	2.71±0.05 f	2.91±0.06 f	3.50±0.06 f
14	-	-	-		2.50±0.04 e	2.71±0.05 f	2.91±0.06 f	3.76±0.07 g

DACT=days after cabbage transplanting; 2009= index of damage caused in 2009, 2010 = index of damage caused in 2010.

(2009) and 12 DACT (2010), while for CSB this was true after 11 DACT in 2009 and at last evaluation in 2010. This data are in accordance with earlier results of [11,30]. Our results are particularly interesting for *Eurydema* genus, which representatives are known as important pests in Far East [31] and Asia Minor [32], while in Europe this insects were up to now much less investigated. With climate changes, which can be in connection with higher air temperatures [33], cabbage stink bugs from *Eurydema* genus will further spread in Europe, therefore their occurrence in new locations in Europe have to be monitored.

In both years between April and September an average air monthly temperatures was above the 30-year average. On the other hand rainfall was more intensive in 2010, when May, June, August and September were more wet compared to the same months in the period 1961-1990. In 2009 only in June and September the rainfall was more intensive than in the period 1961-1990. Therefore the fact that in 'Hinova' hybrid the CSBs were more abundant in 2010 cannot be explained by higher air temperatures neither by lower precipitation – two weather parameters known as the most important abiotic factors in insects abundance [18].

Growth stage of the plants is known as one of the most important factors, which have influence on insect abundance [5]. In 2009, the growth stage of cabbage had weak influence on the extent of damage done by FB ($r = 0.37$), while we explained only 14.01 % variability with the model *flea beetles=1.47609+0.0192354 x growth stage* (if we neglect the influence of the hybrid). On the other side the growth stage of cabbage showed moderate influence on the extent of damage done by CSB ($r = 0.54$), which is explained by the model *cabbage stink bugs=0.26351+0.0374548 x growth stage*. On the hybrid 'Tucana' the correlation between mean indexes of damage done by CSB ($r = 0.34$) and FB ($r = 0.32$) and the growth stages of cabbage was weak, while on the hybrid 'Hinova' the mentioned correlations were weak ($r = 0.40$ for FB) and moderate ($r = 0.64$ for CSB). In 2010 we established a moderate correlation between the extent of damage done by FB (if we neglect the influence of the hybrid) and the growth stage of cabbage (r =

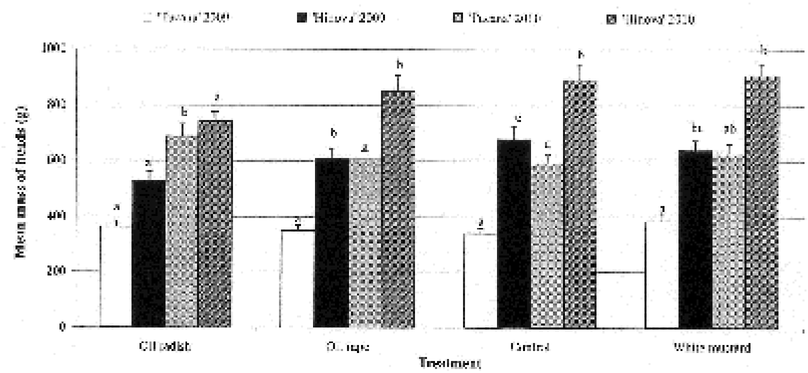


Figure 2. Mean mass of heads of the two cabbage hybrids, 'Tucana' and 'Hinova', in relation to the trap crops. Mean values followed by the same letter do not differ significantly (P≤0.05) according to Student-Newman-Keuls multiple range test.

0.53). With the model *flea beetles=1.4095+0.024319 x growth stage* we explained 27.86 % variability, while with the model *cabbage stink bugs=0.290747+0.045271 x growth stage* we explained 28.33 % variability (r = 0.53). In 2010 we detected on the hybrid 'Tucana' an increase in damage done by FB which was proportionate with higher growth stages (r = 0.54). In 2010 the hybrid 'Tucana' also displayed an increased extent of damage done by CSB which was proportionate with higher growth stages (r = 0.45). Throughout the growth period the extent of damage done by FB (r = 0.52) and CSB (r = 0.62) changes proportionately with the growth of hybrid 'Hinova' plants. [5] reported that damage and abundance of FB on conventional and herbicide-tolerant canola cultivar was similar, therefore our results, where we compared the susceptibility of two cabbage hybrids with different longevity of growing season, are in accordance with their results.

The mean mass of cabbage hybrids was influenced primarily by the selection of hybrid (2009: F = 129.40; df = 1; P<0.0001; 2010: F = 53.63; df = 1; P<0.0001). In 2009, trap crops did not influence the mean yield of the hybrid 'Tucana' (F = 0.51; df = 3; P = 0.6742), while in 2010 we noticed significant influence (F = 5.23; df = 3; P = 0.0015) of trap crops on the yield (Fig. 2). In the second year significantly the highest mass of heads was found in the cabbage which grew near oil radish and white mustard. In 2009, the mean mass of heads in the hybrid 'Hinova' was significantly highest in plants which grew near the control treatment and plots with white mustard, while in 2010 no differences were observed between the influence of oil rape, white mustard and control treatment (F = 1.56; df = 1.56; P = 0.1997).

In 2009 we detected no influence of the distance between trap crops and cabbage on the cabbage yield (F = 1.27; df = 8; P = 0.2577), this distance also had no significant influence (F = 1.19; df = 8; P = 0.3010) on the mean yield in 2010. In 2009 the mass of heads grew as the damage diminishes – particularly due to the hybrid 'Hinova' and white mustard as a trap crop (r = -0.58). In 2010 we confirmed "positive" influence of oil radish (r = -0.24) and oil rape (r = -0.35) on the mean yield, since the extent of damage done by FB on the hybrid 'Hinova' was diminishing while the yield was increasing. The damage done by CSB on oil radish as a trap crop did not reduce the mean yield of the hybrid 'Tucana'. Diversification practices enhance natural enemies in 52 %, reduce pest pressure in 53 %, and increase yield in only 32 % of the cases where this was examined [24]. Our results as well as results of some similar studies [25] are in accordance with these facts.

The damage done by the pests studied was in both years first detected on the trap crops, and the extent of damage on them was considerably higher than on cabbage. Past research shows that young plants of the family Brassicaceae are much more susceptible to FB attack [1,13,22,23], therefore earlier sowing of trap crops in our experiment proved as appropriate. The lowest extent of damage done by FB was observed on white mustard. The reason for this was the fact that white mustard contains higher extent of glucosinolates – the most important substance is sinalbin - which negatively affect nutrition of FB [16,17]. It is known that secondary metabolites content in plants varies during the growth period [7], so the connection between the extent of damage done by the studied groups of harmful pests and the growth stage of trap crops or main crops also changes. On the other hand the extent of damage done by FB to the Brassicas is influenced also by other factors of antixenosis [25], as leaves and stems of white mustard have trichomes which reduce the intensity of attacks by harmful pests.

Oil radish proved as the most suitable plant species for our location. Apart from positive influence on the hybrid 'Tucana' yield, this trap crop displayed no competitiveness [5,7,16] with cabbage. Due to uneven growth, oil radish bloomed through a considerable part of its growth period, which provided food also for useful insects. Since this *Brassica* species is very susceptible to attacks by FB, we efficiently protected the cabbage growing next to it against these important biting insects. The extent of damage done to cabbage by CSB, on the other hand, was successfully controlled by the trap crops of oil rape. We thus believe that the said *Brassica* species are most suitable for trapping the studied harmful pests and simultaneously reducing their harmful effects on cabbage.

Conclusions

When introducing the trap crop method into the plant protection system, one often faces difficulties. Using trap crops can diminish the yield of the main crop [5,6,18]. We can, however, reduce competitiveness between plants of the main crop and those of trap crops by appropriate procedures – watering the main crop, which should cover the majority of field, and treating it with fertilisers during its growth period, selecting trap crops among less competitive plants, choosing fast-growing cultivars etc. [16]. By watering and adding fertilisers during the growth period in the second year of the experiment we secured higher average yield than in the first year of the experiment. Although cabbage requires 65 kg/ha P_2O_5, 280 kg/ha K_2O, 40 kg/ha MgO and 115 kg/ha CaO [30], our treatment with fertilisers in the second year contributed to the enlarged commercial yield of the main crop. Since no insecticides were applied on the cabbage (and on the trap crops), we may conclude that such cabbage would be appreciated on the market as organically produced food. Advanced agrotechnics could further improve our end results.

Acknowledgements

This work was carried out within the V4-0524, a project funded by the Slovenian Research Agency and the Ministry of Agriculture, Food, and Forestry of the Republic of Slovenia. A part of the research was done within the program Horticulture P4-0013, which is funded by the Slovenian Research Agency. Special thanks go to Assist. Prof. Dragan Žnidarčič for producing the white cabbage seedlings, and to Assist. Prof. Matej Vidrih for selecting and sowing the trap crops.

References

[1]Ahuja, I., Rohloff, J. and Bones, A. M. 2010. Defence mechanisms of Brassicaceae: Implications for plant-insect interactions and potential for integrated pest management. A review. Agron. Sustain. Dev. 30:311-348.

[2]Bell, C. E. 1995. Broccoli (*Brassica oleracea* var. *botrytis*) yield loss from Italian ryegrass (*Lolium perenne*) interference. Weed Sci. 43:117-120.

[3]Bommarco, R., Miranda, F., Bylund, H. and Björkman, C. 2011. Insecticides suppress natural enemies and increase pest damage in cabbage. J. Econ. Entomol. 104:782-791.

[4]Bottenberg, H., Masiunas, J., Eastman, C. and Eastburn, D. 1997. Yield and quality constraints of cabbage planted in rye mulch. Biol. Agric. Hortic. 14:323-342.

[5]Brown, J., McCaffrey, J. P., Brown, D. A., Harmon, B. A. and Davis, J. B. 2004. Yield reduction in *Brassica napus*, *B. rapa*, *B. juncea*, and *Sinapis alba* caused by flea beetle (*Phyllotreta cruciferae* (Goeze) (Coleoptera: Chrysomelidae) infestation in Northern Idaho. J. Econ. Entomol. 97:1642-1647.

[6]Cárcamo, H. A. and Blackshaw, R. E. 2007. Insect pest incidence and injury to herbicide tolerant canola in Western Canada. Agron. J. 99:842-846.

[7]Cartea, M. E., Padilla, G., Vilar, M. and Velasco, P. 2009. Incidence of the major Brassica pests in Northwestern Spain. J. Econ. Entomol. 102:767-773.

[8]Cook, S. M., Smart, L. E., Martin, J. L., Murray, D. A., Watts, N. P. and Williams, I. H. 2006. Exploitation of host plant preferences in pest management strategies for oilseed rape (*Brassica napus*). Entomol. Exp. Appl. 119:221-229.

[9]da Costa, F. V., Neves, F. S., Silva, J. O. and Fagundes, M. 2011. Relationship between plant development, tannin concentration and insects associated with *Copaifera langsdorffii* (Fabaceae). Arthropod-Plant Interact. 5:9-18.

[10]Eltez, S. and Karsavuran, Y. 2010. Food preference in the cabbage bug *Eurydema ornatum* (L.) (Heteroptera: Pentatomidae). Pakistan J. Zool. 42:407-412.

[11]Gray, F. A. and Koch, D. W. 2002. Trap crops. In Pimentel, D. (ed.). Encyclopaedia of Pest Management. University of Wyoming, Laramie, Wyoming, pp. 852-864.

[12]Meier, U. (ed.) 2001. Growth Stages of Mono and Dicotyledonous Plants. BBCH Monograph. 2nd edn. Federal Biological Research Centre for Agriculture and Forestry. http://syntechresearch.hu/sites/default/files/publikaciok/bbch.pdf (5.2.2011).

[13]Hatfield, J. L., Boote, K. J., Kimball, B. A., Ziska, L. H., Izaurralde, R. C., Ort, D., Thomson, A. M. and Wolfe, D. 2011. Climate impacts on agriculture: Implications for crop production. Agron. J. 103:351-370.

[14]Henderson, A. E., Hallett, R. H. and Soroka, J. J. 2004. Prefeeding behavior of the crucifer flea beetle, *Phyllotreta cruciferae*, on host and nonhost crucifers. J. Insect. Behav. 17:17-39.

[15]Hoffmann, M. P., Kuhar, T. P., Baird, J. M., Gardner, J., Schwartz, P. and Shelton, A. M. 2001. Nonwoven fiber barriers for control of cabbage maggot and onion maggot (Diptera: Anthomyiidae). J. Econ. Entomol. 94:1485-1491.

[16]Hooks, C. R. R. and Johnson, M. W. 2003. Impact of agricultural diversification on the insect community of cruciferous crops. Crop Prot. 22:223-238.

[17]Hopkins, R. J., Ekbom, B. and Henkow, L. 1998. Glucosinolate content and susceptibility for insect attack of three population of *Sinapis alba*. J. Chem. Ecol. 24:1203-1216.

[18]Leite, G. L. D., Picanco, M., Jham, G. N. and Moreira, M. D. 2005. *Bemisia tabaci*, *Brevicoryne brassicae* and *Thrips tabaci* abundance on *Brassica oleracea* var. *acephala*. Pesq. Agropec. Bras. 40:197-202.

[19]Médiène, S., Valantin-Morison, M., Sarthou, J. P., de Tourdonnet, S., Gosme, M., Bertrand, M., Roger-Estrade, J., Aubertot, J. N., Rusch, A., Motisi, N., Pelosi, C. and Doré, T. 2011. Agroecosystem management and biotic interactions: A review. Agron. Sustain. Dev. 31:491-514.

[20]Moyes, C. L., Collin, H. A., Britton, G. and Raybould, A. F. 2000. Glucosinolates and differential herbivory in wild populations of *Brassica oleracea*. J. Chem. Ecol. 26:2625-2641.

[21]Numata, H. and Nakamura, K. 2002. Photoperiodism and seasonal adaptations in some seed-sucking bugs (Heteroptera) in central Japan. 2002. Eur. J. Entomol. 99:155-161.

[22]OEPP/EPPO 2002. Guidelines for the efficacy evaluation of insecticides *Phyllotreta* spp. on rape. Bull. OEPP/EPPO Bull. 32:361-365.

[23]Palaniswamy, P. and Lamb, R. J. 1992. Host preference of flea beetles *Phyllotreta cruciferae* and *Phyllotreta striolata* (Coleoptera: Chrysomelidae) for crucifer seedlings. J. Econ. Entomol. 85:743-752.

[24]Poveda, K., Gomez, M. I. and Martinez, E. 2008. Diversification practices: Their effect on pest regulation and production. Rev. Col. Entomol. 34:131-144.

[25]Ratnadass, A., Fernandes, P., Avelino, J. and Habib, R. 2011. Plant species diversity for sustainable management of crop pests and diseases in agroecosystems: A review. Agron. Sustain. Dev. 32:273-303.

[26]Soroka, J. J., Holowachuk, J. M., Gruber, M. Y. and Grenkow, L. F. 2011. Feeding by flea beetles (Coleoptera: Chrysomelidae; *Phyllotreta* spp.) is decreased on canola (*Brassica napus*) seedlings with increased trichome density. J. Econ. Entomol. 104:125-136.

[27]Shelton, A. M. and Badenes-Perez, F. R. 2006. Concepts and applications of trap cropping in pest management. Ann. Rev. Entomol. 51:285-308.

[28]Stoner, K. A. and Shelton, A. M. 1988. Effects of planting date and timing of growth stage on damage to cabbage by onion thrips (*Thrips tabaci*). J. Econ. Entomol. 81:329-333.

[29]The list of registered plant protection product on 12.3.11. Ministry of Agriculture, Forestry and Food. Phytosanitary Administration of the Republic of Slovenia. http://spletni2.furs.gov.si/FFS/REGSR/index.htm (13.3.2011)

[30]The Regulations on Integrated Production of Vegetables. 2011. The Republic of Slovenia, Ministry of Agriculture, Forestry and Food. http://www.ikc-mb.si/ikc-mb/images/stories/datoteke/IPZ_TN_2011_zelenjava.pdf (14.6.2011)

[31]Toshova, T. B., Csonka, E., Subchev, M. A. and Tóth, M. 2009. The seasonal activity of flea beetles in Bulgaria. J. Pest. Sci. 82:295-303.

[32]Trdan, S., Valič, N., Žnidarčič, D., Vidrih, M., Bergant, K., Zlatič, E. and Milevoj, L. 2005. The role of Chinese cabbage as a trap crop for flea beetles (Coleoptera: Chrysomelidae) in production of white

cabbage. Sci. Hortic. 106:12-24.

Trdan, S., Žnidarčič, D. and Valič, N. 2006. Field efficacy of three insecticides against cabbage stink bugs (Heteroptera: Pentatomidae) on two cultivars of white cabbage. Int. J. Pest Manage. 52:79-87.

Trdan, S., Valič, N. and Žnidarčič, D. 2007. Field efficacy of deltamethrin in reducing damage caused by *Thrips tabaci* Lindeman (Thysanoptera: Thripidae) on early white cabbage. J. Pest Sci. 80:217-223.

Winqvist, C., Bengtsson, J., Aavik, T., Berendse, F., Clement, L. W., Eggers, S., Fischer, C., Flohre, A., Geiger, F., Liira, J., Paert, T., Thies, C., Tscharntke, T., Weisser, W. W. and Bommarco, R. 2011. Mixed effects of organic farming and landscape complexity on farmland biodiversity and biological control potential across Europe. J. Appl. Entomol. 48:570-579.

Xu, Q. C., Xu, H. L., Qin, F. F., Tan, J. Y., Liu, G. and Fujiyama, S. 2010. Relay-intercropping into tomato decreases cabbage pest incidence. J. Food Agric. Environ. 8(2):1037-1041.

Xu, Q., Fujiyama, S. and Xu, H. 2011. Biological pest control by enhancing populations of natural enemies in organic farming systems. J. Food Agric. Environ. 9(2):455-463.

2.5 GLUKOZINOLATI V VARSTVU RASTLIN – PREGLEDNI ČLANEK

BOHINC, Tanja, GORETA BAN, Smiljana, BAN, Dean in TRDAN, Stanislav
Glucosinolates in plant protection strategies: a review.
Archives of Biological Sciences, 2012, 64: 821-828.
Prejeto: januar 2012 , sprejeto: april 2012

V preglednem članku predstavljamo pomen glukozinolatov v varstvu rastlin. Rastlinske vrste se pred napadom škodljivih organizmov lahko branijo na več načinov. Križnice (Brassicaceae), katerih pridelava (gojenje) je v svetu zelo razširjena, se pred napadom škodljivih organizmov lahko varujejo z glukozinolati in njihovimi razgradnimi produkti. Vsebnost glukozinolatov variira med posameznimi rastlinskimi organi oziroma rastlinskimi vrstami ter glede na razvojni stadij rastline. Med dejavnike, ki vplivajo na vsebnost glukozinolatov, štejemo biotične in abiotične dejavnike. Vrsta glukozinolata oziroma njegova količina (vsebnost) pa vpliva na dovzetnost posamezne skupine škodljivcev. Prenos omenjenih znanj v sisteme pridelovanja kapusnic je lahko otežen predvsem v območjih z zmernim podnebjem, kjer so rastline izpostavljene napadom številnih škodljivih organizmov. Med takšna območja spada tudi Slovenija. Zato bo v prihodnje pomembno še več raziskav nameniti razvijanju novih ali izboljševanju obstoječih načinov varstva križnic pred škodljivimi žuželkami, kjer bodo lahko svoje mesto našli tudi glukozinolati kot eden od dejavnikov naravne odpornosti rastlin.

Arch. Biol. Sci., Belgrade, 64 (3), 821-828, 2012 DOI:10.2298/ABS1203821B

GLUCOSINOLATES IN PLANT PROTECTION STRATEGIES: A REVIEW

TANJA BOHINC[1], SMILJANA GORETA BAN[2], D. BAN[3] and S. TRDAN[1]

[1] *University of Ljubljana, Biotechnical Faculty, Dept. of Agronomy, Chair of Phytomedicine, Agricultural Engineering, Crop Production, Pasture and Grassland Management*, SI-1111 Ljubljana, Slovenia
[2] *Institute for Adriatic Crops*, HR-21000 Split, Croatia
[3] *Institute of Agriculture and Tourism*, HR-52440 Poreč, Croatia

Abstract - This review discusses the importance of glucosinolates in plant protection. The Brassicaceae, which are cultivated worldwide, use glucosinolates and their decomposition products to defend themselves against attacks by harmful organisms. The glucosinolate content varies among individual plant species, plant organs and developmental stages. The glucosinolate content in plants is also affected by biotic and abiotic factors, while the type or quantity of glucosinolate determines the susceptibility of the plants to insect pests. These facts can pose a problem when implementing this knowledge in cultivation of the Brassicaceae, especially in regions with moderate climates where Brassicaceae crops are exposed to attacks by a large number of harmful organisms. Under these circumstances, it is essential to research new, or to improve the existing environmentally acceptable methods of protecting Brassicaceae plants against economically important pests.

Key words: Glucosinolates, generalists, specialists, Brassicaceae, insecticidal effect, biofumigation method

INTRODUCTION

For decades, farmers have been attempting to manage the harmful organisms that feed on Brassicaceae crops by application of synthetic insecticides, which in many cases produce negative consequences, such as the development of resistance and negative influences on natural enemies (Hooks and Johnson, 2003). Because of the socio-economic consequences of the excessive use of synthetic insecticides, the use of new, environmentally acceptable methods for plant protection is gaining ground. These practices often involve such strategies as use different dates for sowing/planting, selecting resistant cultivars and cultivating mixed crops (Hooks and Johnson, 2003; Trdan et al., 2005; Ramalho et al., 2012; Bohinc and Trdan, 2012a).

Plants protect themselves from harmful organisms in two ways: via morphological barriers (Smith et al., 2005; Broekgaarden et al., 2008; Müller, 2008; Trdan et al., 2009) and with chemical substances (secondary metabolites) (Pontoppidan et al., 2003; Broekgaarden et al. 2008; Lucas-Barbosa et al., 2011). For the Brassicaceae, the defense mechanism is predominantly chemical and includes glucosinolates (Björkman et al., 2011) and their decomposition products (Broekgaarden et al., 2008; Pratt et al., 2008). It has been reported that many of the wild plants belonging to the same family as plants of agronomic importance often contain larger quantities of secondary metabolites than their cultivated relatives (Chaplin-Kramer et al., 2011).

The purpose of this report was to collect research findings regarding the influence of individual glucosinolate groups on economically important pests and, as far as possible to promote the use of the natural resistance of Brassicaceae in plant protection and food production.

Versatility of the Brassicaceae

The Brassicaceae (or Cruciferae) comprise 3.200 plant species, including fodder plants, vegetables and ornamental plants; some weed species are also included in this family (Ahuja et al., 2010). Among the Brassicaceae of great agronomic significance are cabbage (*Brassica oleracea* L. var. *capitata*), cauliflower (*Brassica oleracea* L. var. *botrytis*), broccoli (*Brassica oleracea* L. var. *italica*), Brussels sprouts (*Brassica oleracea* var. *gemmifera*), turnip rape (*Brassica rapa* L. ssp. *sylvestris* f. *autumnalis*), different species of mustard (*Brassica juncea*, *Brassica nigra* and *Brassica hirta*) and some other species of leafy vegetables. Production in different climate conditions (Björkman et al., 2011) has enabled the Brassicaceae, which are important from both agronomic (Font et al., 2005; Vaughn and Berhow, 2005; Cartea et al., 2008; Blažević and Mastelić, 2009) and economic aspects (Vaughn and Berhow, 2005), to develop different resistance mechanisms against harmful organisms.

Glucosinolates: characteristic secondary metabolites

Glucosinolates are secondary metabolites (Kliebenstein et al., 2005) that are characteristic of the order Capparales (Al-Gendy et al., 2010; Björkman et al., 2011), primarily represented by the family Brassicaceae (Griffiths et al., 2001; Johnson, 2002; de Villena et al., 2007; Cartea et al., 2007; Blažević and Mastelić, 2009; Al-Gendy et al., 2010; Müller et al., 2010; Björkman et al., 2011; Winde and Wittstock, 2011). These compounds are also produced by 13 other botanical families (Newton et al., 2009). Glucosinolates consist of a β-D-thioglycoside group, a sulfonated oxime functional group and a variable side chain (Beekwilder et al., 2008; Vig et al., 2009; Blažević and Mastelić, 2009; Al-Gendy et al., 2010); based on their side chain, the compounds are divided into aliphatic, indole and aromatic (Cartea and Velasco, 2008; Van Eylen et al., 2009). The presence of glucosinolates varies between individual plant organs (Fahey et al., 2001; Winde and Wittstock, 2011), plant species (Moyes et al., 2000; Chaplin-Kramer et al., 2011), developmental stages (de Villena et al., 2007; Cartea et al., 2008), and also depends on the weather conditions (Velasco et al., 2007; Winde and Wittstock, 2011).

Glucosinolates affect individual groups of pests, generalists or specialists, differently (Lankau, 2007; Müller, 2010), with the activity of these secondary metabolites either stimulating or deterring feeding. When the plant tissue (cells) is damaged, various biotic or abiotic factors cause the hydrolysis of glucosinolates, resulting in the production of isothiocyanates, thiocyanates and nitriles (Broekgaarden et al. 2008; Müller, 2009). Isothiocyanates and nitriles stimulate specialist pests, whereas their effect on generalists is most often considered repellent (Müller, 2009). The insecticidal effect of isothiocyanates on representatives of the order Lepidoptera can be compared to the effects of synthetic insecticides; conversely, the effects of nitriles are less pronounced, and they primarily serve to attract natural enemies (Schramm et al., 2012).

Influence of glucosinolates on monophagous insect pests of the Brassicaceae

To defend themselves against plant secondary metabolites, herbivores have developed several physiological defense mechanisms (Textor and Gershenzon, 2009). Herbivores can reduce the effects of secondary metabolites primarily by rapid enzymatic decomposition, thereby transforming them into less toxic or non-toxic derivates (compounds), or by rapidly excreting them (Poelman et al., 2008; Müller, 2009). Interestingly, certain species of herbivores can employ glucosinolates for their own defense. Although these species are primarily a small group of specialists (Pontoppidan et al., 2003; Broekgaarden et al. 2008; Chaplin-Kramer et al., 2011), glucosinolates exert toxic effects on some specialists (Poelman et al., 2008). To defend itself against natural enemies, the cabbage aphid (*Brevicoryne brassicae* [L.]) produces the enzyme myrosinase which degrades plant glucosinolates (Broekgaarden et al. 2008). A similar defense mechanism is used by the mustard aphid (*Lipaphis erysimi* [Kaltenbach]) (Pratt et al., 2008) which occasionally infests oilseed rape and certain mustard plants (Rana, 2005).

Caterpillars of the small white butterfly (*Pieris rapae* [L.]) can degrade glucosinolates through a specific protein found in their intestines. The protein transforms unstable aglycone into nitriles which the larvae then excrete from their bodies. The transformation of these toxic isothiocyanates into less-toxic or non-toxic nitriles also occurs in other species of the genus *Pieris*, *Pieris virginiensis* (Edwards), the green-veined white (*Pieris napi* [L.]), the large white (*Pieris brassicae* [L.]) and also in the orange tip (*Anthocharis cardamines* [L.]). Stimulating effects of glucosinolates on the adult females of the small white (*Pieris rapae* [L.]) and the large white (*Pieris brassicae* [L.]) have also been established in addition to the stimulating effects on the feeding of large white and green-veined white caterpillars (Smallegange et al., 2007). The glucosinolate concentration also significantly influences the duration of the developmental stages of these butterflies (Smallegange et al., 2007).

Caterpillars of the diamondback moth (*Plutella xylostella* [L.]) contain the enzyme sulphatase which transforms the glucosinolates into desulphoglucosinolates; the caterpillars then excrete the desulphoglucosinolates. A similar system of decomposition was found in the desert locust (*Schistocerca gregaria* [Forskål]) (Müller, 2009; Textor and Gershenzon, 2009). Caterpillars of the turnip sawfly (*Athalia rosae* [L.]) use many aliphatic and aromatic glucosinolates to protect themselves against the predatory European paper wasp (*Polistes dominulus* [Christ]), common wasp (*Vespula vulgaris* [L.]) and spined soldier bug (*Podisus maculiventris* [Say]) (Müller et al., 2001; Müller, 2009). Glucosinolates also protect caterpillars of the turnip sawfly against attacks by the European fire ant (*Myrmica rubra* [L.]) (Müller, 2009). Soler et al. (2007) reported on the negative influence of high glucosinolate concentrations on the development of cabbage fly larvae (*Delia radicum* [L.]), whereas the negative influence of isothiocyanates on eggs was detected for the Brassica pod midge (*Dasineura brassicae* [Winn.]) (Åhman, 1985; Björkman et al., 2011) and on the feeding of rape beetle (*Meligethes aeneus* [Fabricius]) imagos (Cook et al., 2006).

The accessibility of different Brassicaceae species within a specific area has also influenced the different extents of damage caused by cabbage stink bugs (*Eurydema* spp.), and it was reported that the glucosinolates in oilseed rape had the greatest stimulating effect on the feeding behavior of cabbage stink bugs (Bohinc et al., 2012).

Influence of glucosinolates on polyphagous insect pests feeding on the Brassicaceae

The influence of individual glucosinolates can significantly affect the feeding behavior of the bertha armyworm (*Mamestra configurata* [Walker]). Indeed, the ability of a plant to defend itself against attacks by this species is affected by the presence of sinalbin and sinigrin (McCloskey et al., 1993; Ulmer et al., 2001): the higher the content of these glucosinolates, the less likely it is that *Mamestra configurata* (Walker) will feed on the plant. One study established that the green peach aphid (*Myzus persicae* [Sulzer]) excretes glucosinolates in its honeydew (Kos et al., 2011) and thus avoids the insecticidal effects of the secondary metabolites; however, this is not the case for all of the glucosinolate groups. Plants that contain only indole glucosinolates are much more resistant to attack by the green peach aphid (*Myzus persicae*) (Kim et al., 2008), which is a very significant pest of peach, tobacco, vegetables and flowers (Vučetić et al., 2008).

Although monophagous caterpillars of the order Lepidoptera can adjust to glucosinolates (they use them for defending themselves), polyphagous caterpillars of the same order have no such adjustment mechanism when feeding (Schramm et al., 2012).

The Brassicaceae are not the most important hosts for the beet armyworm (*Spodoptera exigua* [Hübner]) and the African cotton leafworm (*Spodoptera littoralis*), yet these insects can still complete their developmental cycle. In contrast, the appearance of the cabbage moth (*Mamestra brassicae* [L.]) and the cabbage looper (*Trichoplusia ni* [Hübner]) can represent a serious problem in the cultivation of Brassicaceae (Schramm et al., 2012). It is known that higher glucosinolate content reduces the extent of cabbage looper (*Trichoplusia ni*) feeding (Kliebenstein et al., 2002). An important factor in the feeding behavior of the cabbage moth (*Mamestra brassicae*) is also the selection of the plant variety, as some Brassicaceae varieties are much more susceptible to attack by these harmful pests (Cartea et al., 2010), an observation that is attributed to the lower glucosinolate content of these varieties.

Influence of glucosinolates on harmful soil organisms (the process of biofumigation)

The term "biofumigation" normally means the suppression of harmful organisms in the soil (herbivores, nematodes and fungi) using plant species (most frequently the Brassicaceae) that contain glucosinolates (Elberson et al. 1996; Matthiessen and Shackleton, 2005; Gimsing and Kirkegaard, 2009). Glucosinolate decomposition products can also influence the ability of weeds to germinate and grow (Bangarwa et al. 2011; Boydston et al. 2011). The influence of products created during the hydrolysis of glucosinolates can successfully be used as an alternative for methyl bromide (Lazzeri et al., 2004).

The effect of biofumigation can be reached in several ways: by ploughing in fresh plant mass or Brassicaceae seed meal (a side-product of the pressing of seeds to produce oil). Biocidal effects can also be achieved by ploughing in dry plant mass, which contains a proportion of the active isothiocyanates of the living plants (Gimsing and Kirkegaard, 2009).

The use of seed meal has proven successful in suppressing wireworms (*Agriotes* spp.) (Elberson et al. 1996; Furlan et al. 2010), and nematicidal (Lazzeri et al., 2009; Zasada et al., 2009) and herbicidal properties have been reported (Noswothy et al. 2005; Handiseni et al., 2011). The nematicidal effects of glucosinolate decomposition products have been reported for the species *Meloidogyne javanica* (Treub [Chitwood]) (McLeod and Steel, 1999, Wu et al., 2011), and a high *in vitro* efficiency against potato nematode (*Globodera rostochiensis* (Woll [Behrens]) has also been confirmed (Serra et al., 2002; Aires et al., 2009).

The results of recent research show that the biofumigation method is also successful in suppressing soil pathogens (Mattner et al., 2008; Motisi et al., 2009), including *Fusarium* spp. (Martínez et al., 2011), Texas root rot (*Phymatotrichopsis omnivora*, [Duggar] Hennebert) (Hu et al., 2011), *Verticillium dahliae* (Kleb.) (Larkin et al., 2011), *Rhizoctonia solani* (Kühn) and take-all (*Gaeumannomyces graminis* var. *tritici* [Walker]) (Motisi et al., 2009).

Influence of glucosinolates on useful organisms

The activities of natural enemies are influenced by both the kind of prey and the plant genotype (Kos et al., 2011). The glucosinolate content in the body of the cabbage aphid can influence natural enemies differently. On the one hand, the negative influence on the predators marmalade hoverfly (*Episyrphus balteatus* [De Geer]) (Kos et al., 2012) and common green lacewing (*Chrysoperla carnea* [Stephens]) (Kos et al., 2011) were described; on the other hand, a stimulating influence of isothiocyanates on the species *Diaeretiella rapae* (M' Intosh) was also reported (Bradburne and Mithen, 2000; Kos et al., 2012).

The vitality of the two-spot ladybird (*Adalia bipunctata* [L.]) and the seven-spot ladybird (*Coccinella septempunctata* [L.]), which feed on cabbage aphid larvae, depends on the sinigrin content (Prat et al., 2008). Sinigrin negatively influences the species *A. bipunctata*, whereas no influence has been detected for the species *C. septempunctata* (Prat et al., 2008).

The application of Brassicaceae seed meal can have a whole spectrum of positive properties that affect harmful organisms in the soil, yet it can also negatively influence non-target organisms, for example, entomopathogenic nematodes of the genus *Steinernema*, as glucosinolate decomposition products prevent the activities of these biotic agents (Henderson et al., 2009).

CONCLUSIONS

Our review has outlined the specificity of the effects of individual glucosinolates on different groups of harmful and useful organisms (Chaplin-Kramer et al., 2011). Because the number of registered synthetic insecticides is continuously decreasing (Stojanović et al., 2007; The list of registered …, 2012), more research has been focused on the study of the natural resistance of plants which is manifested by ennobling programs (Müller, 2009; Ban et al., 2006). The utilization of the inherent defense mechanisms of plants will gain more importance in the future.

The fact that the glucosinolate content varies between individual plant species (Meyes et al., 2000; Chaplin-Kramer et al., 2011), between organs of the same plant species and between the developmental stages (de Villena et al., 2007; Cartea et al., 2008) of individual plant species, suggests that the same species and glucosinolate concentration may differentially influence (repel or stimulate) various species of harmful organisms. We believe that all of the listed attributes can represent problems for the implementation of this knowledge in Brassicaceae production, especially in areas with a moderate climate where the Brassicaceae are exposed to attack by numerous harmful insects and other organisms. This situation can undoubtedly be an additional reason to research new methods or to improve the existing methods of the environmentally acceptable protection of Brassicaceae against economically important harmful organisms.

Acknowledgments - The present work was performed within project V4-1067 which was financed by the Slovenian Research Agency and the Ministry of Agriculture and Environment of the Republic of Slovenia. A portion of the present research was funded by Horticulture N° P4-0013-0481, a program funded by the Slovenian Research Agency.

REFERENCES

Aires, A., Carvalho, R., Barbosa, M. D., and *E. Rosa* (2009). Supressing potato cyst nematode, *Globodera rostochiensis*, with extracts of Brassicacea plants. *Am. J. Potato Res.* **86**, 327-333.

Al-Gendy, A.A., El-gindi, O.D., Hafez, A.S., and *A.M. Ateya* (2010). Glucosinolates, volatile constituents and biological activities of *Erysimum corinthium* Boiss. (Brassicaceae). *Food Chem.* 118, 519-524.

Åhman, I (1986). Toxities of host secondary compounds to eggs of the *Brassica* specialist *Dasineura brassicae*. *J. Chem. Ecol.* 12. 1481-1488.

Ahuja, I., Rohloff, J., and *A.M. Bones* (2010). Defence Mechanisms of Brassicaceae: implications for plan-insect interactions and potencial for integrated pest management. In: Sustainable Agriculture Volume 2 (Eds. Lichtfouse E.), 623-666. Springer Science+Business Media, Netherlands.

Ban, D., Goreta, S., and *J. Borošić* (2006). Plant spacing and cultivar affect melon growth and yield components. *Sci. Hortic.* 109, 238-243.

Bangarwa, S.K., Norsworthy, J.K., Mattice, J.D., and *E.E. Gbur* (2011). Glucosinolate and isothiocyanate production from Brassicaceae cover crops in a plasticulture production system. *Weed Sci.* 59, 247-254.

Beekwilder, J., van Leeuwen, W., van Dam, N.M., Bertossi, M., Grandi, V., Mizzi, L., Soloviev, M., Szabados, L., Molthoff, J.W., Schipper, B., Verbocht, H., de Vos, R.C.H., Morandini, P., Aarts, M.G.M., and *A. Bovy* (2008). The impact of the absence of aliphatic glucosinolates on insect herbivory in Arabidopsis. *Plos One* 3: e2068. doi:10.1371/journal.pone.0002068.

Björkman, M., Klingen, I., Birch, A.N.E., Bones, A.M., Bruce, T.J.A., Johansen, T.J., Meadow, R., Mølmann, J., Seljåsen, R., Smart, L.E., and *D. Stewart* (2011). Phytochemicals of Brassicaceae in plant protection and human health – Influences of climate, environment and agronomic practice. *Phytochem.* 72, 538-556.

Bohinc, T., and *S. Trdan* (2012)a. Trap crops for reducing damage caused by cabbage stink bugs (*Eurydema* spp.) and flea beetles (*Phyllotreta* spp.) on white cabbage: fact or fantasy. *J. Food Agric. Environ.* **10**. (in press)

Bohinc, T., and *S. Trdan* (2012)b. Environmental factors affecting the glucosinolate content in *Brassicaceae*. *J. Food Agric. and Environ.* **10**. (in press).

Bohinc, T., Hrastar, R., Košir, I.J. and *S. Trdan* (2012). Association between glucosinolate concentration and injuries caused by cabbage stink bugs *Eurydema* spp. (Heteroptera: Pentatomidae) on different Brassicas. *Acta Sci.-Agron.* **34** in press

Blažević, I., and *J. Mastelić* (2009). Glucosinolate degradation products and other bound and free volatiles in the leaves and roots of radish (*Raphanus sativus* L.). *Food Chem.* 113, 96-102.

Boydston, R.A., Morra, M. J., Borek, V., Clayton, L., and *S.F. Vaughn* (2011). Onion and weed response to mustard (*Sinapis alba*) seed meal. *Weed Sci.* **59**, 546-552.

Bradburne, R.P. and *R. Mithen* (2000). Glucosinolate genetics and the attraction of the aphid parasitoid *Diaeretiella rapae* to Brassica. *Proc. R. Soc. Lon. Sci. Ser. B.* **266**: 89-95.

Broekgaarden, C., Poelman, E.H., Steenhuis, G., Voorrips, R.E., Dicke, M., and *B. Vosman* (2008). Responses of *Brassica oleracea* cultivars to infestation by the aphid *Brevicoryne brassicae*: an ecological and molecular approach. *Plant, Cell and Environ.* **31**, 1592-1605.

Cartea, M.E., Velasco, P., Obregon, S., Padilla, G., and *A. de Haro* (2008). Seasonal variation in glucosinolate content in *Brassica oleracea* crops grown in northwestern Spain. *Phytochem.* **69**, 403-410.

Cartea, M.E., and *P. Velasco* (2008). Glucosinolates in Brassica foods: bioavability in food and significance for human health. *Phytochem. Rev.* 7, 213-229.

Cartea, M.E., Francisco, M., Lema, M., Soengas, P. and *P. Velasco* (2010). Resistance of cabbage (*Brassica oleracea capitata* group) crops to *Mamestra brassicae*. *J. Econ. Entomol.* **103**, 1866-1874.

Chaplin-Kramer, R., Kliebenstein, D.J., Chiem, A., Morrill, E., Mills, N.J., and *C. Kremen* (2011). Chemically mediated tritrophic interactions: opposing effects of glucosinolates on a specialist herbivore and its predators. *J. Appl. Ecol.* 48, 880-887.

Cook, S.M., Smart, L.E., Martin, J.L., Murray, D.A., Watts, N.P., and *I.H. Williams* (2006). Exploitation of host plant preferences in pest management strategies for oilseed rape (*Brassica napus*). *Entomol. Exp. Appl.* **119**, 221-229.

de Villena, I.A., Fritz, V.A., Cohen, J.D., and *W.D. Hutchison* (2007). Changes in gluconasturtiin concentrations in chinese cabbage with increasing cabbage looper density. *HortSci.* 42, 1337-1340.

Elberson, L.R., Borek, V., McCaffrey, J.P., and *M.J. Morra* (1996). Toxity of rapeseed meal-amended soil to wireworms, *Limonius californicus* (Coleoptera: Elateridae). *J. Agric. Entomol.* 13, 323-330.

Fahey, J.W., Zalcmann, A.T., and *P. Talalay* (2001). The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. *Phytochem.* 56: 5-51.

Furlan, L., Bonetto, C., Finotto, A., Lazzeri, L., Malaguti, L., Patalano, G., and *W. Parker* (2010). The efficacy of biofumigant meals and plants to control wireworm populations. *Ind. Crops Prod.* 31, 245-254.

Font, R., del Rio-Celestino, M., Cartea, E., and *A. de Haro-Bailón* (2005). Quantification of glucosinolates in leaves of leaf rape (*Brassica napus* ssp. *pabularia*) by near-infrared spectroscopy. *Phytochem.* **66**, 175-185.

Gimsing, A. L., and *J.A. Kirkegaard* (2009). Glucosinolates and biofumigation: fate of glucosinolates and their hydrolysis products in soil. *Phytochem. Rev.* 8, 299-310.

Griffiths, D.W., Deighton, N., Birch, A.N.E., Patrian, B., Baur, R. and *E. Städler* (2001). Identification of glucosinolates on the leaf surface of plants from the cruciferae and other closely related species. *Phytochem.* 57, 693-700.

Handiseni, M., Brown, J., Zemetra, R., and *M. Mazzola* (2011). Herbicidal activity of brassiceae seed meal on wild oat (*Avena fatua*), italian ryegrass (*Lolium multiflorum*), redroot pigweed (*Amarathus retroflexus*), and prickly lettuce (*Lactuca serriola*). *Weed Tech.* 25, 127-134.

Henderson, D.R., Riga, E., Ramirez, R.A., Wilson, J., and E. Snyder (2009). Mustard biofumigation disrupts biological control by *Steinernema* spp. nematodes in the soil. *Biol. Control* 48, 316-322.

Hu, P., Wang, A.S., Engledow, A. S., Hollister, E.B., Rothlisberger, K. L., Matocha, J.E., Zuberer, D. A., Provin, T. L., Hons, F.M., and *T.J. Gentry* (2011). Inhibition of the germination and growth of *Phymatotrichopsis omnivora* (cotton root rot) by oilseed meals and isothiocyanates. *Appl. Soil Ecol.* **49**, 68-75.

Hooks, C.R.R., and *M.W. Johnson* (2003). Impact of agricultural diversification on the insect community of cruciferous crops. *Crop Prot.* 22, 223-238.

Johnson, I.T. (2002). Glucosinolates in the human diet. Bioavailability and implications for health. *Phytochem. Rev.* **1**, 183-188.

Lankau, R. A. (2007). Specialist and generalist herbivores exert opposing selection on a chemical defense. *New Phytol.* **175**, 176-184.

Larkin, R. P., Honeycutt, C. W., and *O.M. Olanya* (2011). Management of *Verticillium* wilt of potato with disease-suppressive green manures and as affected by previous cropping history. *Plant Dis.* 95, 568-576.

Lazzeri, L., Leoni, O., and *L.M. Manici* (2004). Biocidal plant dried pellets for biofumigation. *Ind. Crops Prod.* **20**, 59-65.

Lazzeri, L., Curto, G., Dallavalle, E., D'Avino, L., Malaguti, L., Santi, R., and *G. Patalano* (2009). Nematicidal efficacy of biofumigation by defatted brassicaceae meal for control of *Meloidogyne incognita* (Kofoid et White) Chitw. on full field zucchini crop. *J. Sustain. Agric.* 33, 349-358.

Lucas-Barbosa, D., van Loon, J.J.A., and *M. Dicke* (2011). The effects of herbivore induced plant volatiles on interactions between plants and flower-visiting insects. *Phytochem.* 72, 1647-1654.

Kim, J. H., Lee, B. W., Schroeder, F. C., and G. Jander (2008). Identification of indole glucosinolate breakdown products with antifeedant effects on *Myzus persicae* (green peach aphid). *Plant J.* 54, 1015-1026.

Kliebenstein, D. J., Pedersen, D., Barker, B., and T. Mitchell-Olds (2002). Comparative analysis of quatative trait loci controlling glucosinolates, myrosinase and insect resistance in *Arabidopsis thaliana*. Genetics 161, 325-332.

Kliebenstein, D. J., Kroymann, J., and T. Mitchell-Olds (2005). The glucosinolate-myrosinase system in an ecological and evolutionary context. *Curr. Opinion Plant Biol.* 8, 264-271.

Kos, M., Kabouw, P., Noordam, R., Hendriks, K., Vet, L.E.M., van Loon, J.J.A., and M. Dicke (2011). Prey mediated effects of glucosinolates on aphid predators. *Ecol. Entomol.* 36, 377-388.

Kos, M., Houshyani, B., Achhami, B.B., Wietsma, R., Gols, R., Weldegergis, B.T., Kabouw, P., Bouwmeester, H.J., Vet, L.E.M., Dicke, M., and J.J.A. van Loon (2012). Herbivore-mediated effects of glucosinolates on different natural enemies of a specialist aphid. *J. Chem. Ecol.* 38, 100-115.

Martínez, M. A., Martínez, M.C., Bielza, P., Tello, J., and A. Lacasa (2011). Effect of biofumigation with manure amendments and repeated biosolarization on *Fusarium* densities in pepper crops. *J. Ind. Microbiol. Biotechnol.* 38, 3-11.

Matthiessen, J. N., and M. A. Shackleton (2005). Biofumigation: environmental impacts on the biological activity of diverse pure and plant-derived isothiocyanates. *Pest Manage. Sci.* 61, 1043-1051.

Mattner, S. W., Porter, I. J., Gounder, R.K., Shanks, A.L., Wren, D. J., and D. Allen (2008). Factors that impact on the ability of biofumigants to supress fungal pathogens and weeds of strawberry. *Crop Prot.* 27, 1165-1173.

McCloskey, C., and M.B. Isman (1993). Influence of foliar glucosinolates in oilseed rape and mustard on feeding and growth of the bertha armyworm, *Mamestra configurata* Walker. *J. Chem. Ecology* 19, 249-266.

McLeod, R. W., and C.C. Steel (1999). Effects of brassica-leaf green manures and crops on activity and reproduction of *Meloidogyne javanica*. *Nematol.* 1, 613-624.

Mewis, C.L., Collier, H.A., Britton, G., and A.F. Raybould (2002). Glucosinolates and differential herbivory in wild populations of *Brassica oleracea*. *J. Chem. Ecol.* 26, 2625-2641.

Motisi, N., Montfort, F., Doré, T., Romillac, N., and P. Lucas (2009). Duration of control of two soilborne pathogens following incorporation of above- and below-ground residues of *Brassica juncea* into soil. *Plant Pathol.* 58, 470-478.

Müller, C., Agerbirk, N., Olsen, C.E., Boevé, J.L., Schaffner, U., and P.M. Brakefield (2001). Sequestration of host plant glucosinolates in the defensive hemolymph of the sawfly *Athalia rosae*. *J. Chem. Ecol.* 27, 2505-2516.

Müller, C. (2008). Resistance at the plant cuticule. In: Induced plant resistance to herbivory (Eds. A. Schaller), 107-129. Springer Science+Business Media. Netherlands.

Müller, C. (2009). Interactions between glucosinolate- and myrosinase-containig plants and the sawfly *Athalia rosae*. *Phytochem. Rev.* 8, 121-134.

Müller, R., de Vos, M., Sun, J.Y., Sønderby, I.E., Halkier, B.A., Wittstock, U., and G. Jander (2010). Differential effects of indole and aliphatic glucosinolates on Lepidopteran herbivores. *J. Chem. Ecol.* 36, 905-913.

Newton, E.L., Bullock, J.M., and D.J. Hodgson (2009). Glucosinolate polymorphism in wild cabbage (*Brassica oleracea*) influences the structure of herbivore communities. *Oecologia* **160**, 63-76.

Norsworthy, J.K., and J.T. Meehan (2005). Use of isothiocyanates for suppression of palmer amaranth (*Amaranthus palmeri*), pitted morningglory (*Ipomoea lacunosa*) and yellow nutsedge (*Cyperus esculentus*). *Weed Sci.* 53, 884-890.

Poelman, E. H., Galiart, R.J.F.H., Raaijmakers, C.E., van Loon, J.J.A., and N.M. van Dam (2008). Performance of specialist and generalist herbivores feeding on cabbage cultivars is not explained by glucosinolate profiles. *Entomol. Exp. Appl.* 127, 218-228.

Pontoppidan, B., Hopkins, R., Rask, L., and J. Meijer (2003). Infestation by cabbage aphid (*Brevicoryne brassicae*) on oilseed rape (*Brassica napus*) causes a long lasting induction of the myrosinase system. *Entomol. Exp. Appl.* 109, 55-62.

Pratt, C., Pope, T.W., Powell, G., and J.T. Rossiter (2008). Accumulation of glucosinolates by the cabbage aphid *Brevicoryne brassicaceae* as a defense against two coccinellid species. *J. Chem. Ecol.* 34, 323-329.

Rana, J.S. (2005). Performance of *Lipaphis erysimi* (Homoptera: Aphididae) on different *Brassica* species in a tropical environment. *J. Pest Sci.* 78, 155-160.

Ramalho, F.S., Fernandes, F.S., Nascimento, A.R.B., Nascimento, J.L., Malaquias, J.B., and C. A. D. Silva (2012). Feeding damage from cotton aphids, *Aphis gossypii* glover (Hemiptera: Heteroptera: Aphididae) in cotton with colored fiber intercropped with fennel. *Ann. Entomol. Soc. America.* 105, 20-27.

Schramm, K., Giddings Vassão, D., Reichelt, M., Gershenzon, J., and U. Wittstock (2012). Metabolism of glucosinolate-derived isothiocyanates to glutathione conjugates in generalist lepidopteran herbivores. *Insect Biochem. and Mol. Biol.* 42, 174-182.

Serra, B., Rosa, E., Iori, R., Barillari, J., Cardoso, A., Abreu, C., and *P. Rollin* (2002). In vitro activity of 2-phenylethyl glucosinolate, and its hydrolis derivates on the root-knot nematode *Globodera rostochiensis* (Woll.). *Sci. Hortic.* **92**, 75-81.

Smallegange, R.C., van Loon, J.J.A., Blatt, S.E., Harvey, J.A., Agerbirk, N., and *M. Dicke* (2007). Flower vs. leaf feeding by *Pieris brassicae*: glucosinolate rich flower tissues are preferred an sustain higher growth rate. *J. Chem. Ecol.* **33**, 1831-1844.

Soler, R., Bezemer, T.M., Cortesero, A.M., van der Putten, W.H., Vet, L.E.M., and *J.A. Harvey* (2007). Impact of foliar herbivory on the development of a root-feeding insect and its parasitoid. *Oecologia* **152**, 257-264.

Stojanović, M., Karaman, S. and *T. Milutinović* (2007). Herbicide and pesticide effects on the earthworm species *Eisenia foetida* (Savigny, 1826) (Oligochaeta, Lumbricidae). *Arch. Biol. Sci. Belgrade*. **59**, 25-26.

Textor, S., and *J. Gershenzon* (2009). Herbivore induction of the glucosinolate-myrosinase defense system: major trends, biochemical bases and ecological significance. *Phytochem. Rev.* **8**, 149-170.

The list of registered plant protection product on 28.03.12. Ministry of Agriculture, Forestry and Food. Phytosanitary Administration of the Republic of Slovenia http://spletni2.furs.gov.si/FFS/REGSR/index.htm (28.03.2012)

Trdan, S., Valič, N., Žnidarčič, D., Vidrih, M., Bergant, K., Zlatič, E., and *L. Milevoj* (2005). The role of Chinese cabbage as a trap crop for flea beetles (Coleoptera: Chrysomelidae) in production of white cabbage. *Sci. Hortic.* **106**, 12-24.

Trdan, S., Valič, N., Vovk, I., Martelanc, M., Simonovska, B., Vidrih, R., Vidrih, M., and *D. Žnidarčič* (2009). Natural resistance of cabbage against three insect pests. In: Integrated Protection of Field Vegetables, Proceedings of the meeting. IOBC/wprs Bulletin (Eds. R. Collier), 93-106. Porto, Portugal.

Ulmer, B., Gillott, C., and *M. Erlandson* (2001). Feeding preferences, growth, and development of *Mamestra configurata* (Lepidoptera: Noctuidae) on Brassicaceae. Can. Entomol. 133, 509-519.

van Eylen, D., Bellostas, N., Strobel, B.W., Oey, I., Hendrickx, M., Van Loey, A., Sørensen, H., and *J.C. Sørensen* (2009). Influence of pressure/temperature treatments on glucosinolate conversion in broccoli (*Brassica oleracea* L. cv. *italica*). *Food Chem.* 112, 646-653.

Vaughn, S.F., and *M.A. Berhow* (2005). Glucosinolate hydrolisis products from various plant sources: Ph effects, isolation, and purification. *Ind. Crop Prod.* 21, 693-202.

Velasco, P., Cartea, M.E., Gonzales, C., Vilar, M., and *A. Ordas* (2007). Factors affecting the glucosinolate content of kale (*Brassica oleracea acephala* group). *J. Agric. Food Chem.* 55, 955-963.

Vig, A.P., Rampal, G., Thind, T.S., and *S. Arora* (2009). Bio-protective effects of glucosinolates – a review. *LWT – Food Sci. Techn.* **42**, 1561-1572.

Vučetić, A., Petrović-Obradović, O., Margaritopoulos, J. and *P. Skouras* (2008). Establishing the resistance of *Myzus persicae* (Sulzer) by molecular methods. *Arch. Biol. Sci. Belgrade*. 60, 3, 493-499.

Winde, I., and *U. Wittstock* (2011). Insect herbivore counter adaptions to the plant glucosinolate-myrosinase system. *Phytochem.* 72, 1566-1575.

Wu, H., Wang, C. J., Bian, X. W., Zeng, S. Y., Lin, K. C., Wu, B., Zhang, G. A., and *X. Zhang* (2011). Nematicidal efficacy isothiocyanates against root-knot nematode *Meloidogyne javanica* in cucumber. *Crop Prot.* **30**, 33-37.

Zasada, I.A., Meyer, S.L.L., and *M.J. Morra* (2009). Brassicaceous seed meals as soil amendments to supress the plant-parasitic nematodes *Pratylenchus penetrans* and *Meloidogyne incognita*. *J. Nematol.* **41**, 221-227.

2.6 ZA ZMANJŠEVANJE POŠKODB KAPUSOVIH BOLHAČEV (*Phyllotreta* spp.) NA ZELJU SE PRIPOROČA SETEV MEŠANIH PRIVABILNIH POSEVKOV KRIŽNIC

BOHINC, Tanja, TRDAN, Stanislav
Sowing mixtures of Brassica trap crops is recommended to reduce *Phyllotreta* beetles injury to cabbage.
Acta Agriculturae Scandinavica Section B – Soil and Plant Science, 2013, 63: 297-303.
Prejeto: 28.08.2012, sprejeto: 19.11.2012

V dvoletnem poljskem poskusu (2009-2010) smo na dveh lokacijah v Sloveniji preučevali obseg poškodb zaradi odraslih osebkov kapusovih bolhačev na štirih različnih vrstah križnic. Zanimala nas je možnost uporabe krmne ogrščice, bele gorjušice in oljne redkve, kot potencialnih privabilnih posevkov za omenjene škodljivce, z namenom obvarovanja glavne rastlinske vrste, zelja. Na obeh lokacijah poskusa smo ugotovili signifikanten vpliv vrste križnic na obseg poškodb kapusovih bolhačev. Obseg poškodb na oljni redkvi je bil signifikatno najvišji skozi večino rastne dobe, medtem ko je bila preferenca do krmne ogrščice in bele gorjušice izražena samo v določenih obdobjih. Prvi obseg poškodb smo zabeležili v prvi polovici maja, medtem ko je bil obseg poškodb signifikantno najvišji v začetku julija. Rezultati naše raziskave kažejo, da je obravnavana metoda uporabna v pridelavi srednjepoznih genotipov zelja. Ker v naši raziskavi nismo zabeležili izrazite preference kapusovih bolhačev do izbranih vrst križnic, predlagamo uporabo mešanih privabilnih posevkov v pridelovalnih sistemih zelja. Z omenjenim načinom bo vsaka od treh obravnavanih vrst križnic privabljala odrasle osebke kapusovih bolhačev v določenem delu rastne robe zelja.

Acta Agriculturae Scandinavica, Section B - Soil & Plant Science

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/sagb20

Sowing mixtures of Brassica trap crops is recommended to reduce Phyllotreta beetles injury to cabbage

Tanja Bohinc [a] & Stanislav Trdan [a]

[a] Department of Agronomy, Biotechnical Faculty , University of Ljubljana , Ljubljana , Slovenia

Accepted author version posted online: 30 Nov 2012.Published online: 25 Mar 2013.

To cite this article: Tanja Bohinc & Stanislav Trdan (2013): Sowing mixtures of Brassica trap crops is recommended to reduce Phyllotreta beetles injury to cabbage, Acta Agriculturae Scandinavica, Section B - Soil & Plant Science, 63:4, 297-303

To link to this article: http://dx.doi.org/10.1080/09064710.2012.752858

Acta Agriculturae Scandinavica, Section B - Soil & Plant Science, 2013
Vol. 63, No. 4, 297–303, http://dx.doi.org/10.1080/09064710.2012.752858

Taylor & Francis

ORIGINAL ARTICLE

Sowing mixtures of Brassica trap crops is recommended to reduce *Phyllotreta* beetles injury to cabbage

Tanja Bohinc* and Stanislav Trdan

Department of Agronomy, Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia

(Received 28 August 2012; final version received 16 November 2012; accepted 19 November 2012)

We studied the extent of damage by cabbage flea beetles on four different Brassica species in a two-year field experiment (2009–2010) at two locations in Slovenia. The entire experiment was based on testing oilseed rape, white mustard and oil radish as potential trap crops to protect cabbage from cabbage flea beetles. A significant influence of the Brassica species on the feeding by the flea beetles was confirmed at both locations. The damage index on oil radish was the highest throughout most of the growth period, whereas oilseed rape and white mustard were preferred only during a certain growth period. The initial damage by the cabbage flea beetles occurred in the first half of May, whereas the greatest damage occurred at the beginning of July. The research shows that the onset of cabbage flea beetle feeding can be controlled in a medium late cabbage cultivar using trap cropping. However, because none of the tested trap crops strongly attracted the flea beetles throughout the entire growing period of the crop, we recommend sowing mixtures of crops for cabbage production; thus, each of the three Brassica species would attract phytophagous insects during a particular part of the cabbage growing season.

Keywords: Alternative methods; Brassica; cabbage; flea beetles; trap cropping

Introduction

Food producers strive to maximise crop production, and this has brought about many side effects. Food production in monocultures is difficult without using pesticides, which leads to negative consequences (Tahvanainen & Root, 1972; Bommarco et al., 2011). However, the reduced number of insecticides registered for suppressing harmful organisms (The list of registered plant protection product on 08.08.12, 2012) has contributed to more sustainable plant protection (George et al., 2009).

Cabbage (*Brassica oleracea* L. convar. *capitata* [L.] Alef. var. *alba* DC), an important vegetable in Europe, is subject to attack by a wide variety of harmful pests (Trdan et al., 2006; Reddy, 2010). Cabbage flea beetles (Coleoptera; Chrysomelidae; *Phyllotreta*) are a key group of harmful pests on Brassica (Trdan et al., 2005b; Andersen et al., 2006), and *Phyllotreta armoraciae* Koch, *Phyllotreta cruciferae* Goeze, *Phyllotreta undulata* Kutschera, *Phyllotreta nemorum* L., *Phyllotreta striolata* Fabricius, *Phyllotreta atra* Fabricius and *Phyllotreta vittula* Redtenbacher have been reported as the main flea beetle species on cabbage in Slovenia (Brelih et al., 2003). In Central Europe and in continental parts of Southern Europe, cabbage flea beetles usually have one generation per year (Trdan et al., 2005b), thus the development of resistance to certain insecticides is not surprising (Tansey et al., 2009; Trdan et al., 2005b). Feeding on seedlings can cause plant death (Palaniswamy & Lamb, 1992; Trdan et al., 2008c), whereas feeding on later developmental stages can reduce the quantity or quantity of production due to the uneven growth of the plants and reduced quantity of seed or marketable product (Tansey et al., 2009).

Using trap crops is a way to decrease the use of insecticides if these traps crops maintain harmful pest populations below the threshold of economic damage (Trdan et al., 2005b; George et al., 2009). Although some species of trap crops from the Brassica family have proven successful in reducing the intensity of cabbage feeding by cabbage flea beetles (Trdan et al., 2005b), diamondback moths

*Corresponding author. Email: tanja.bohinc@bf.uni-lj.si

(Musser et al., 2005; George et al., 2009) and stink bugs (Bohinc & Trdan, 2012), no single ideal trap crop candidate has been identified to date.

The purpose of our research was to study the cabbage flea beetle's preference for cabbage, oilseed rape, oil radish and white mustard in an effort to determine the most appropriate trap crop for protecting cabbage against the studied harmful pests.

Materials and methods

Study site and material

The two-year field experiment (2009–2010) was conducted at two locations: at the Laboratory Field of the Biotechnical Faculty in Ljubljana (46°04′N latitude, 14°31′E longitude, 300 m above sea level) (Site One) and in the village of Zgornja Lipnica in the Gorenjska region (46°19′ N latitude, 14°10′ E longitude, 511 m above sea level) (Site Two).

The following trap crops were used in the experiment: white mustard (*Sinapis alba* [L.]), cv. Zlata (supplier: Semenarna Ljubljana, d. d., Ljubljana, Slovenia), oilseed rape (*Brassica napus* [L.] ssp. *oleifera* f. *biennis*), cv. Daniela (supplier: Semenarna Ljubljana, d. d., Ljubljana, Slovenia) and oil radish (*Raphanus sativus* [L.] var. *oleiformis*), cv. Apoll (supplier: Semenarna Ljubljana, d. d., Ljubljana, Slovenia).

The intensity of feeding by cabbage flea beetles was studied using a medium-late cabbage cultivar ('Hinova F1') (growth period, 120 days; breeder, Bejo Seeds, Inc., Warmenhuizen, The Netherlands). Transplants were grown in a greenhouse in plant trays with commercial compost and fed and irrigated according to standard practices (Trdan et al., 2008b).

Field experiment

The setting of the field experiment at Site Two was identical to the setting described in Bohinc and Trdan (2012). The experiment was performed using an area of 528 m^2 that was divided into four blocks of dimensions of 11 m × 12 m. The experiment at Site One was performed using a plot of 234 m^2. The field was divided into four blocks of dimensions of 4.5 m × 13 m. The above-mentioned trap crops were seeded in different treatments within each block, with the fourth treatment being the bare surface control. Cabbage cultivar 'Hinova' was selected because of previous work (Bohinc & Trdan, 2012) that showed a strong susceptibility to flea beetle feeding. Our aim was to determine the influence of two different environments on the feeding behaviour.

Field observations and evaluation

The damage inflicted by the cabbage flea beetles was assessed on the trap crops and the cabbage at approximately 10-day intervals. In the first year of the experiment, we performed 6 assessments at Site One and 13 at Site Two; in 2010, we performed 9 assessments at Site One and 14 at Site Two. In both years of the experiment at Site One, we analysed the results of assessments on five different dates: 3-May, corresponding to the last 10 days of May; 1-June, corresponding to the first 10 days of June; 2-June, the second 10 days of June; 3-June, the last 10 days of June; and 1-July, corresponding to the first 10 days of July. Similar sampling periods were assessed at Site Two (Zgornja Lipnica), with three additional sampling intervals: 3-July, corresponding to the last 10 days of July; 2-August, the second 10 days of August; and 2-September, the second 10 days of September. To facilitate the comparison between the two locations, only the specific dates of assessments were used. The damage due to the cabbage flea beetles was evaluated using the 5-grade European and Mediterranean Plant Protection Organization (EPPO) scale (OEPP/EPPO, 2002). The plants were evaluated on a scale from 1 (no damage) to 5 (more than 25% leaf area eaten), as follows: (2) up to 2% leaf area eaten, (3) between 3% and 10% leaf area eaten and (4) 11–25% leaf area eaten. The growth stages of the trap crops were identified using the Biologische Bundesanstalt, Bundessortenamt and Chemical Industry (BBCH) scale for the growth stages of oilseed rape (Weber & Bleiholder, 1990; Lancashire et al., 1991), whereas the 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). The BBCH scale for leafy vegetables (forming heads) includes the main growth stages, as follows: (0) germination, (1) leaf development (main shoot), (4) development of harvestable vegetative plant parts and (5) inflorescence emergence. The BBCH scale for oilseed rape includes the following growth stages: (0) germination, (1) leaf development, (2) formation of side shoots, (3) stem elongation, (5) inflorescence emergence, (6) flowering, (7) development of fruit, (8) ripening and (9) senescence (Growth stages of mono- and dicotyledonous plants, 2001).

Data analysis

The differences in the damage due to feeding on the leaves of cabbage and three trap crops were analysed using a general ANOVA (one-way). Prior to analysis, each variable was tested for homogeneity of the variance (Bartlett's test), and the data found to be non-homogenous were transformed to log (Y) prior to the ANOVA. Kruskal–Wallis tests were also applied to analyse the impact of different factors on the damage level. The differences ($P<.05$) between the mean values were identified using Student–Newman–Keuls multiple range test. All the statistical analyses were performed using Statgraphics Centurion XVI (2009). The data are presented as untransformed means ± SE.

Results

Level of injury by cabbage flea beetles (Phyllotreta spp.) in 2009

The general analysis showed that the level of cabbage flea beetle feeding on the trap crops at Site One was determined by the date of assessment (ANOVA, $F=76.66$; Df = 4; $P<.0001$; Kruskal–Wallis, $H=119.38$; Df = 4, $P<.05$) and the trap crop type (ANOVA, $F=7.07$, Df = 2; $P=.0010$; Kruskal–Wallis test, $H=16.41$; Df = 2; $P<.05$). In contrast, the level of feeding was not affected by the developmental stage of the trap crops (ANOVA, $F=1.33$; Df = 27; $P=.1403$; Kruskal–Wallis test, $H=180.15$; Df = 27; $P=1.36$). The level of feeding on the cabbage 'Hinova' was significantly influenced by the date of assessment (ANOVA, $F=19.54$; Df = 4; $P<.0001$; Kruskal–Wallis test, $H=69.94$; Df = 4; $P<.05$) and the developmental stage (ANOVA, $F=5.23$, Df = 9; $P<.0001$; Kruskal–Wallis, $H=43.64$; Df = 9; $P<.05$). The average damage index in the last 10 days of May (3-May) was significantly highest for the oil radish (2.69 ± 0.12) (BBCH stages 49–50), whereas the damage index on cabbage was the lowest at 1.90 ± 0.07 (Figure 1A) (BBCH stages 13–15). The average indices of damage reached their highest values in the first 10 days of July (1-July). In this time period, we recorded 3.81 ± 0.10 as the average index of damage on oil radish (BBCH stages 65–71), 3.68 ± 0.12 as the average damage index on oilseed rape (BBCH stages 53–54), and 3.62 ± 0.12 on white mustard (BBCH stages 52–54), whereas the average damage index for cabbage flea beetles on cabbage (BBCH stages 42–46) was significantly lowest at 2.50 + 0.06 (Figure 1A).

The analysis of the data collected at Site Two confirmed that the level of appearance of flea beetles was influenced by the date of assessment (ANOVA, $F=53.12$; Df = 7; $P<.0001$; Kruskal–Wallis, $H=253.12$; Df = 7; $P<.05$), the type of trap crop (ANOVA, $F=3.00$; Df = 2, $P=.0003$; Kruskal–

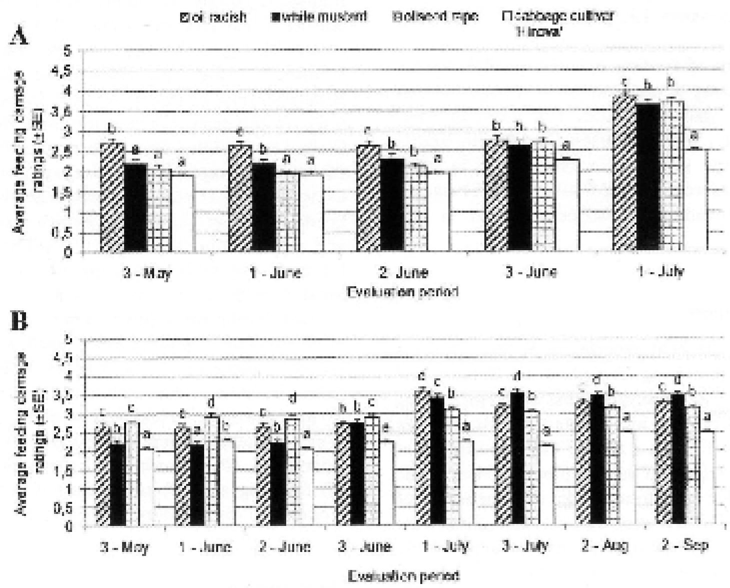


Figure 1. (A) Average indices (±SE) of feeding damage caused by cabbage flea beetles during the growing season in 2009 in Ljubljana (Site One). (B) Average indices (±SE) of feeding damage caused by cabbage flea beetles during the growing season in 2009 in Zgornja Lipnica (Site Two). Average values belonging to a specific date evaluation, followed by the same lowercase letter, are not significantly different according to Student–Newman–Keuls multiple range test ($P<.05$). The bars represent the SE of the mean.

Wallis, $H=55.11$; Df = 2; $P<.05$) and the developmental stage of the trap crops (ANOVA, $F=5.90$; Df = 48; $P<.0001$; Kruskal–Wallis, $H=217.24$; Df = 48; $P<.05$). The analysis of the damage to the hybrid 'Hinova' confirmed the significant influence of the cabbage developmental stage (ANOVA, $F=7.34$; Df = 13; $P<.0001$; Kruskal–Wallis, $H=98.56$; Df = 13; $P<.05$). In the last 10 days of May (3-May), the highest damage index was found on oilseed rape (2.78 ± 0.07) (BBCH stages 12–15) and oil radish (2.69 ± 0.09) (BBCH stages 15–35), whereas the average damage index on the cabbage 'Hinova' (2.08 + 0.06) (BBCH stages 16–18) was significantly the lowest among the studied species of Brassica. The intensity of feeding by adult specimens of *Phyllotreta* spp. was significantly highest on oil radish (3.59 ± 0.10) in the first 10 days of July (1-July) (BBCH stages 25–57), whereas the intensity of feeding on white mustard was significantly highest in the last 10 days of July (3-July) (3.56 ± 0.09) (BBCH stages 57–65), second 10 days of August (2-August) (3.47 ± 0.09) (BBCH stages 65–69) and second 10 days of September (2-September) (3.47 + 0.09) (Figure 1B) (BBCH stages 81–85).

Level of injury by cabbage flea beetles (Phyllotreta spp.) in 2010

The analysis of the results for the extent of damage to the Brassica species at Site One showed a significant influence of the assessment date (ANOVA, $F=7.39$; Df = 4; $P<.0001$; Kruskal–Wallis, $H=28.54$, Df = 4; $P<.05$), the developmental stage (ANOVA, $F=3.36$; Df = 30; $P<.0001$; Kruskal–Wallis, $H=75.89$; Df = 30; $P<.05$) and the type of trap crops (ANOVA, $F=16.28$; Df = 2; $P<.0001$; Kruskal–Wallis, $H=28.47$; Df = 2; $P<.05$). The seasonal dynamics of feeding by cabbage flea beetles on cabbage was determined by the date of assessment (ANOVA, $F=8.89$; Df = 4; $P<.0001$; Kruskal–Wallis, $H=39.44$; Df = 4; $P<.05$), whereas no influence of the developmental stage was detected (ANOVA, $F=1.69$; Df = 3; $P=.1677$; Kruskal–Wallis, $H=179.14$; Df = 3; $P=1.13$). The significantly lowest average damage indices were found on the plants of the main crop (cabbage cultivar 'Hinova'), which varied from 2.44 ± 0.07 in the last 10 days of May (3-May) (BBCH stages 14–39) to 3.00 ± 0.00 in the first 10 days of July (1-July) (BBCH stages 62–65). The damage indices for oil radish varied from 4.12 ± 0.20 in the last 10 days of May (3-May) to 3.94 ± 0.10 (BBCH stages 14–39) in the first 10 days of July (1-July) (Figure 2A).

The results of the general analysis confirmed that the intensity of feeding by *Phyllotreta* spp. at Site Two was significantly influenced by the date of assessment (ANOVA, $F=52.97$; Df = 7; $P<.0001$; Kruskal–Wallis, $H=267.45$; Df = 7; $P<.05$), the plant species (ANOVA, $F=64.34$; Df = 2; $P<.0001$; Kruskal–Wallis, $H=115.45$; Df = 2; $P<.05$) and the developmental stage of trap crops (ANOVA, $F=17.38$; Df = 40; $P<.0001$; Kruskal–Wallis, $H=413.17$; Df = 40; $P<.05$). The seasonal nature of the appearance of cabbage flea beetles on the cabbage hybrid was determined by the date of assessment (ANOVA, $F=17.13$; Df = 7; $P<.0001$; Kruskal–Wallis; $H=98.71$; Df = 7; $P<.05$) and the developmental stage of the cabbage (ANOVA, $F=14.87$; Df = 14; $P<.0001$; Kruskal–Wallis, $H=156.14$; Df = 14; $P<.05$). The cultivar 'Hinova' displayed the significantly lowest damage indices, varying from 2.16 + 0.04 in the last 10 days of May (3-May) (BBCH stages 17–19) to 2.71 ± 0.05 in the second 10 days of September (2-September) (BBCH stages 47–49) (Figure 2B). In the first 10 days of July (1-July), we recorded a characteristic increase in the damage index on the three non-cabbage species. Oil radish was the most susceptible to attack by the cabbage flea beetles. The average damage indices on oil radish varied from 2.75 ± 0.10 in the last 10 days of May (3-May) (BBCH stages 19–30) to 4.25 ± 0.10 in the second 10 days of September (2-September) (BBCH stages 67–75) (Figure 2B).

Discussion

The results of our research show a distinct preference of cabbage flea beetles (*Phyllotreta* spp.) for particular species of Brassica, as was also shown by other authors (Bodnaryk, 1992a; Brown et al., 2004; Štolcová, 2009; Bohinc & Trdan, 2012). In our experiment, white mustard represented the least attractive or appropriate species for cabbage flea beetles. White mustard was also the least attractive to *Phyllotreta* spp. feeding in the experiment performed by Brandt and Lamb (1993). The susceptibility of cabbage flea beetle feeding on white mustard is also described by Bohinc and Trdan (2012). This plant can defend itself against attack by harmful organisms in several ways: by means of glucosinolates (chemically) (Bodnaryk, 1991, 1992b) or by means of trichomes (morphologically) (Soroka et al., 2011). Other Brassica also use glucosinolates to defend themselves against harmful organisms (Bohinc et al., 2012).

Of the species tested, we noted that white mustard was the first to reach the ripening stage (BBCH stage 80–89) (Hassan & Arif, 2012), and the low susceptibility of white mustard to damage by *Phyllotreta* spp. found in the beginning of the growth period in 2009 at the field in the Gorenjska region was very distinct. We can thus conclude that the developmental stage of the plant is an important factor determining the

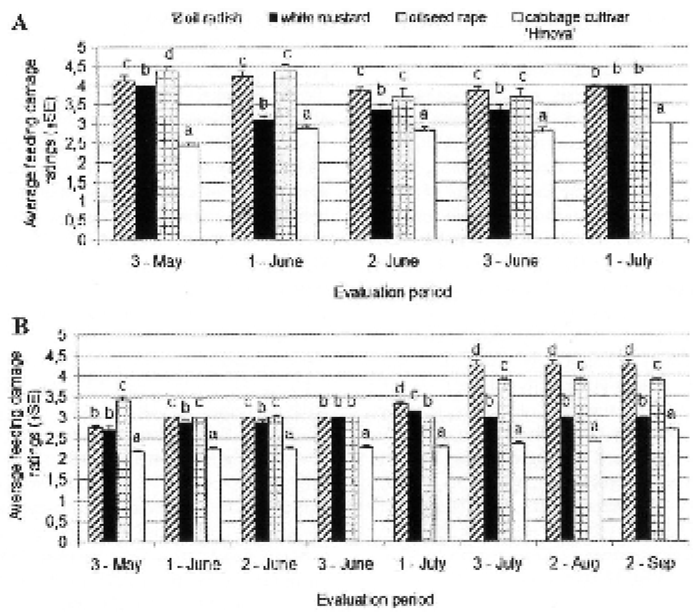


Figure 3. (A) Average indices (±SE) of feeding damage caused by cabbage flea beetles during the growing season in 2010 in Ljubljana (Site One). (B) Average indices (±SE) of feeding damage caused by cabbage flea beetles during the growing season in 2010 in Zgornja Lipnica (Site Two). Average values belonging to a specific date evaluation, followed by the same lowercase letter, are not significantly different according to Student–Newman–Keuls multiple range test ($P < .05$). The bars represent the SE of the mean.

intensity of feeding by insects, as mentioned in Bohinc and Trdan (2012). The trap crops in the Gorenjska region were most susceptible to attack by cabbage flea beetles during blossoming (BBCH stages 60–67) at which time the main crop was developing vegetative parts suitable for harvesting (BBCH stages 41–49). The feeding by cabbage flea beetles on the blossoms was recorded in other studies (Leavitt & Robertson, 2006).

The studied population of harmful pests in Slovenia characteristically appears in large numbers during the first days of July (Trdan et al., 2008a). Their feeding becomes increasingly intense until the end of the month, which was confirmed in our research despite the fact that we noticed the first damage at the beginning of May. During the last 10 days of May 2010, we recorded a higher damage index in Ljubljana (Site One) than at the field in the Gorenjska region (Site Two), and this was attributed to higher average temperatures (Slovenian Environment Agency, 2010). The average daytime temperature in the last 10 days of May (3-May) in the Gorenjska region was 16.07°C, whereas 17.35°C was recorded in Ljubljana. The influence of the average daytime temperature on the development of cabbage flea beetles in 2009 was not established. The comparison of the average daytime temperature across the decades with regard to the location showed that the average daytime temperature in Ljubljana is significantly higher than in the Gorenjska region (Slovenian Environment Agency, 2010); in our opinion, this situation affected the ecology of the harmful pest (Toshova et al., 2009). Consequently, the higher average daytime temperatures facilitated the more rapid development of the studied Brassica species in Ljubljana (Cárcamo et al., 2008).

We found that the average damage indexes in the second year of our experiment were slightly higher at both locations, which can be attributed to the effect of crop rotation (Andersen et al., 2006). We emphasise that the location in Gorenjska had no major Brassica crop before 2009, thus the harmful pest was not present in large numbers; conversely, its numbers in Ljubljana were higher due to years of successive Brassica production (Trdan et al., 2005a, b, 2006, 2008a, b).

On the basis of the two years of field experiments, we conclude that the trap crop method is a successful alternative protection for cabbage against cabbage flea beetles, which is particularly important because the number of insecticides registered for the suppression of cabbage flea beetles is constantly decreasing (The list of registered plant protection product on 08.08.12, 2012). By seeding various Brassica species in our plots, we reduced the intensity of damage to the main crop. The results of our experiment confirm that cabbage flea beetles begin to appear in this part of Europe in the first half of May (Trdan et al., 2005b) or are already present in April (Toshova et al., 2009), and the population reaches its highest numbers in the first half of July (Trdan et al., 2008a; Toshova et al., 2009). By choosing different species of Brassica as trap crops, we reduced the extent of cabbage flea beetle-induced damage to an important vegetable crop, cabbage (Altieri & Gliessman, 1983; Trdan et al., 2005b).

By evaluating different trap crops, we successfully showed that the average damage indexes for all three species of trap crops were significantly higher than for the main crop. Due to the different susceptibility of the trap crops to attack by cabbage flea beetles during the growth period, we cannot propose a single plant species that would be suitable for protecting cabbage throughout its crop cycle. However, one option for environmentally acceptable systems of cabbage production is multiple trap cropping, whereby each of the three Brassica species would attract harmful pests at a certain stage of the growth. For example, a mixture of Chinese cabbage, marigold, rapes and sunflower has been successfully applied as a trap crop for the pollen beetle (*Meligethes aeneus*) in cauliflower fields in Finland (Hokkanen, 1989). The results of our research indicated that the simultaneous sowing of oil radish, white mustard and oilseed rape as multiple trap crops can be effective in controlling cabbage flea beetles in cabbage; however, additional field trials should be performed to confirm the results.

Acknowledgements

This work was performed within V4-0524 and V4-1067, projects funded by the Slovenian Research Agency and the Ministry of Agriculture and Environment of Republic of Slovenia. A portion of the research was performed within the programme Horticulture P4-0013, which is funded by the Slovenian Research Agency. Special thanks are extended to Assist. Prof. Dragan Žnidarčič for producing the white cabbage seedlings and to Assist. Prof. Matej Vidrih for selecting and sowing the trap crops.

References

Altieri MA, Gliessman SR. 1983. Effects of plant diversity on the density and herbivory of the flea beetle, *Phyllotreta cruciferae* Goeze, in California collard (-*rassica oleracea*) *cropping systems*. Crop Protection 2:497–501.

Andersen CL, Hazzard R., van Driesche R., Mangan FX. 2006. Alternative management tactics for control of *Phyllotreta cruciferae* and *Phyllotreta striolata* (Coleoptera: Chrysomelidae) on *Brassica rapa* in Massachusetts. Journal of Economic Entomology 99:803–810.

Bodnaryk RP. 1991. Developmental profile of sinalbin (p-hydroxybenzyl glucosinolate) in mustard seedlings, Sinapis alba L., and its relationship to insect resistance. Journal of Chemical Ecology 17:1543–1556.

Bodnaryk RP. 1992a. Distinctive leaf feeding patterns on oilseed rapes and related Brassicaceae by flea beetles, *Phyllotreta cruciferae* (Goeze) (Coleoptera: Chrysomelidae). Canadian Journal of Plant Science 72:575–581.

Bodnaryk RP. 1992b. Effects of wounding of glucosinolates in the cotyledons of oilseed rape and mustard. Phytochemistry 31:2671–2677.

Bohinc T., Goreta Ban S., Ban D., Trdan S. 2012. Glucosinolates in plant protection strategies. Archives of Biological Sciences 64:821–828.

Bohinc T, Trdan S. 2012. Trap crops for reducing damage caused by cabbage stink bugs (*Eurydema* spp.) and flea beetles (*Phyllotreta* spp.) on white cabbage: Fact or fantasy? Journal of Food, Agriculture and Environment 10:1365–1370.

Bommarco R, Miranda F, Bylund H, Björkman C. 2011. Insecticides suppress natural enemies and increase pest damage in cabbage. Journal of Economic Entomology 104:782–791.

Brandt RN, Lamb RJ. 1993. Distribution of feeding damage by *Phyllotreta cruciferae* (Goeze) (Coleoptera, Chrysomelidae) on oilseed rape and mustard seedlings in relation to crop resistance. Canadian Entomologist 125:1011–1021.

Brelih, S, Döberl, M, Drovenik, B & Pirnat, A. (2003). Gradivo za favno hroščev (Coleoptera) Slovenije. 1. Prispevek. Polyphaga: Chrysomeloidea (Phytophaga): Chrysomelidae: Alticinae. Materialien zur Käferfauna (Coleoptera) Slowenien. 1. Beitrag: Polyphaga: Chrysomeloidea (Phytophaga): Chrysomelidae: Alticinae. Scopolia 50, 1–279. [in Slovenian]

Brown J, McCaffrey JP, Brown DA, Harmon BL, Davis JB. 2004. Yield reduction in *Brassica napus, B. rapa, B. juncea,* and *Sinapis alba* caused by flea beetle (*Phyllotreta cruciferae* (Goeze) (Coleoptera: Chrysomelidae)) infestation in Northern Idaho. Journal of Economic Entomology 97:1642–1647.

Cárcamo HA, Otani JK, Dosdall LM, Blackshaw RE, Clayton GW, Harker KN, O'Donovan JT, Entz T. 2008. Effects of seeding date and canola species on seedling damage by flea beetles in three ecoregions. Journal of Applied Entomology 132:623–631.

Feller C, Bleiholder H, Buhr L, Hack H, Hess M, Klose R, Meier U, Stauss R, van den Boom T, Weber E. 1995. Phänologische Entwicklungsstadien von Gemüsepflanzen: IZiebel-, Wurzel-, Knollen- und Blattgemüse. Nachrichtenbl Deut Pflanzenschutzd 47:217–232.

George DR, Collier R, Port G. 2009. Testing and improving the effectiveness of trap crops for management of the diamondback moth *Plutella xylostella* (L.): A laboratory-based study. Pest Management Science 65:1219–1227.

Growth stages of mono- and dicotyledonous plants (2001). In U Meier, editor BBCMnograph. 2nd Ed. (Braunshweig, Germany: Federal Biological Research Centre for Agriculture and Forestry). Accessed 8 August 2012, available at: http://syntechresearch.hu/sites/default/files/publikaciok/bbch.pdf

Hassan FU, Arif M. 2012. Response of white mustard (*Sinapis alba* L) to spacing under rainfed conditions.

The Journal of Animal and Plant Sciences 22:137–141.

Hokkanen HMT. 1989. Biological and agrotechnical control of the rape blossom beetle *Meligethes aeneus* (Coleoptera: Nitidulidae). Acta Entomogica Fennica 53:25–30.

Lancashire PD, Bleiholder H, Van Den Boom T, Langelüddeke P, Strauss P, Weber E, Witzenberger A. 1991. A uniform decimal code for stages of crops and weeds. Annals of Applied Biology 119:561–601.

Leavitt H, Robertson IC. 2006. Petal herbivory by chrysomelid beetles (*Phyllotreta* sp.) is determinal to pollination and seed production in *Lepidium papilliferum* (Brassicacene). Ecological Entomology 31:657–660.

Musser FR, Nault BA, Nyrop JP, Shelton AM. 2005. Impact of a glossy collard trap crop on diamondback moth adult movement, oviposition, and larval survival. Entomologia Experimentalis et Applicata 117:71–81.

OEPP/EPPO. (2002). Guidelines for the efficiency evaluation of insecticides. *Phyllotreta* spp. on rape. OEPP/EPPBlletin 32, 361–365.

Palaniswamy P, Lamb RJ. 1992. Host preferences of the flea beetles *Phyllotreta cruciferae* and *P. striolata* (Coleoptera: Chrysomelidae) for crucifer seedlings. Journal of Economic Entomology 85:743–752.

Reddy GVP. 2010. Comparative effect of integrated pest management and farmers' standard pest control practice for managing insect pests on cabbage (*Brassica* spp.). Pest Management Science 67:980–985.

Slovenian Environment Agency (2010). Republic of Slovenia. Ministry of Agriculture and Environment. Accessed 27 August 2012, available at: http://www.arso.gov.si/en/

Soroka JJ, Holowachuk JM, Gruber MY, Grenkow LF. 2011. Feeding by flea beetles (Coleoptera: Chrysomelidae; *Phyllotreta* spp.) is decreased on canola (*Brassica napus*) seedlings with increased trichome density. Journal of Economic Entomology 104:125–136.

Statgraphics Centurion XVI. (2009). Warrenton, Virginia: Statpoint Technologies, Inc.

Štolcová J. 2009. Feeding preferences of *Phyllotreta* herbivores to winter rape and chosen weeds. Plant Protection Science 45:156–160.

Tahvanainen JO, Root RB. 1972. The influence of vegetational diversity of the population ecology of a specialized herbivore, *Phyllotreta cruciferae* (Coleoptera: Chrysomelidae). Oecologia 10:321–346.

Tansey JA, Dosdall LM, Keddie BA. 2009. *Phyllotreta cruciferae* and *Phyllotreta striolata* responses to insecticidal seed treatments with different modes of action. Journal of Applied Entomology 133:201–209.

The list of registered plant protection product on 08.08.12 (2012). Ministry of Agriculture and Environment. Phytosanitary Administration of the Republic of Slovenia Accessed 08 August 2012, available at: http://spletni2.furs.gov.si/FFS/REGSR/index.htm

Toshova TB, Csonka É, Subchev M, Tóth M. 2009. The seasonal activity of flea beetles in Bulgaria. Journal of Pest Science 82:295–303.

Trdan S, Milevoj L, Žežlina I, Raspudić E, Andjus L, Vidrih M, Bergant K, Valič N, Žnidarčič D. 2005a. Feeding damage by onion thrips, *Thrips tabaci* Lindeman (Thysanoptera, Thripidae), on early white cabbage grown under insecticide-free conditions. African Entomology 13:85–95.

Trdan S, Valič N, Žnidarčič D, Vidrih M, Bergant K, Zlatič E, Milevoj L. 2005b. The role of Chinese cabbage as a trap crop for flea beetles (Coleoptera: Chrysomelidae) in production of white cabbage. Scientia Horticulturae 106:12–24.

Trdan S, Vidrih M, Bobnar A. 2008a. Seasonal dynamics of three insect pests in the cabbage field in central Slovenia. Communications in Agricultural and Applied Biological Sciences 73:557–561.

Trdan S, Žnidarčič D, Valič N. 2006. Field efficacy of three insecticides against cabbage stink bugs (Heteroptera: Pentatomidae) on two cultivars of white cabbage. International Journal of Pest Management 52:79–87.

Trdan S, Vidrih M, Valič N, Laznik Ž. 2008c. Impact of entomopathogenic nematodes on adults of *Phyllotreta* spp. (Coleoptera: Chrysomelidae) under laboratory conditions. Acta Agriculturae Scandinavica Section B – Soil and Plant Science 58:169–175.

Trdan S, Žnidarčič D, Kač M, Vidrih M. 2008b. Yield of early white cabbage grown under mulch and non-mulch conditions with low populations of onion thrips (*Thrips tabaci* Lindeman). International Journal of Pest Management 54:309–318.

Weber E, Bleiholder H. 1990. Explanations of the BBCH decimal codes for the growth stages of maize, rape, faba beans, sunflowers and peas – With illustrations. Gesunde Pflanzen 9:308–321.

3 RAZPRAVA IN SKLEPI

3.1 RAZPRAVA

Alternativne metode zatiranja škodljivih organizmov vse bolj pridobivajo na pomenu, vedno večja pozornost pa se pripisuje tudi naravni odpornosti rastlin, ki je pogojena z morfološkimi in kemičnimi dejavniki. Križnicam za obrambo pred škodljivimi organizmi tako lahko služijo različni dejavniki. Ugotovljeno je bilo, da trdnejše (bolj zbite glave) zelja (Trdan in sod., 2005a) in višja vsebnost epikutikularnega voska (Trdan in sod., 2009) vplivata na manjši obseg poškodb tobakovega resarja (*Thrips tabaci* Lindeman) na zelju. Debelejša plast epikutikularnega voska lahko negativno vpliva tudi na hranjenje kapusovih bolhačev (*Phyllotreta* spp.) in kapusovih stenic (*Eurydema* spp.) (Trdan in sod., 2009). V naši raziskavi smo se osredotočili na preučevanje vsebnosti glukozinolatov v različnih vrstah križnic in njihovega delovanja na ciljni skupini škodljivih organizmov, kapusove bolhače in kapusove stenice.

Kar so že navajali Moyes in sod. (2000), namreč, da je vsebnost omenjenih sekundarnih metabolitov različna pri posameznih rastlinskih vrstah, se je pokazalo tudi v naši raziskavi. V vzorcih preučevanih vrst križnic smo prisotnost samo enega od glukozinolatov, glukobrasicina, zabeležili v vseh rastlinskih vrstah. Ugotovili smo, da je vsebnost glukobrasicina v vzorcih oljne redkve negativno vplivala na preučevani skupini škodljivih organizmov, tako kapusovih bolhačev kot kapusovih stenic. Kljub temu, da je bila povezava med vsebnostjo glukobrasicina in obsegom poškodb kapusovih bolhačev (r=-0,30) in kapusovih stenic (r=-0,32) negativna, pa o identičnem vplivu skozi celo rastno dobo ne moremo govoriti. To je posledično pogojeno z dejstvom, da je vsebnost glukozinolatov v križnicah variirala tako med rastno dobo, kot tudi med posameznimi rastlinskimi organi. Zanimiva je tudi ugotovitev, da smo v cvetovih oljne redkve določili več glukobrasicina kot v listih.

Smallegange in sod. (2007) navajajo višje vsebnosti petih različnih glukozinolatov v cvetovih črne gorjušice (*Brassica nigra*) kot v listih omenjene rastlinske vrste. V naši raziskavi omenjena trditev velja za glukobrasicin in glukorafenin v vzorcih oljne redkve in za sinalbin ter epiprogoitrin v vzorcih bele gorjušice. Ahuja in sod. (2010) poročajo, da vsebnost glukozinolatov lahko variira tudi znotraj posameznih genotipov iste rastlinske vrste, kar se je izkazalo tudi v naši raziskavi. Med izbranima hibridoma zelja smo namreč ugotovili signifikatne razlike v vsebnosti progoitrina in glukobrasicina. V vzorcih hibrida 'Hinova' smo ugotovili več progoitrina, medtem ko je bil višji delež glukobrasicina prisoten v hibridu 'Tucana'. Od 9 glukozinolatov, ki smo jih analizirali v rastlinah glavnega posevka, smo v obeh hibridih zelja prisotnost štirih (sinalbina, glukonasturtiina, glukorafenina in progoitrina) potrdili le v sledovih (pod mejo detekcije).

Visoke koncentracije alifatskih glukozinolatov v križnicah so lahko pomemben dejavnik odpornosti omenjenih rastlin pred škodljivimi organizmi (Beekwilder in sod., 2008). Ugotovljeno pa je tudi bilo, da je progoitrin v oljni redkvi in beli gorjušici pod mejo detekcije, to pa omogoča širši spekter uporabnosti teh rastlinskih vrst, saj lahko omenjeni alifatski glukozinolat povzroča negativne učinke pri prehrani živali (Padilla in sod., 2007).

Kljub predhodno dokazanemu nematicidnemu delovanje sinigrina (Branca in sod., 2002), pa je v našem poskusu ta glukozinolat v srednje poznem hibridu zelja na hranjenje kapusovih stenic deloval stimulativno, s tem pa je vplival na večji obseg poškodb. Ker je bila vsebnost progoitrina v vzorcih krmne ogrščice najvišja v zadnjem terminu ocenjevanja (31. avgusta), avtorji ugotavljajo, da lahko ta snov potencialno negativno vpliva na živino, če krmno ogrščico uporabimo kot krmo (Padilla in sod.,, 2007). Izrazita preferenca različnih škodljivcev do krmne ogrščice (v našem primeru velja za obe leti poskusa), pa velikokrat botruje dejstvu, da se kmetje ne odločajo za pridelavo omenjene rastlinske vrste (Vallantin-Morison in sod., 2007).

Glede na rezultate naše raziskave lahko sklepamo, da bi bila vsebnost progoitrina v hibridih zelja v prihodnje lahko pomemben dejavnik izbire hibridov za pridelavo zelja v naših razmerah. V raziskavi, ki so jo izvedli na Nizozemskem (van Doorn in sod., 1999) so ugotovili, da vsebnost sinigrina in progoitrina vpliva na okus brstičnega ohrovta. S tem namenom je bilo veliko dela vloženega v zmanjšanje vsebnosti omenjenih glukozinolatov oziroma žlahtnenje (van Doorn in sod., 1999). Kljub temu da poročajo o negativnem vplivu progoitrina v krmi za živino, pa o negativnem vplivu glukozinolatov omenjenega glukozinolata in ostalih glukozinolatov na prehrano ljudi ne moremo govoriti (Sun in sod., 2011). Čeprav so v preteklosti obstajali sumi tudi o negativnem delovanju glukozinolatov na prehrano ljudi (Hill in sod., 2003), pa so bili ti pozneje ovrženi (Sun in sod., 2011; Dinkova-Kostova in Kostov, 2012).

Potencialni negativni vpliv glukobrasicina na prehranjevanje kapusovih bolhačev smo ugotovili v vseh rastlinskih vrstah, razen v krmni ogrščici. Na eni strani ima tako ta glukozinolat negativen vpliv na vrste iz rodu *Phyllotreta*, na drugi strani pa ima pozitiven (antikarcinogen) vpliv na zdravle ljudi (Sun in sod., 2011). To sta zato »dobri« lastnosti tega sekundarnega metabolita. Kljub temu pa se je glukobrasicin izkazal za zelo podvženega okoljskim dejavnikom, kar izpostavljamo v nadaljevanju naše razprave.

Potrdili smo, da sta bila med alifatskimi glukozinolati v vzorcih oljne redkve signifikatno najbolj zastopana glukorafenin in sinalbin, medtem ko je bil med indol glukozinolati prisoten glukobrasicin. Vsebnost sinalbina je bila med obravnavanimi rastlinskimi vrstami križnic najvišja v vzorcih bele gorjušice (30,12±5,52 µmol/ g mase suhega semena) in krmne ogrščice (11,16±6,50 µmol/g mase suhega semena). Glede na zgornjo ugotovitev, da se vsebnost glukozinolatov med posameznimi vrstami križnic razlikuje, ugotavljamo, da med vrstami križnic obstajajo tudi razlike glede njihove ustreznosti za prehrano kapusovih bolhačev in kapusovih stenic. Medtem ko smo pri hibridih 'Tucana' in 'Hinova' ter beli gorjušici in oljni redkvi ugotovili negativen vpliv glukobrasicina na obseg poškodb kapusovih stenic, pa smo v vzorcih krmne ogrščice ugotovili pozitiven vpliv glukobrasicina na obseg poškodb. Glukonapin je tudi eden od glukozinolatov, ki so v naši raziskavi stimulirali hranjenje vrst iz rodu *Eurydema*.

Na vsebnost sekundarnih metabolitov v rastlinah vplivajo tudi dejavniki okolja, kar ugotavljata Tiwari in Cumins (2013), to dejstvo pa se je pokazalo tudi v naši raziskavi. Tako se znanje o vplivu posameznih parametrov okolja na vsebnost glukozinolatov v rastlinah postopoma nadgrajuje. Francisco in sod. (2012) poročajo o pozitivnem vplivu ekstremnih temperatur na vsebnost omenjenih sekundarnih metabolitov. Vpliv temperature naj bi bil bolj izražen pri indol glukozinolatih (Bones in Rossiter, 2006), kar je bilo potrjeno tudi v naši raziskavi, v kateri smo ugotovili, da je vsebnost glukobrasicina pogojena predvsem z povprečno dnevno in najvišjo dnevno temperaturo zraka.

Zelo malo pa vemo o vplivu posameznih okoljskih dejavnikov na vsebnost alifatskih in aromatskih glukozinolatov. V naši raziskavi ugotavljamo, da se vpliv okolja na posamezen glukozinolat iz omenjenih dveh skupin zelo razlikuje. Tako ne moremo govoriti o uniformnem delovanju okolja na vrste glukozinolatov. Strokovnjaki predvidevajo, da je lahko razlog za različen odziv glukozinolatov na delovanje okolja v tem, da pri nastanku specifičnih glukozinolatov sodelujejo specifični encimi, ki so različni od encimov, ki sodelujejo pri nastanku drugih glukozinolatov (Schonhof in sod., 2007) .

Kot smo že poudarili, je pomen alternativnih metod v varstvu rastlin vedno večji in v tej zvezi z veseljem ugotavljamo, da se je tudi metoda privabilnih posevkov, ki smo jo preučevali v okviru naše disertacije, na obeh lokacijah poljskega poskusa izkazala za uspešno. Tako so bile rastline privabilnih posevkov veliko bolj dovzetne za poškodbe kapusovih stenic (*Eurydema* spp.) in kapusovih bolhačev (*Phyllotreta* spp), v primerjavi z zeljem. Na Gorenjskem je bila krmna ogrščica najbolj dovzetna za hranjenje kapusovih stenic, preferenco kapusovih bolhačev pa smo na isti lokaciji zaznali pri oljni redkvi. Tudi v Ljubljani smo v letu 2009 ugotovili signifikantno najvišji obseg poškodb vrst iz rodu *Eurydema* na krmni ogrščici, medtem ko v letu 2010 med privabilnimi posevki nismo ugotovili razlik. Kljub temu pa ugotavljamo, da dovzetnost različnih križnic za napad preučevanih škodljvih žuželk med rastno dobo variira.

Na njivi na Gorenjskem smo (Priloga B1) v letu 2009 največji obseg poškodb zaradi kapusovih stenic ugotovili na krmni ogrščici (povprečni indeks poškodb 3,38±0,05), v drugem letu poskusa pa je bila za poškodbe kapusovih stenic najbolj dovzetna krmna ogrščica (3,58±0,02). Pri beli gorjušici smo v prvem (2,72±0,04) in drugem (2,56±0,05) letu poskusa ugotovili signifikantno najnižji indeks poškodb kapusovih stenic. V drugem letu poskusa smo največjo dovzetnost za poškodbe kapusovih bolhačev ugotovili na oljni redkvi (3,5±2,82), medtem ko je bil obseg poškodb na beli gorjušici signifikantno najnižji (2,82±0,02).

Van Dorn in sod. (1999) navajajo, da je vsebnost glukozinolatov v križnicah pogojena tudi s hranjenjem škodljivih organizmov. Ti s svojim prehranjevanjem - s tem, ko poškodujejo celično strukturo - uravnavajo vsebnost glukozinolatov. V naši raziskavi smo ugotovili, da se povezava med vsebnostjo glukozinolata in obsegom poškodb vrst iz rodov *Phyllotreta* oziroma *Eurydema* razlikuje med različnimi glukozinolati. Kot tudi, da posamezni glukozinolati, različno vplivajo na obseg poškodb.

Rastlinske vrste, ki smo jih kot privabilne posevke uporabili v naši raziskavi, so v Sloveniji največkrat uporabljene kot krmni dosevki (Krmni dosevki, 2013). Krmna ogrščica velja med obravnavanimi privabilnimi posevki za rastlinsko vrsto, ki je zelo občutljiva na napad škodljivih organizmov in je zato posledično manj priljubljena v ekološki pridelavi (Valantin-Morison in sod., 2007). Izrazita dovzetnost krmne ogrščice za poškodbe kapusovih stenic je vidna v Prilogi A4. Če primerjamo indekse poškodb kapusovih stenic na različnih vrstah križnic, ugotavljamo, da so bili na omenjeni rastlinski vrsti najvišji. Iz vidika možnosti implementacije križnic kot privabilne posevke v sisteme pridelave zelja, pa predstavlja bela gorjušica najmanj ustrezno vrsto, saj smo jo potrdili kot najmanj dovzetno za hranjenje kapusovih bolhačev. Bela gorjušica je v raziskavi, ki so jo izvedli Brown in sod. (2004) navedena kot rastlinska vrsta z zelo visoko naravno odpornostjo na napad bolhačev. Kot ugotavljajo, je povrhnjica, gosto poraščena s trihomi, glavni razlog odpornosti rastlin, kar potrjuje tudi raziskava, ki so jo izvedli Soroka in sod. (2011).

S tem, ko smo privabilne posevke sejali pred glavno rastlinsko vrsto, smo slednjo (zelje) obvarovali pred zgodnjim napadom kapusovih bolhačev in kapusovih stenic. Znano je, da so kapusovi bolhači, ki se pri nas pojavijo v začetku maja, zelo škodljivi mladim rastlinam križnic, ki imajo takrat majhno skupno površino listov (Trdan in sod., 2005b). Starost rastlin je eden od pomembnejši dejavnikov, ki vplivajo na obseg poškodb vrst iz rodu *Phyllotreta*. Že Knodel in sod. (2008) v svoji raziskavi navajajo uspešnost zmanjšanja obsega poškodb na zelju zaradi vrst iz rodu *Phyllotreta* z uporabo različnih terminov sajenja.

Ugotovili smo, da obstaja negativna korelacija med rastlinami privabilnih posevkov in obsegom poškodb kapusovih bolhačev v začetku razvojnega stadija razvijanja listov (BBCH 12-14). Glede na rezultate naše raziskave lahko sklepamo, da se odrasli osebki kapusovih stenic spomladi pojavijo v drugi polovici maja ali v začetku junija. V omenjenem obdobju smo namreč zabeležili prvi interval poškodb (Bohinc in Trdan, 2010). Poškodbe smo najprej ugotovili na privabilnih posevkih in šele pozneje na glavnem posevku. Omenjeno je veljalo za obe lokaciji poskusa.

Hibrid zelja 'Hinova' se je v vseh primerjavah izkazal za veliko bolj dovzetnega za poškodbe kapusovih stenic in kapusovih bolhačev (Bohinc in Trdan, 2011b), v primerjavi s hibridom 'Tucana'. Kljub temu pa ugotavljamo, da dovzetnost križnic v posameznih obdobjih rastne dobe med genotipi zelja variira, in tako smo v nekaterih obdobjih zaznavnejše poškodbe zabeležili tudi na hibridu 'Tucana'. Tako so bile poškodbe kapusovih bolhačev na kultivarju 'Tucana' bolj obsežne v začetku rastne dobe, v fazi razvijanja listov, ko so razgrnile 4-7 pravih listov. Večji obseg poškodb je bil pozneje zabeležen na kultivarju 'Hinova'. Za poškodbe kapusovih stenic srednji pozni hibrid zelja ni bil tako dovzeten. Omenjena ugotovitev je bila omenjena že v raziskavi Trdan in sod. (2006). Povezavo lahko najdemo v interakciji med dolžino rastne dobe hibrida in bionomijo preučevanega škodljivca. V času, ko se vrsti *Eurydema ventrale* in *Eurydema oleracea* šele začneta masovno pojavljati smo namreč pridelek zgodnjega hibrida že pospravili.

Ugotavljamo, da je uporabnost izbrane alternativne metode v varstvu rastlin pogojena z izbiro ustreznega glavnega posevka oziroma ustreznega hibrida. Na povprečni pridelek zelja je vplivala izbira kultivarja, medtem ko izrazitih razlik v povprečnem pridelku med posameznimi obravnavanji (vrstami privabilnih posevkov) nismo zasledili. Primerjali smo tudi poškodbe zaradi vrst iz rodov *Phyllotreta* in *Eurydema* na glavnem posevku glede na razdaljo od privabilnega posevka. Ugotovili smo, da se kapusove stenice najraje pojavljajo na rastlinah zelja, ki so od privabilnih posevkov najbolj oddaljene;,medtem ko vpliva oddaljenosti rastlin na poškodbe kapusovih bolhačev nismo ugotovili. 'Hinova' se je v naših pridelovalnih razmerah izkazala za tisti hibrid, ki je prinašal večji povprečni pridelek, izbira zgodnjega hibrida 'Tucana' pa se ni izkazala za ustrezno. Glede na rezultate naše raziskave lahko rečemo, da se sklada z ugotovitvami avtorjev Bell (1995) ter Cárcamo in Blackshaw (2007), ki navajajo, da alternativne metode (vmesni posevki, zelene prekrivke) niso dosegali želenega cilja.

S tem, ko bi intenzivnejše skrbeli za glavni posevek (namakanje med rastno dobo, dognojevanje,…), bi lahko poskrbeli za višji pridelek. Vsem je dobro znano, da metodo privabilnih posevkov lahko izvedemo na več načinov. Eden od teh je tudi, da bi privabilne posevke pospravili sredi rastne dobe, kjer smo izvajali poskus (Shelton in Badenes-Perez, 2006). Obenem bi tvegali, da se preučevani skupini škodljivcev množično preselita na glavni posevek (Shelton in Badenes-Perez, 2006). Lahko pa bi privabilne posevke tretirali z insekticidi in tako poskrbeli za tako imenovani »dead-end trap crop«. Vendar bi z uporabo insekticidov naredili več škode kot koristi (Bommarco in sod., 2011). Vrste, ki smo jih uporabili kot privabilne posevke so namreč del rastne dobe tudi cvetele in s tem privabljale koristne organizme. Vzroke za različno dovzetnost preučevanih vrst križnic lahko iščemo v naravni odpornosti rastlin. Glukozinolati, ki so za nekatere pomemben dejavnik odpornosti rastlin na škodljive organizme, spet drugi pa jim v tej zvezi ne pripisujejo večjega pomena, lahko pri obrambi rastlin sodelujejo stimulativno ali zaviralno. Medtem, ko glukonasturtiin v krmni ogrščici deluje zaviralno (r=-0,99) na prehranjevanje kapusovih bolhačev, o povezavi omenjenega glukozinolata v beli gorjušici in oljni redkvi ne moremo govoriti. Glede na omenjene raziskave lahko sklepamo, da je delovanje teh sekundarnih metabolitov na škodljive žuželke zelo kompleksno. O univerzalnem vplivu treh skupin glukozinolatov na škodljive organizme tako ne moremo govoriti, ampak je potrebno vpliv vsalega posameznega glukozinolata analizirati individualno.

Glukonasturtiin v krmni ogrščici deluje negativno na prehranjevanje kapusovih bolhačev (r=-0,99) in kapusovih stenic (r=-0,98). Prav tako na prehranjevanje kapusovih bolhačev (r=-0,80) in kapusovih stenic (r=-0,99) na omenjeni rastlinski vrsti močno vpliva vsebnost epiprogoitrina. Vsebnost glukoiberina v vzorcih krmne ogrščice je negativno vplivala na prehranjevanje kapusovih bolhačev (r=-1) in kapusovih stenic (r=-1), progoitrin v vzorcih krmne ogrščice pa na prehranjevanje kapusovih stenic (r=0,51) in kapusovih bolhačev (r=0,51) deluje stimulativno, medtem ko je delovanje v vzorcih 'Tucana' na prehranjevanje kapusovih stenic negativno (r=-1,0). Glukonapiin je edini glukozinolat, ki v rastlinah krmne ogrščice deluje stimulativno na prehranjevanje kapusovih stenic (r=0,64) in kapusovih bolhačev (r=0,67). Izrazitega vpliva preučevanih glukozinolatov na prehranjevanje vrst iz rodu *Phyllotreta*. v vzorcih oljne redkve nismo ugotovili. Lahko le sklepamo, da je komaj zaznavna prisotnost glukonasturtiina, glukoiberina, glukonapiina najverjetneje pripomogla k višjemu indeksu poškodb kapusovih bolhačev na preučevanih vrstah križnic.

Glede na doslej zbrane ugotovitve, da se vsebnost glukozinolatov med rastno dobo in glede na rastlinsko vrsto spreminja, nas je zanimala primerjava obsega poškodb kapusovih bolhačev tudi glede na lokaciji poskusov.

V Sloveniji se kapusovi bolhači zanajštevilčneje pojavljajo v juliju (Trdan in sod., 2008), to pa smo potrdili tudi v naši raziskavi. Na hranjenje kapusovih bolhačev vpliva tudi povprečna dnevna temperatura (Toshova in sod., 2009), kar smo prav tako ugotovili v naši raziskavi. Med rastno dobo smo v obeh letih poskusa ugotovili več takih primerov. Tako smo v letu 2010 v zadnjih desetih dneh maja na Laboratorijskem polju Biotehniške fakultete v Ljubljani zabeležili višje povprečne dnevne temperature kot na lokaciji poskusa na Gorenjskem.

Indeksi poškodb zaradi obeh skupin škodljivcev so bili v drugem letu poskusa izrazito višji, kar potrjuje dejstvo, da med načela uspešne kmetijske prakse spada tudi uporaba kolobarja (Duda in Liste, 1991; Andersen in sod., 2006; Charles in sod., 2011). Tako smo v obeh letih poskusa višji obseg poškodb zaznali na Laboratorijskem polju Biotehniške fakultete in na njivi na Gorenjskem. Pomen kolobarja se je pokazal zlasti na Gorenjskem, kjer nekaj let pred našim poskusom ni bilo v bližini večje njive s kapusnicami, zato je bil napad obeh skupin škodljivcev v prvem letu poskusa relativno šibek. Na Laboratorijskem polju Biotehniške fakultete kapusnice vsako leto pridelujejo že daljše časovno obdobje, posledično je bil napad kapusovih bolhačev in kapusovih stenic močan že v prvem letu poskusa. Trditvam, da na znatno manjši obseg poškodb škodljivcev vpliva tudi kolobar (Dosdall in sod., 2012; Mazzi in Dorn, 2012), lahko pritrdimo tudi v naši raziskavi.

Povprečna dnevna temperatura 17,4°C na Laboratorijskem polju Biotehniške fakultete v Ljubljani je tako stimulativno vplivala na skupini preučevanih škodljivcev, medtem ko je povprečna dnevna temperatura v istem terminu na Gorenjskem znašala 16,1°C.

Ugotavljamo, da je bela gorjušica najhitreje dosegla stadij zorenja (BBCH 80-89) (Hassan in Arif, 2012), hkrati pa se je dovzetnost te rastlinske vrste za napad preučevanih škodljivcev zmanjšala. Majhna dovzetnost bele gorjušice za poškodbe vrst iz rodu *Phyllotreta* v začetku rastne dobe je bila še izrazito poudarjena leta 2009 na Gorenjskem. Tako lahko povzamemo, da je razvojni stadij rastlin eden od pomembnejših dejavnikov prehranjevanja kapusovih bolhačev in kapusovih stenic. Na Gorenjskem so bili privabilni posevki za kapusove bolhače najbolj dovzetni v času cvetenja (BBCH 60-67), takrat pa je zelje prehajalo v stadij razvoja vegetativnih delov rastlin, ustreznih za pridelek (oblikovanje glav). Prehranjevanje kapusovih bolhačev na cvetovih rastlin je bilo sicer zabeleženo že tudi v drugih raziskavah (Leavitt in Robertson, 2006).

3.2 SKLEPI

V letih 2009-2010 smo v dveh poljskih poskusih ugotavljali učinkovitost metode privabilnih posevkov za zmanjševanje obsega poškodb kapusovih bolhačev (*Phyllotreta* spp.) in kapusovih stenic (*Eurydema* spp.) na glavni rastlinski vrsti, zelju. Glede na to, da je bil obseg poškodb signifikatno višji na rastlinah privabilnih posevkov, kot tudi, da smo poškodbe najprej zabeležili na rastlinah privabilnih posevkov, lahko rečemo, da se je izbrana alternativna metoda varstva rastlin izkazala za učinkovito.

Prvi pojav kapusovih bolhačev smo v naši raziskavi ugotovili v začetku maja, medtem ko so se kapusove stenice začele pojavljati v drugi polovici maja. Omenjeni ugotovitvi sta v skladu z rezultati bionomije preučevanih skupin škodljivcev v srednjem delu Evrope.

Prehranjevanje kapusovih bolhačev in kapusovih stenic smo zabeležili na vseh izbranih rastlinskih vrstah. Glede na zbrane podatke ugotavljamo, da kažejo kapusove stenice posebno preferenco do hranjenja na krmni ogrščici, medtem ko so se kapusovi bolhači večinoma prehranjevali na oljni redkvi, ki se je izkazala za zanje najbolj privlačnega gostitelja.

Metodo privabilnih posevkov, ki smo jo preučevali v naši raziskavi, bo mogoče uporabiti zlasti v sistemih pridelovanja srednje poznega zelja. Na povprečno maso zelja je vplivala predvsem izbira hibrida. Srednje pozni hibrid 'Hinova' se je v naši raziskavi izkazal za bolj produktivnega in posledično za ustreznejšaga za pridelavo v naših rastnih razmerah.

Na podlagi rezultatov naše raziskave, ki smo jih pridobili na dveh različnih lokacijah, lahko sklepamo, da ima temperatura zraka pomemben vpliv na obseg poškodb kapusovih bolhačev na križnicah. Z višanjem povprečne dnevne temperature zraka je bil namreč višji tudi obseg poškodb kapusovih bolhačev na preučevanih vrstah križnic.

Različna preferenca preučevanih skupin škodljivcev je pogojena tudi z naravno odpornostjo rastlin. Eden od dejavnikov naravne odpornosti križnic je tudi vsebnost glukozinolatov. Glukozinolati so se v naši raziskavi pokazali za pomemben, a variabilen dejavnik naravne odpornosti križnic na napad kapusovih bolhačev in kapusovih stenic. Ugotovili smo, da je variabilnost glukozinolatov pogojena z rastlinsko vrsto, vsebnost teh snovi pa se precej razlikuje tudi med različnimi organi iste rastlinske vrste. V naši raziskavi je prišla do izraza tudi variabilnost v vsebnosti glukozinolatov med posameznimi genotipi iste rastlinske vrste, tj. med posameznimi genotipi zelja. V vzorcih oljne redkve smo ugotovili največ glukorafenina (8,66±1,81 µmol/ g mase suhega semena), v vzorcih krmne ogrščice in bele gorjušice pa je bilo signifikantno največ sinalbina. Kljub temu, da je analiza potrdila sinalbin kot najbolj pogost glukozinolat v vzorcih bele gorjušice, pa nadaljnja analiza podatkov kaže na šibko korelacijo (r=0,36) med njegovo vsebnostjo v križnicah in obsegom poškodb zaradi kapusovih stenic na njih.

Vzorčenje različnih rastlinskih delov je pokazalo, da je vsebnost določenih glukozinolatov, na primer glukobrasicina in glukorafenina v cvetovih oljne redkve ali sinalbina in epiprogoitrina v cvetovih bele gorjušice, veliko višja kot v listih preučevanih rastlinskih vrst. Ugotovili smo, da so se kapusovi bolhači v času cvetenja zelo intenzivno prehranjevali na rastlinah bele gorjušice in oljne redkev.

Ugotavljamo, da na vsebnost glukozinolatov v rastlinah vplivajo predvsem temperaturni ekstremi. Rezultati dosedanjih raziskav delovanja teh sekundarnih metabolitov govorijo v prid delovanja, ki je specifično za posamezno skupino teh sekundarnih metabolitov; vendar smo ugotovili, da tudi znotraj posameznih skupin glukozinolatov prihaja do razlik v delovanju. Glukobrasicin, edini zaznavin glukozinolat v vseh preučevanih rastlinskih vrstah, spada med indol glukozinolate. Ugotavljamo, da na obseg poškodb vrst iz rodov *Phyllotreta* in *Eurydema* ta glukozinolat v oljni redkvi deluje negativno. Omenjena snov spada v skupino tistih, na katere so signifikatno najbolj vplivali okoljski parametri, predvsem povprečna dnevna in najvišja temperatura zraka. Glukonasturtiin in epiprogoitrin sta v krmni ogrščici na obe preučevani skupini škodljivcev delovala negativno., sinalbin pa je na prehranjevanje kapusovih stenic v vzorcih krmne ogrščice deloval negativno, na kapusove bolhače pa je imel stimulativno delovanje.

Pridobljeni podatki na dveh različnih lokacijah so pokazali, da lahko obseg poškodb kapusovih bolhačev uspešno nadziramo z uporabo mešanih posevkov križnic, ki smo jih uporabili v našem poskusu. Omenjena kombinacija rastlinskih vrst bi bila glede na naše ugotovitve ustrezna za obe lokaciji, na katerih smo izvajali poskus, pa tudi za druga območja, na katerih v Sloveniji prideljujejo zelje in kjer se pridelovalci srečujejo s škodljivci, ki smo jih preučevali v naši raziskavi. S tem, ko smo v obeh letih poskusa rastline privabilnih posevkov sejali pred glavnim posevkom, smo v precejšnji meri vplivali na to, da so se poškodbe vrst iz rodov *Phyllotreta* in *Eurydema* najprej pojavile na privabilnih posevkih.

Z izbrano metodo privabilnih posevkov smo uspešno nadzirali pojav vrst iz rodov *Phyllotreta* in *Eurydema* na zelju. Ker je za zatiranje kapusovih bolhačev oziroma kapusovih stenic na voljo zelo malo oziroma nič sintetičnih pripravkov, so alternativne metode potencialna izbira. Glede na to, da se kmetijska zemljišča, namenjena pridelavi kapusnic v zadnjih letih spet povečujejo, predstavljajo nove metode v varstvu pridelka nove možnosti za okoljsko sprejemljivo pridelavo zelja. Omenjena metoda je uporabna predvsem v ekološki in integrirani pridelavi. Tržna potrošnja nas uči, da vse bolj zaupamo hrani, ki je bila pridelana v naši bližini, po možnosti brez uporabe insekticidov. Res je, da v našem poskusu nismo dosegli količine pridelka, ki bi bila tržno zanimiva, pridelali pa smo zelje brez uporabe insekticidov in s tem bo mogoče še bolj kot doslej spodbuditi zanimanje potrošnikov, ki se želijo prehranjevati zdravo. Da bi dosegli zmanjšanje številčnosti vrst iz rodov *Phyllotreta* in *Eurydema* na zelju se zdi uporaba mešanih posevkov križnic povsem realna. In kot kaže naša raziskava, je metoda bolj učinkovita v pridelovalnih sistemih, kjer prideljujejo srednjepozne genotipe zelja. Kot smo ugotovili, se zaradi spektra različnih dejavnikov (med drugim tudi vsebnosti glukozinolatov) dovzetnost rastlin za poškodbe škodljivih organizmov med rastno dobo spreminja. S tem, ko bi uporabili mešane prispevke kot vir zmanjševanja poškodb na glavnem posevku, bi lahko omenjeno metodo uporabili v različnih območjih Slovenije, in tako zanemarili vpliv okoljskih dejavnikov na privabilne posevke, kot tudi samo bionomijo obravnavanih škodljivih vrst. Kot privabilne posevke bi lahko uporabili druge vrste križnic, vendar je to predmet že druge raziskave.

4 POVZETEK (SUMMARY)

4.1 POVZETEK

V letih 2009 in 2010 smo preučevali uporabnost metode privabilnih posevkov, kot alternativne metode zmanjševanja poškodb kapusovih bolhačev (*Phyllotreta* spp.) in kapusovih stenic (*Eurydema* spp.) na zelju, kot glavni rastlinski vrsti. Poskus je potekal na dveh različnih lokacijah, na Laboratorijskem polju Biotehniške fakultete Univerze v Ljubljani, in na njivi v vasi Zgornja Lipnica v občini Radovljica. V poskusu smo kot privabilne posevke uporabili krmno ogrščico (*Brassica napus* [L.] ssp. *oleifera* f. *biennis*, kultivar 'Daniela'), belo gorjušico (*Sinapis alba* [L.], kultivar 'Zlata') in oljno redkev (*Raphanus sativus* [L.] var. *oleiformis*, kultivar 'Apoll'). Omenjene rastlinske vrste smo posejali v dveh ločenih pasovih, znotraj katerih smo posadili glavni posevek, tj. dva kultivarja zelja – zgodnjega 'Tucana' in srednje poznega 'Hinova'. Poskus je potekal v štirih blokih, znotraj katerih smo naključno posejali rastline privabilnih posevkov. Obseg poškodb smo med rastno dobo ocenjevali v 10-dnevnih intervalih oziroma trikrat mesečno. Poškodbe kapusovih bolhačev smo ocenjevali s 5-stopenjsko lestvico EPPO (OEPP/EPPO, 2002), poškodbe kapusovih stenic pa smo ovrednotili na osnovi 6-stopenjske lestvice Stonerjeve in Sheltona (1988). Ugotovili smo, da obstaja preferenca preučevanih skupin škodljivcev do posamezne rastlinske vrste. Obseg poškodb kapusovih bolhačev je bil skozi celoten poskus največji na oljni redkvi in je v letu 2009 variiral od 2,86±0,07 na Laboratorijskem polju Biotehniške fakultete do 3,00±0,03 na njivi na Gorenjskem. V letu 2010 je bil obseg poškodb na oljni redkvi na Laboratorijskem polju BF 3,87±0,06; medtem ko smo na oljni redkvi na Gorenjskem zabeležili obseg poškodb, ki je znašal 3,50±0,04. Kapusove stenice so s svojim hranjenjem največ poškodb povzročale na rastlinah krmne ogrščice. Tako smo v prvem letu poskusa na rastlinah krmne ogrščice na njivi na Gorenjskem zabeležili povprečni indeks, ki je znašal 3,38±0.04, v drugem letu poskusa pa 3,58±0,08. Povprečni indeksi poškodb so bili na rastlinah glavnega posevka na obeh lokacijah v obeh letih poskusa signifikatno nižji. Kljub temu pa se je obseg poškodb na preučevanih hibridih zelja razlikoval, kar lahko pripišemo vplivu rastne dobe rastlin. Srednjepozni hibrid zelja se je v naši raziskavi izkazal za veliko bolj dovzetnega za poškodbe preučevanih skupin škodljivcev

Kapusovi bolhači so se v naši raziskavi začeli pojavljati v začetku maja, medtem ko smo kapusovo stenico (*Eurydema oleracea*) in pisano stenico (*Eurydema ventrale*) prvič opazili v drugi polovici maja. Naše ugotovitve se ujemajo s predhodnimi raziskavami, da se začnejo kapusovi bolhači v tem delu Evrope množično pojavljati v začetku julija.

Izrazitega vpliva privabilnih posevkov na pridelek zelja nismo ugotovili. Smo pa ugotovili, da je bil povprečni pridelek večji pri srednje poznem kultivarju zelja. Glede na to, da v našem poskusu nismo izvajali izrazitega dodatnega dognojevanja in namakanja, so se omenjeni ukrepi poznali tudi na končnem pridelku. Če bi uporabili insekticide na privabilnih posevkih, bi s tem vplivali na populacijo škodljivcev, obenem pa bi škodovali tudi koristnim organizmom, ki se prehranjujejo na rastlinah privabilnih posevkov med cvetenjem.

Ugotovili smo, da je intenzivnost hranjenja kapusovih bolhačev in kapusovih stenic povezana z razvojnim stadijem rastlin. Tako je bila v začetku rastne dobe zabeležena visoka dovzetnost privabilnih posevkov za obe skupini škodljivih žuželk. Dovzetnost rastlin pa se je med rastno dobo spreminjala. Razlog, da se je dovzetnost rastlin med rastno dobo spreminjala, lahko pripišemo naravni odpornosti rastlin.
V naši raziskavi smo ocenjevali glukozinolate kot parametre naravne odpornosti križnic. Te sekundarne metabolite, ki so značilni predvsem za družino križnic, smo ocenjevali v drugem letu poskusa, in sicer v rastlinah, ki so bile gojene na njivi na Gorenjskem. Med rastno dobro rastlin smo vzorčenje za potrebe glukozinolatov izvedli v različnih terminih. Pri rastlinah bele gorjušice in oljne redkve smo vzorčili tudi cvetove rastlin. V obravnavanih rastlinskih vrstah smo ovrednotili vsebnost devetih različnih glukozinolatov, ki smo jih uvrstili v tri različne skupine. Tako nas je med alifatskimi glukozinolati zanimala vsebnost glukoiberina, progoitrina, epiprogoitrina, sinigrina, glukonapina in glukorafenina, med indol glukozinolati vsebnost glukobrasicina. Med aromatskimi glukozinolati nas je zanimala vsebnost glukonasturtiina in sinalbina. Vrednotenje glukozinolatov je potekalo po standardu ISO 9167:1 – (1992) in je opisano tekom naše raziskave. Primer vzorca, namenjenega analizi glukozinolatov je prikazan v Prilogi C1. Uporabljena enota prikaza je bila µmol/ g mase suhega semena.

Glukozinolati, kot tipični sekundarni metaboliti, lahko različno vplivajo na škodljive organizme. Tako lahko gosenice repnega belina (*Pieris rapae* [L.]) glukozinolate s pomočjo njihovega metabolizma izločijo, medtem ko višja vsebnost sinigrina in sinalbina deluje zaviralno na hranjenje sovke *Mamestra configurata*. Literatura navaja, da škodljivi organizmi glukozinolate rastlin lahko izrabijo za svojo obrambo.

Glede na povprečni indeks poškodb vrst iz rodov *Phyllotreta* in *Eurydema* o delovanju glukozinolatov lahko govorimo o zmanjševanju/povečanju obsega hranjenja. Vendar delovanje posameznega glukozinolata na *Phyllotreta* spp. in *Eurydema* spp. ni identično med rastlinskimi vrstami, Ugotovili smo namreč, da v tej zvezi prihaja do razlik. Zanimivo bi bilo ugotoviti mehanizem delovanja preučevanih glukozinolatov na metabolizem žuželk iz rodov *Phyllotreta* in *Eurydema*.

Naša raziskava potrjuje ugotovitve, da vsebnost glukozinolatov variira med rastno dobo, med rastlinskimi vrstami, kot tudi med posameznimi deli rastlin. Ugotovili smo, da je glukobrasicin kot edini glukozinolat prisoten v vseh rastlinskih vrstah, njegova vsebnost pa je najvišja v rastlinah oljne redkve (3,24±0,86 µmol/g mase suhega semena). Sinalbin najdemo najpogosteje v vzorcih krmne ogrščice (11,16±6,50 µmol/g mase suhega semena) in bele gorjušice (30,12±5,52 µmol/g mase suhega semena). Prav tako lahko rečemo, da se vsebnost glukozinolatov razlikuje tudi med posameznimi kultivarji. V srednje poznem kultivarju zelja 'Hinova' smo zabeležili višje vrednosti progoitrina, medtem ko je bila vsebnost glukobrasicina višja v zgodnjem hibridu 'Tucana'. Ugotovili smo tudi razlike v vsebnosti glukozinolatov med posameznimi deli rastlin iste vrste. Tako je vsebnost sinalbina in epiprogoitrina višja v cvetovih bele gorjušice, glukorafenin in glukobrasicin pa sta bolj prisotna v cvetovih kot v listih oljne redkve.

Negativen vpliv glukonasturtiina v vzorcih krmne ogrščice je bil zabeležen tako na kapusove stenice (r=-0,98) kot kapusove bolhače (r=-0,99). Epiprogoitrin v vzorcih krmne ogrščice na prehranjevanje kapusovih stenic (r=-0,99) in kapusovih bolhačev (r=-0,80) deluje izrazito negativno. Višja vsebnost epiprogoitrina v vzorcih krmne ogrščice na prehranjevanje kapusovih stenic in kapusovih stenic deluje negativno. Domnevamo, da bi lahko preferenco kapusovih bolhačev (*Phyllotreta* spp.) do oljne redkve povezali z vsebnostjo dotičnih glukozinolatov. Ker so glukonasturtiin, glukoiberin, progoitrin v vzorcih oljne redkve prisotni v sledovih in imajo v krmni ogrščici in beli gorjušici veliko večjo vlogo, bi lahko vsebnost omenjenih glukozinolatov v večjih količinah pripomogla k drugačnemu obsegu poškodb.

Poleg izrazite variabilnosti glukozinolatov med posameznimi rastlinskimi vrstami uporabljenimi v raziskavi, smo ugotovili, da imajo na vsebnostteh sekundarnih metabolitov v križnicah vpliv tudi dejavniki okolja. Ne moremo govoriti o uniformnem delovanju okolja na skupino glukozinolatov, lahko pa se opis delovanja naveže na dotičen glukozinolat. Znotraj skupin alifatskih in aromatskih glukozinolatov okoljski dejavniki različno vplivajo na izraženo delovanje. Skupina indol glukozinolatov je tista skupina, na katero ima temperatura zraka največji vpliv. V naši raziskavi ugotovljen negativen vpliv povprečne dnevne temperature in maksimalne dnevne temperature se sklada s pričakovanji.

Z manjšo dostopnostjo insekticidov se je uporabnost izbrane alternativne metode samo še povečala. Res, da nismo dosegali tržno zanimive količine pridelka zelja, vendar rezultati naše raziskave omogočajo uporabo v ekološki oziroma integrirani pridelavi kapusnic. S pravilnim terminom setve/sajenja smo vplivali na bionomijo škodljivca, in poskrbeli, da poškodbe vrst iz rodov *Phyllotreta* in *Eurydema* na zelju v začetku rastne dobe niso bile tako izrazite, kar je eden od ključnih elementov v pridelovalnih sistemih. Trdimo, da so glukozinolati pomemben parameter naravne odpornosti rastlin, vendar je njihovo delovanje zelo variiabilno in pogojeno s spektrom dejavnikov. Med drugim tudi okoljskih. Potrebno bo še veliko več raziskav, da bi lahko z večjo gotovostjo govorili o njihovem vplivu. Že danes pa lahko trdimo, da je mogoče s hkratno setvijo večjega števila vrst privabilnih posevkov uspešno uravnavati številčnost preučevanih skupin škodljivcev na zelju.

Naša raziskava je zgled, da je mogoče z metodo privabilnih posevkov zmanjšati obseg poškodb različnih škodljivih žuželk, ki se pojavljajo na zelju. Izbrana metoda varstva rastlin namreč omogoča možnost izbire različnih vrst privabilnih posevkov, ki se lahko med seboj razlikujejo tako po botaničnih lastnostih kot morfoloških lastnostih.

4.2 SUMMARY

In 2009 and 2010, we studied the applicability of the trap crop method as an alternative method for reducing the damage done by cabbage flea beetles (*Phyllotreta* spp.) and cabbage stink bugs (*Eurydema* spp.) on cabbage as the main plant species. The experiment was carried out at two different locations – at the Laboratory field of the Biotechnical Faculty in Ljubljana and at a field in the village Zgornja Lipnica in the municipality Radovljica. The trap crops used in the experiment were oil rape (*Brassica napus* [L.] ssp. *oleifera* f. *biennis*, the cultivar 'Daniela'), white mustard (*Sinapis alba* [L.], the cultivar 'Zlata') and oil radish (*Raphanus sativus* [L.] var. *oleiformis*, the cultivar 'Apoll'). The said plant species were sowed in two separate bands, within which we planted the main crop, i.e. the two cabbage cultivars – the early 'Tucana' and the mid-late 'Hinova'. The experiment was carried out in the four blocks within which we randomly sowed the trap crop plants. The extent of damage was during the growth period assessed in 10-day intervals or three times per month. The damage done by cabbage flea beetles was assessed by the 5-grade EPPO-scale (OEPP/EPPO, 2002), while the damage done by cabbage stink bugs was evaluated on the basis of the 6-grade visual scale (Stoner and Shelton, 1988). We established the preference of the studied groups of harmful pests for individual plant species. The extent of damage caused by cabbage flea beetles was throughout the experiment highest on oil radish, in 2009 it varied from 2.86±0.07 at the Laboratory Field of the Biotechnical Faculty to 3.00±0.03 at the field in the Gorenjska region. In 2010 the extent of damage on oil radish at the Laboratory field of the Biotechnical Faculty was 3.87±0.06; while on oil radish in the Gorenjska region we recorded the damage extent of 3.50±0.04. The highest damage caused by feeding of cabbage stink bugs was observed on the plants of oil rape. In the first year of the experiment we thus recorded the average index of 3.38±0.04 on the plants of oil rape at the field in the Gorenjska region, while in the second year of the experiment it was 3.58±0.08. The average indexes of damage were on the main crop plants at both locations in both years of the experiment significantly lower. Despite this, the damage extent on the studied cabbage hybrids differed, which can be attributed to the influence of the plants' growth period. The mid-late cabbage hybrid in our study proved as much more susceptible to damage caused by the studied groups of harmful pests. The cabbage flea beetles in our study began appearing in the beginning of May, while rape bugs (*Eurydema oleracea*) and cabbage stink bugs (*Eurydema ventrale*) were first detected in the second half of May. Our findings correspond to those of some previous studies – cabbage flea beetles in this part of Europe begin to appear massively in the beginning of July.

No pronounced influence of the trap crops on the production of cabbage was established. We have, however, found out that the average production was higher in the mid-late cabbage cultivar. Since our experiment did not include any significant additional manuring and irrigating, the said measures affected also the final crops. If we used insecticides on the

trap crops, we would influence the population of harmful pests, and at the same time harm useful organisms which feed on the trap crop plants during blossoming.

We found out that the intensity of feeding by cabbage flea beetles and cabbage stink bugs is connected with the plants' developmental stage. In the beginning of the growth period we thus recorded high susceptibility of trap crops for both groups of harmful insects. The susceptibility of plants varied during the growth period. The changing susceptibility of plants during the growth period can be attributed to the plants' natural resistance.

In our study we assessed glucosinolates as parameters of natural resistance in Brassicaceae. These secondary metabolites, which are characteristic primarily for the family Brassicaceae, were assessed in the second year of the experiment, in the plants which were cultivated at the field in the Gorenjska region. During the plants' growth period we at different intervals carried out sampling to determine glucosinolate content. In the white mustard and oil radish plants we sampled also blossoms. For the studied plant species we evaluated the content of nine different glucosinolates, which were classified into three different groups. Among aliphatic glucosinolates we measured the content of glucoiberin, progoitrin, epiprogoitrin, sinigrin, gluconapin and glucoraphenin; among indole glucosinolates we measured the content of glucobrassicin. Among aromatic glucosinolates we measured the content of gluconasturtiin and sinalbin. Evaluation of glucosinolates was carried out according to the standard ISO 9167:1 - 1992. Entire procedure has been described during our survey. The applied presentation unit was µmol/g of dry seed mass. Glucosinolates, as typical secondary metabolites, can differently influence harmful organisms. The caterpillars of the small white butterfly (*Pieris rapae*) can thus metabolise and excrete glucosinolates, while higher contents of sinigrin and sinalbin inhibit the feeding of bertha armyworms (*Mamestra configurata)*. The literature says that harmful organisms can use glucosinolates in plants for their own defence.

In view of the average index of damage caused by the species from the genera *Phyllotreta* and *Eurydema*, glucosinolates either reduce or increase the extent of feeding. However, the effects of an individual glucosinolate on *Phyllotreta* spp. and *Eurydema* spp. is not identical among plant species. We found out that certain differences occur. It would be interesting to identify the mechanism with which the studied glucosinolates affect the metabolism of the insects from the genera *Phyllotreta* and *Eurydema*.

Our study confirms the finding that the content of glucosinolates varies during the growth period, between plant species, as well as between individual parts of a plant. We found out that glucobrassicin is the only glucosinolate present in all plant species, while its content is highest in plants of oil radish (3.24±0.86 µmol/g of dry seed mass). Sinalbin is most frequently found in the samples of oil rape (11.16±6.50 µmol/g of dry seed mass) and white mustard (30.12±5.52 µmol/g of dry seed mass). We can also say that the content of glucosinolates differs between individual cultivars. In the mid-late cabbage cultivar 'Hinova' we recorded higher values of progoitrin, while the content of glucobrassicin was higher in the early hybrid 'Tucana'. We also established differences in the content of glucosinolates between individual parts of plants in the same species. The contents of sinalbin and epiprogoitrin are thus higher in the blossoms of white mustard, while glucoraphenin and glucobrassicin are present to a larger extent in blossoms than in leaves of oil radish.

Negative influence of gluconasturtiin in the samples of oil rape was recorded both in cabbage stink bugs (r=-0.98) and flea beetles (r=-0.99). Epiprogoitrin in the samples of oil rape markedly negatively affects the feeding of cabbage stink bugs (r=-0.99) and cabbage flea beetles (r=-0.80). Higher contents of epiprogoitrin in the samples of oil rape negatively affect the feeding of cabbage stink bugs and flea beetles. We assume that the preference of cabbage flea beetles (*Phyllotreta* spp.) for oil radish could be related to the contents of the said glucosinolates. Since gluconasturtiin, glucoiberin and progoitrin in the samples of oil radish are present in traces and have a considerably larger role in oil rape and white mustard, the presence of the said glucosinolates in larger quantities could affect the extent of damage.

Besides the marked variability of glucosinolates between the individual plant species used in the study, we also identified a pronounced influence of environmental factors. We cannot talk about any uniform environmental influence on the group of glucosinolates, yet the description of functioning can be linked to the glucosinolate in question. Environmental factors differently influence the manifested functioning within the groups of aliphatic and aromatic glucosinolates. The group of indole glucosinolates is the one most markedly affected by temperature. The negative influence of the average daily temperature and the maximum daily temperature established in our study corresponds to the expectations. Limited accessibility of insecticides further increased the applicability of the selected alternative method. It is true that we have not achieved produce in terms of market significance, but the results of our study enable the use in ecological or integral production of Brassicas. By the proper timing of sowing/planting we influenced the bionomics of the harmful pest and saw to it that the damage done by *Phyllotreta* spp. and *Eurydema* spp. on cabbage in the beginning of the growth period was not so pronounced, which is one of the key elements in the systems of production. We believe that glucosinolates are an important parameter of plants' natural resistance, yet their functioning is very variable and conditioned by a numerous factors, among others also by environmental factors. Several further studies are required in order to establish their influence with more certainty. It is clear that by selecting a large number of host species serving as trap crops we can successfully regulate the susceptibility of the main crops.

Our study sets an example that with the help of trap crops we could reduce the extent of damage done by other groups of harmful organisms which appear on cabbage, since the chosen method of plant protection comprises several possibilities for selecting trap crops, which may differ in regard to both botanical and morphological characteristics.

5 VIRI

Al-Gendy A.A., El-gindi O.D., Hafez A.S., Ateya A.M. 2010. Glucosinolates, volatile constituents and biological activities of *Erysimum corinthium* Boiss. (Brassicaceae). Food Chemistry, 118:519-524

Ahuja I., Rohloff J., Bones A.M. 2010. Defence mechanisms of Brassicaceae: implications for plant-insect interactions and potential for integrated pest management. A review. Agronomy for Sustainable Development, 30: 311-348

Andersen C.L., Hazzard R., van Driesche R., Mangan F.X. 2006. Alternative management tactics for control of *Phyllotreta cruciferae* and *Phyllotreta striolata* (Coleoptera: Chrysomelidae) on Brassica rapa in Massachusetts. Journal of Economic Entomology, 99: 803-810

Bell C.E. 1995. Broccoli (*Brassica oleracea* var. *botrytis*) yield loss from Italian ryegrass (Lollium perenne) interference. Weed Science, 43: 117-120

Björkman M., Klingen I., Birch A.N.E., Bones A.M., Bruce T.J.A., Johansen T.J., Meadow R., Mølmann J., Seljåsen R., Smart L.E., Stewart D. 2011. Phytochemicals of Brassicaceae in plant protection and human health – Influences of climate, environment and agronomic practice. Phytochemistry, 72: 538-556

Bohinc T., Trdan S. 2010. Uporaba treh privabilnih posevkov z namenom odvračanja kapusovih stenic (*Eurydema* spp.) in kapusovih bolhačev (*Phyllotreta* spp.) od belega zelja. V: Novi izzivi v poljedelstvu 2010. New challenges in field crop production 2010. Kocjan Ačko D. in Čeh B. (ur.). Ljubljana. Slovensko agronomsko društvo: 259-265

Bohinc T., Trdan S. 2011a. Ščitaste stenice (Pentatomidae) kot škodljivci gojenih rastlin in načini njihovega zatiranja. Acta agriculturae Slovenica, 97: 63-72

Bohinc T., Trdan S. 2011b. Privabilni posevki kot metoda zmanjševanja škodljivosti kapusovih stenic (*Eurydema* spp.) in kapusovih bolhačev (*Phyllotreta* spp.) na belem zelju – primerjava rezultatov poljskega poskusa v letih 2009 in 2010. V: Zbornik predavanj in referatov 10. Slovenskega posvetovanja o varstvu rastlin. Podčetrtek, 1.-2. marec. Trdan S. in Maček J. (ur.). Ljubljana, Društvo za varstvo rastlin Slovenije: 97-106

Bones A.M., Rossiter J.T. 2006. The enzymic and chemically induced decomposition of glucosinolates. Phytochemistry, 67: 1053-1067

Branca F., Li G., Goyal S., Quiros C.F. 2002. Survey of aliphatic glucosinolates in Sicilian wild and cultivated Brassicaceae. Phytochemistry, 59: 717-724

Bommarco R., Miranda R., Bylund H., Björkman C. 2011. Insecticides suppress natural enemies and increase pest damage in cabbage. Journal of Economic Entomology, 104: 782-791

Brelih S., Dőberl M., Drovenik B., Pirnat A. 2003. Gradivo za favno hroščev (Coleoptera) Slovenije. 1. Prispevek Polyphaga: Chrysomeloidea (Phytophaga): Chrysomelidae: Alticinae. Materialien zur Käferfauna (Coleoptera) Slowenien. 1. Beitrag: Polyphaga: Chrysomeloidea (Phytophaga): Chrysomelidae: Alticinae. Scopolia, 50: 279 str.

Brown J., McCaffrey J.P., Brown D.A., Harmon B.L., Davis J.B. 2004. Yield reduction in *Brassica napus, B. rapa, B. juncea*, and *Sinapis alba* caused by flea beetle (*Phyllotreta cruciferae* (Goeze) (Coleoptera: Chrysomelidae)) infestation in nothern Idaho. Journal of Economic Entomology, 97:1642-1647

Cárcamo H.A., Blackshaw R.E. 2007. Insect pest incidence and injury to herbicide-tolerant canola in Western Canad. Agronomy Journal, 99: 842-846

Charles R., Cholley E., Frei P., Mascher F. 2011. Crop rotation, soil tillage, variety and fungicide protection in cereal production. Agrarfoschung Schweiz, 2: 264-271

Chemical Book. 2010.
www.chemicalbook.com (7.5.2013)

Chen C., Qian YZ., Liu X.J., Tao C.J., Liang Y., Li Y. 2012. Risk assessment of chlorpyrifos on rice and cabbage in China. Regulatory Toxicology and Pharmacology, 62: 125-130

Cook S. M., Smart L. E., Martin J. L., Murray D. A., Watts N. P., Williams I.H. 2006. Explotation of host plant preferences in pest management strategies for oilseed rape (*Brassica napus*). Entomologia Experimentalis et Applicata, 119: 221-229

Dinkova-Kostova A., Kostov R.V. 2012. Glucosinolates and isothiocyanates in health and disease. Trends in Molecular Medicine, 18: 337-347

Dixon G.R. 2007. Vegetable Brassicas and related crucifers. Biddles Ltd. UK: 314 str.

Dosdall L.M., Harker K.N., O'Donovan J.T., Blackshaw R.E., Kutcher H.R., Gan Y., Johnson E.N. 2012. Crop sequence effects on root maggot (Diptera: Anthomyiidae: *Delia* spp.) infestations in canola. Journal of Economic Entomology, 105: 1261-1267

Duda A., Liste H.J. 1991. Measures of crop-rotation for limiting the damage caused by the beet cyst nematode. Bodenkultur, 42: 253-260

Fahey J.W., Zalcmann A.T., Talalay P. 2001. The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. Phytochemistry, 56: 5-51

Francisco M., Cartea M.E., Butron A.M., Sotelo L., Velasco P. 2012. Environmental and genetic effects on yield and secondary metabolite production in *Brassica rapa* crops. Journal of Agricultural and Food Chemistry, 60: 5507-5514

Gimsing A.L., Kirkegaard J.A. 2009. Glucosinolates and biofumigation: fate of glucosinolates and their hydrolysis products in soil. Phytochemistry Review, 8: 299-310

Gray F.A., Koch D. W. 2002. Trap crops. V: Encyclopedia of pest management. Pimental D. (ur.). Wyoming, Lacraime, University of Wyoming: 852-864

Hadacek F. 2002. Secondary metabolites as plant traits: current assessment and future perspectives. Critical Reviews in Plant Sciences, 21:273-322

Hopkins R. J., van Dam N., van Loon J. J. A. 2009. Role of glucosinolates in insect-plant relationship and multitrophic interactions. Annual Review of Entomogy, 54: 57-83

Hill J., Lethenborg P., Li P.W., Rahman M.H., Sørensen H., Sørensen J.C. 2003. Inheritance of progoitrin and total aliphatic glucosinolates in oilseed rape (*Brassica napus* L.). Euphytica, 134: 179-187

ISO 9167-1. Rapeseed – determination of glucosinolates content. Part 1: Method using high-performance liquid chromatography. 1992: 9 str.

Janežič F. 1951. Varstvo rastlin pred boleznimi in škodljivci. Ljubljana, Državna založba Slovenije: 567 str.

Kos M., Houshyani B., Achhami B.B., Wietsma R., Gols R., Weldegergis BT, Kaboum P., Bouwmeester H.J., Vet L.E.M., Dicke M., Loon J.J.A. 2012. Herbivore-mediated effects of glucosinolates on different natural enemies of a specialist aphid. Journal of Chemical Ecology, 38: 100-115

Knodel J.J., Olson D.L., Hanson B.K., Henson R.A. 2008. Impact of planting dates and insecticide strategies for managing crucifer flea beetles (Coleoptera: Chrysomelidae) in spring-planted canola. Journal of Economic Entomology, 101: 810-821

Krmni dosevki. 2013. Semenarna Ljubljana. http://www.semenarna.si/krmne-poljscine/category/krmni-dosevki (28.4.2013)

Hassan F.U., Arif M. 2012. Response of white mustard (*Sinapis alba* L) to spacing under rainfed conditions. The Journal of Animal and Plant Sciences, 22: 137-141

Lambdon P. W., Hassall M., Boar R.R., Mithen R. 2003. Asynchrony in the nitrogen and glucosinolate leaf-age profiles of *Brassica*: is this a defensive strategy against generalist herbivores? Agriculture, Ecosystems and Environment, 97: 205-214

Leavitt H., Robertson I.C. 2006. Petal herbivory by chrysomelid beetles (*Phyllotreta* spp.) is determinal to pollination and seed production in *Lepidium papilliferum* (Brassicaceae). Ecological Entomology, 31: 657-660

Maiti R., Satya P., Rajkumar D., Ramaswamy A. 2012. Crop plant anatomy. CPI Group, Velika Britanija: 313 str.

Mazzi D., Dorn S. 2012. Movement of insect pests in agricultural landscapes. Annals of Applied Biology, 160: 97-113

Moyes C.L., Collin H.A., Britton G., Raybould A.F. 2000. Glucosinolates and differential herbivory in wild populations of *Brassica oleracea*. Journal of Chemical Ecology, 26: 2625-2641

Newton E.L., Bullock J.M., Hodgson D.J. 2009. Glucosinolate polymorphism in wild cabbage (*Brassica oleracea*) influences the structure of herbivore communities. Oecologia, 160: 63-76

Olson D.M., Webster T.M., Scully B.T., Strickland T.C., Davis R.F., Knoll J.E., Anderson W.F. 2012. Use of winter legumes as banker plants for beneficial insect species in as sorghum and cotton rotation system. Journal of Entomological Science, 47: 350-359

OEPP/EPPO. 2002. Guidelines for the efficiency evaluation of insecticides. *Phyllotreta* spp. on rape. OEPP/EPPO Bulletin, 32: 361-365

Osvald J., Kogoj Osvald M. 2005. Vrtnarstvo. Splošno vrtnarstvo in zelenjadarstvo. Ljubljana, Univerza v Ljubljani, Biotehniška fakulteta, Oddelek za agronomijo: 591 str.

Padilla G., Cartea M. E., Velasco P., de Haro A., Ordás A. 2007. Variation of glucosinolates in vegetable crops of *Brassica rapa*. Phytochemistry, 68: 536-545

Palaniswamy P., Lamb R.J. 1992. Host preference of the flea beetles *Phyllotreta cruciferae* and *P. striolata* (Coleoptera: Chrysomelidae) for crucifer seedlings. Journal of Economic Entomology, 85: 743-752

Poelman E.H., Galiart R.J.F.H., Raaijmakers C.E., van Loon J.J.A., vam Dam N.M. 2008. Performance of specialist and generalist herbivores feeding on cabbage cultivars is not explained by glucosinolate profiles. Entomologia Experimentalis et Applicata, 127: 218-228

Popis tržnega vrtnarstva. 2010. Statistični urad Republike Slovenije. http://www.stat.si/novica_prikazi.aspx?id=4555 (24.4.2013)

Pratt C., Pope T.W., Powel G., Rossiter J.T. 2008. Accumulation of glucosinolates by the cabbage aphid *Brevicoryne brassicaceae* as a defense against two coccinellid species. Journal of Chemical Ecology, 34: 323-329

Safraz M., Dosdall L.M., Keddie B.A. 2007. Resistance of some cultivated Brassicaceae to infestations by *Plutella xylostella* (Lepidoptera: Plutellidae). Journal of Economic Entomology, 100: 215-224

Schaefer C.W., Panizzi A.R. 2000. Heteroptera of economic importance. Boca raton, CRC Press: 828 str.

Schonhof I., Kläring H.P., Krumbein A., Claußen W., Schreiner M. 2007. Effect of temperature increase under low radiation conditions on phytochemicals and ascorbic acid in greenhouse grown broccoli. Agriculture, Ecosystems & Environment, 119: 103-111

Seznam registriranih fitofarmacevtskih sredstev na dan 23.4.2013. Ministrstvo za kmetijstvo in okolje. Uprava RS za varno hrano, veterinarstvo in varstvo rastlin. http://spletni2.furs.gov.si/FFS/REGSR/index.htm (24.4.2013)

Shelton A.M., Badenes-Perez F.R. 2006. Concepts and applications of trap cropping in pest management. Annual Review of Entomology, 51: 285-308

Smallegange R.C., van Loon J.J.A., Blatt S.E., Harvey J.A., Agerbirk N., Dicke M. 2007. Flower vs. leaf feeding by *Pieris brassicae*: Glucosinolate-rich flower tissues are preferred and sustain higher growth rate. Journal of Chemical Ecology, 33: 1831-1844

Soroka J.J., Bartelt R.J., Zilkowski B.W., Cosse A.A. 2005. Response of flea beetle *Phyllotreta cruciferae* to synthetic aggregation pheromone components and host plant volatiles in field trials. Journal of Chemical Ecology, 31: 1829-1843

Soroka J.J., Holowachuk J.M., Gruber M.Y., Grenkow L.F. 2011. Feeding by flea beetles (Coleoptera: Chrysomelidae; *Phyllotreta* spp.) is decrease on canola (*Brassica napus*) seedlings with increased trichome density. Journal of Economic Entomology, 104: 125-136

Stoner K.A. 1992a. Glossy leaf wax and plant resistance to insects in *Brassica oleracea* under natural infestation. Environmental Entomology, 19: 730-739

Stoner K.A. 1992b. Density of imported cabbageworms (Lepidoptera, Pieridae), cabbage aphids (Homoptera, Aphididae), and flea beetles (Coleoptera, Chrysomelidae) on glossy and trichome-bearing lines of *Brassica oleracea*. Journal of Economic Entomology, 85: 1023-1030.

Stoner K.A., Shelton A.M. 1988. Effects of planting date and timing of growth stage on damage to cabbage by onion thrips (*Thrips tabaci*). Journal of Economic Entomology, 91: 329-333

Sun B, Liu N, Zhao Y, Yan H., Wang Q. 2011. Variation of glucosinolates in three edible parts of Chinese kale (*Brassica alboglabra* Bailey) varieties. Food Chemistry, 124: 941-947

Talgre L., Lauringson E., Makke A., Lauk R. 2011. Biomass production and nutrient binding of catch crops. Zemdirbyste-Agriculture, 98: 251-258

Tansey J.A., Dosdall L.M., Keddie B.A. 2009. *Phyllotreta cruciferae* and *Phyllotreta striolata* responses to insecticidal seed treatments with different modes of action. Journal of Applied Entomology, 133: 201-209

Tansey J.A., Dosdall L.M., Keddie A., Fletcher R.S., Kott L.S. 2010. Antixenosis and antibiosis resistance to *Ceutorhynchus obstrictus* in novel germplasm derived from *Sinapis alba* x *Brassica napus*. Canadian Entomologist, 142: 212-221

Tehnološka navodila za integrirano pridelavo zelenjave. 2013. Ministrstvo za kmetijstvo in okolje. http://www.mko.gov.si/fileadmin/mko.gov.si/pageuploads/podrocja/Kmetijstvo/Integrirana_pridelava/TN_zelenjava_2013.pdf (24.4.2013)

Tian D., Tooker J., Pfeiffer M., Chung S.H., Felton G. 2012. Role of trichomes in defence against herbivores: comparison of herbivore response to wolly and hairless trichome mutants in tomato (*Solanum lycopersicum*). Planta, 236: 1053-1066

Tiwari U., Cummins E. 2013. Factors influencing levels of phytochemicals in selected druit and vegetables during pre- and post- harvest food processing operations. Food Research International, 50: 497-506

Toshova T. B., Csonka E., Subschev M. A., Tóth M. 2009. The seasonal activity of flea beetles in Bulgaria. Journal of Pest Science, 82: 295-303

Trdan S., Milevoj L., Žežlina I., Raspudić E., Andjus L., Vidrih M., Bergant K., Valič N., Žnidarčič D. 2005a. Feeding damage by onion thrips, *Thrips tabaci* Lindeman (Thysanoptera: Thripidae), on early white cabbage grown under insecticide-free conditions. African Entomology, 13: 85-95

Trdan S., Valič N., Žnidarčič D., Vidrih M., Bergant K., Zlatič K., Milevoj L. 2005b. The role of Chinese cabbage as a trap crop for flea beetles (Coleoptera: Chrysomelidae) in production of white cabbage. Scientia Horticulturae, 106: 12-24

Trdan S., Žnidarčič D., Valič N. 2006. Field efficacy of three insecticides against cabbage stink bugs (Heteroptera: Pentatomidae) on two cultivars of white cabbage. International Journal of Pest Management, 52: 79-87

Trdan S., Vidrih M., Bobnar A. 2008. Seasonal dynamics of three insect pests in the cabbage field in Central Slovenia. V: Proceedings 60th International symposium on crop protection. Spanoghe, P. (ur.). Communications in Agricultural and Applied Biological Sciences, 73: 557-561

Trdan S., Valič N., Vovk I., Martelanc M., Simonovska B., Vidrih R., Vidrih M., Žnidarčič D. 2009. Natural resistance of cabbage against three insect pests. V: Integrated Protection of Field Vegetables. Collier, R. (ur.). IOBC/WPRS Bulletin, 51: 93-106

Vaughn S.F., Berhow M.A. 2005. Glucosinolate hydrolisis products from various plant sources: Ph effects, isolation, and purification. Industrial Crops and Products, 21: 693-202

Valantin-Morison M., Meynard J.M., Doré T. 2007. Effects of crop management and surrounding field environment on insect incidence in organic winter oilseed rape (*Brassica napus* L.). Crop Protection, 26: 1108-1120

Van Doorn J.E., van der Kruk G.C., van Holst G.J., Schoofs M., Broer J.B., Nijs J.J.M. 1999. Quantitive inheritance of the progoitrin and singrin content in Brussels sprouts. Euphytica, 108: 41-52

Velasco P., Cartea M.E., Gonzalez C., Vilar M., Ordas S. 2007. Factors affecting the glucosinolate content of kale (*Brassica oleracea acephala* group). Journal of Agricultural and Food Chemistry, 55: 955-962

Vrabl S. 1992. Škodljivci poljščin. Ljubljana, Kmečki glas: 142 str.

Wallingford A.K., Kuhar T.P., Pfeiffer D.G., Tholl D.B., Freeman J.H., Doughty H.B., Schultz P.B. 2013. Host plant preference of harlequin bug (Hemiptera: Pentatomidae), and evaluation of a trap cropping strategy for its control in collard. Journal of Economic Entomology, 106: 283-288

ZAHVALA

Zahvaljujem se prof. dr. Stanislavu Trdanu, mentorju te doktorske naloge. Hvala za vse napotke in spodbude na dodiplomskem kot tudi podiplomskem študiju! HVALA!!!

Hvala staršem, ki me že od osnovne šole spodbujajo, da počnem tisto kar me veseli. Privzgojene delavne navade so mi v letih študija velikokrat pomagale. Včasih sem potrebovala ravno tisto malo pozitivne energije, na katero sem sem lahko zanesla pri vama. HVALA!!!

Hvala ekipi Katedre za fitomedicino, kmetijsko tehniko, poljedelstvo, pašništvo in travništvo (Jaka Rupnik, Matej Vidrih, Žiga Laznik, …) za vso pomoč pri izvajanju poskusa, kot tudi ostalem delu!!!

Hvala Kristini in vsem kolegom za vse pozitivne misli!

Hvala zaposlenim na Oddelku za agrokemijo in pivovarstvo na Inštitutu za hmeljarstvo in pivovarstvo Slovenije v Žalcu za izvedene analize glukozinolatov.

Zahvaljujem se tudi organizacijam, ki so omenjeno raziskavo finančno podprle (Ministrstvo za kmetijstvo in okolje, Javna agencija za raziskovalno dejavnost Republike Slovenije).

Hvala vsem imenovanim, kot vsem neimenovanim, ki ste sodelovali pri izvedbi naloge in me spodbujali!

PRILOGA A

Slikovno gradivo – preučevane škodljive vrste in tip poškodb

Priloga A1: Parjenje kapusovih stenic (*Eurydema oleracea*) na cvetu bele gorjušice (foto: T. Bohinc)

Priloga A2: Poškodbe kapusovih bolhačev (*Phyllotreta* spp.) na oljni redkvi (foto: T. Bohinc)

Priloga A3: Ličinki (levo) in odrasel osebek (desno) pisane stenice (*Eurydema ventrale*) (foto: T. Bohinc)

Priloga A4: Poškodbe kapusovih stenic na krmni ogrščici v letu 2010 na njivi na Gorenjskem (foto: T. Bohinc)

Priloga A5: Poškodbe kapusovih bolhačev na listu oljne redkve (Foto: T. Bohinc)

PRILOGA B

Slikovno gradivo – lokacije poskusa

Priloga B1: Poljski poskus na njivi na Gorenjskem v letu 2010 (foto: T. Bohinc)

Priloga B2: Poljski poskus na Laboratorijskem polju Biotehniške fakultete v Ljubljani (foto: T. Bohinc)

PRILOGA C

Slikovno gradivo – vzorci namenjeni liofiliziranju in posledično analizi glukozinolatov

Priloga C1: Vzorec cvetov bele gorjušice namenjen liofiliziranju, in posledično analizi glukozinolatov (foto: T. Bohinc)

PRILOGA D

Dovolje založbe »EDUEM«

Editora da Universidade Estadual de Maringa - EDUEM
Av. Colombo, 5790, bloco 40
87020-900-Maringa PR/Brasil

Maringa, 10th May 2013

Tanja Bohinc
Zgornja Lipnica 9a
4246 Kamna Gorica

Dear Tanja Bohinc,

herewith we confirm that we agree with using manuscript

»Association between glucosinolate concentration and injuries caused by cabbage stink bugs *Eurydema* spp. (Heteroptera: Pentatomidae) on different Brassicas«,

which was published by Acta Scientiarum Agronomy (v. 35, no. 1, p. 1-8) as part of doctoral dissertation.

Paper mentioned above can be used in paper and electronical version of doctoral dissertation »Interactions of cabbage flea beetles (Phyllotreta spp.) and cabbage stink bugs (Eurydema spp.) with cabbage and selected trap crops«.

Yours sincerely,

Prof. Dr. Alessandro Lucca Braccini
Diretor da Eduem

Priloga D1: Dovoljenje, da je članek »Association between glucosinolate concentration and injuries caused by cabbage stink bugs *Eurydema* spp. … on different Brassicas« lahko del doktorske disertacije, ki bo predstavljena v papirnati in elektronski obliki.

PRILOGA E

Dovoljenje založbe »Institute of Agriculture Lithuanian Research Centre for Agriculture and Forestry«

INSTITUTE OF AGRICULTURE
LITHUANIAN RESEARCH CENTRE
FOR AGRICULTURE AND FORESTRY

Instituto alėja 1, Akademija, LT-58344 Kėdainiai distr., LITHUANIA
Tel. +370 347 37 057 / 37 271, faks. +370 347 37 096, e-mail: lammc@lammc.lt, http://www.lammc.lt
Enterprise code 302471203, VAT code LT 100005122310

2013-05-14 No. CS-4/185

Tanja Bohinc
Zgornja Lipnica 9a
4246 Kamna Gorica, Slovenia

Re: acknowledgement of the formal consent of the copyrights owner of scientific journal "Žemdirbystė-Agriculture"

With this document we certify that we permit Tanja Bohinc from University of Ljubliana to reproduce the listed below paper in her PhD thesis:

Bohinc T., Košir I. J., Trdan S. 2013. Glucosinolates as arsenal for defending *Brassicas* against cabbage flea beetles attack. Žemdirbystė-Agriculture, 100 (2) (in press).

Director of Lithuanian Research Centre
for Agriculture and Forestry,
Chairperson of the Editorial Board
of the scientific journal "Žemdirbystė-Agriculture"

Zenonas Dabkevičius

Daiva Puidokiene,
tel. +370 347 37 135, +370 676 12 791

Priloga E1: Dovoljenje, da je lahko članek »Glucosinolates as arsenal for defending Brassicas against cabbage flea beetles attack« del doktorske disertacije, ki bo predstavljena v elektronski in papirnati obliki.

PRILOGA F

Prošnja in dovoljenje za uporabo člankov, naslovljeno na WFL Publisher

Tanja Bohinc
Zgornja Lipnica 9a
4246 Kamna Gorica

Ljubljana, 8th May 2013

Dear Publisher,

I am PhD student. For the purpose of finishing Postgraduate study program of Biological and Biotechical Sciences (University of Ljubljana) and because my doctoral dissertation is composed of six scientific papers , I would like to ask you kindly, if you could please send me letter of permission for paper

»Environmental factors affecting the glucosinolate content in Brassicaceae«,

written by authors (Tanja Bohinc and Stanislav Trdan);

which was published by International Journal of Food, Agriculture and Environment, v. 10, No. 2., pages 357-380.

Letter of permission will be needed due to a fact that doctoral dissertation will be published in paper and electronical version (one year after defending).

Thank you in advance,

Tanja Bohinc
corresponding author
Bohinc Tanja

Priloga F1: Prošnja, naslovljena na WFL Publisher, ki se nanaša na distribucijo članka »Environmental factors affecting the glucosinolate content in Brassicaceae« v okviru doktorske naloge.

Tanja Bohinc
Zgornja Lipnica 9a
4246 Kamna Gorica

Ljubljana, 8th May 2013

Dear Publisher,

I am PhD student. For the purpose of finishing Postgraduate study program of Biological and Biotechical Sciences (University of Ljubljana) and because my doctoral dissertation is composed of six scientific papers , I would like to ask you kindly, if you could please send me letter of permission for paper

»Trap crops for reducing damage caused by cabbage stink bugs (*Eurydema* spp.) and flea beetles (*Phyllotreta* spp.) on white cabbage: fact of fantasy?«,

written by authors (Tanja Bohinc and Stanislav Trdan);

which was published by International Journal of Food, Agriculture and Environment, v. 10, No. 2., pages 1365-1370.

Letter of permission will be needed due to a fact that doctoral dissertation will be published in paper and electronical version (one year after defending).

Thank you in advance,

Tanja Bohinc
corresponding author
Bohinc Tanja

Priloga F2: Prošnja, naslovljena na WFL Publisher, ki se nanaša na distribucijo članka »Trap crops for reducing damage caused by cabbage stink bugs (*Eurydema* spp.) and flea beetles (*Phyllotreta* spp.) on white cabbage: fact or fantasy?« v okviru doktorske naloge.

17. 05. 13 Gmail - kind appeal

Gmail by Google

Tanja Bohinc <tanja.bohinc@gmail.com>

kind appeal

JFAE-Editorial Office <info@world-food.net> 17. maj 2013 15:50
Za: Bohinc Tanja <tanja.bohinc@gmail.com>

Ms.Bohinc Tanja
In fact if your both papers hav been published already in JFAE that means
that you may use them and show them to the comitee board..

Please use this mail as a confirmation.
Niskanen
Editor

--
JFAE-Editorial Office
Department of Editors
Meri-Rastilantie 3 B
FIN-00980 Helsinki, Finland
Mobile: 00 368 50 50 505 11 35
Tel/Fax: 00 358 9 75 92 775
email:info@world-food.net
http://www.isfae.org/scientificjournal.php
[Citirano besedilo skrito]
--
JFAE-Editorial Office
Department of Editors
Meri-Rastilantie 3 B
FIN-00980 Helsinki, Finland
Mobile: 00 358 50 50 505 11 35
Tel/Fax: 00 358 9 75 92 775
email: info@world-food.net
http://www.isfae.org/scientificjournal.php

CONFIDENTIALITY NOTICE
This e-mail and any files transmitted with it are confidential and solely for the use of the intended recipient. It may contain information which is covered by professional or other privilege. If you are neither the intended recipient of this e-mail nor the person responsible for delivering it to the intended recipient, be advised that you have received this e-mail in error and that any use of it is strictly prohibited. Please notify the sender immediately by reply e-mail and then delete it from your system. WFL Publisher accepts no liability for any loss or damage suffered by any person arising from the use of this e-mail.
�: Co. Right & Policy, �: Intelectual Property;
�: Terms and Conditions).

- Please consider the environment before printing this email.

[illegible] 1/1

Priloga F3: Dovoljenje, ki se navezuje na članka, omenjena v prošnjah v Prilogi F1 in Prilogi F2.

PRILOGA G

Dovoljenje »Archives of Biological Sciences«

ARCHIVES OF BIOLOGICAL SCIENCES, BELGRADE

International and Multidisciplinary Journal Established in 1948
Official Journal of the Serbian Biological Society
Editor-in-Chief: Prof. Dr. Božidar P. M. Ćurčić
Institute of Zoology, Faculty of Biology, University of Belgrade
Studentski Trg 16, 11000 Belgrade, Serbia
Tel.: + 381 11 3281 789 and 2187 266 ext. 103; fax: + 381 11 2638 500
E-mail: bcurcic@bio.bg.ac.rs and bpmcurcic@yahoo.com

Belgrade, 12 May 2013

Permission

This is to confirm that Miss Tanja Bohinc from 4246 Kamna Gorica, Slovenia, has published the paper "Glucosinolates in plant protection strategies" together with Smiljana Goreta Ban, Dean Ban, and Stanislav Trdan. The paper has appeared in the Archives of Biological Sciences, vol. 64, issue 3, 821-828, 2012.
The permission of ABS is granted to Miss Bohinc since her doctoral dissertation has to be published in paper and electronical version.

Thank you for your consideration,

Prof. Dr. Božidar Ćurčić, Editor ABS,
President, Serbian Biological Society

Priloga G1: Dovoljenje, da lahko članek »Glucosinolates in plant protection strategies« uporabimo v doktorski nalogi, ki bo dostopna v elektronski in papirnati verziji

PRILOGA H

Dokumentacija založbe »Taylor & Francis«

Tanja Bohinc
Zgornja Lipnica 9a
4246 Kamna Gorica

Ljubljana, 8th May 2013

Dear Publisher,

I am PhD student. For the purpose of finishing Postgraduate study program of Biological and Biotechnical Sciences (University of Ljubljana) and because my doctoral dissertation is composed of six scientific papers, I would like to ask you kindly, if you could please send me letter of permission for paper

»Sowing mixtures of Brassica trap crops is recommended to reduce *Phyllotreta* beetles injury to cabbage«,

written by authors (Tanja Bohinc and Stanislav Trdan);

which will published by Acta Agriculturae Scandinavica, Section B – Soil & Plant Sciences, doi: 10.1080/09064710.2012.752858.

Letter of permission will be needed due to a fact that doctoral dissertation will be published in paper and electronical version (one year after defending).

Thank you in advance,

Tanja Bohinc
corresponding author
Bohinc Tanja

Priloga H1: Prošnja naslovljena na založbo »Taylor & Francis«, v zvezi z uporabo članka »Sowing mixtures of Brassica trap crops is recommended to reduce *Phyllotreta* beetles injury to cabbage«, kot del doktorske naloge v elektronski in papirnati obliki.

15. 05. 13 Gmail - kind request

Gmail by Google

Tanja Bohinc <tanja.bohinc@gmail.com>

kind request

Woodbridge-Johns, David <David.Woodbridge-Johns@tandf.co.uk> 08. maj 2013 15:13
Za: tanja.bohinc@gmail.com

Dear Tanja,

I have had a response from the editorial department;

No special letters are needed for permissions to publish in a dissertation. This is built-in to the copyright agreement, please see http://journalauthors.tandf.co.uk/permissions/reusingOwnWork.asp under point 3.2.

Kind regards,

David.

David Woodbridge-Johns – Production Editor

Taylor & Francis Group

4 Park Square, Milton Park, Abingdon, Oxon, OX14 4RN, UK

Tel: +44 (0)20 7017 6000

Fax: +44 (0)20 7017 6336

Web: www.tandfonline.com

e-mail: david.woodbridge-johns@informa.com

Taylor & Francis is a trading name of Informa UK Limited, registered in England under no. 1072954

---

https://mail.google.com/mail/u/0/?ui=2&ik=54d801c46f&view=pt&search=inbox&msg=13e84461a187a4c2 1/2

Priloga H2: Odgovor založbe »Taylor & Francis, v zvezi s prošnjo v Prilogi H1.

3. Copyright

We recommend that authors assign copyright in journal articles to Taylor & Francis or the journal proprietor (such as a learned society on whose behalf we publish). Our belief is that the assignment of copyright in an article by the author to us or to the proprietor of a journal on whose behalf we publish remains the best course of action for proprietor and author alike, as assignment allows Taylor & Francis, without ambiguity, to assure the integrity of the Version of Scholarly Record, founded on rigorous and independent peer review. Moreover, given a journal is a single copyright entity composed of multiple contributions, assignment allows us to properly manage both an author's and proprietor's intellectual property rights (IPR) associated with the article, and to act on an author's and proprietor's behalf when resolving allegations of plagiarism, abuse of moral rights, or infringements of copyright. Assignment also eliminates confusion over copyright in multiple-author articles, and facilitates our ability to negotiate subsidiary licenses with and administer permissions and reuse requests from third parties. Most importantly, we believe assignment enhances the reputation and prestige of the journal, its proprietor, its editors and editorial board, its peer review processes, and the added value we bring. You will be sent the assignment automatically, via our Central Article Tracking System (CATS), in the form of your Author Publishing Agreement.

For the avoidance of doubt, any patent rights, trademark rights, or rights to any process, product, or procedure described in an article are not assigned or licensed by you to us by virtue of the Author Publishing Agreement.

3.1 Moral rights

Similarly, you expressly reserve and assert your right to be identified as the Author of the Article, such right arising under section 77 of the Copyright, Designs and Patents Act 1988, and all your other moral rights arising under the Act and, so far as is legally possible, any broadly equivalent rights you may have in any territory of the world.

3.2 Retained rights

In assigning Taylor & Francis or the journal proprietor copyright, or granting an exclusive license to publish, you retain:

- the right to share on a non-commercial basis with colleagues in print or digital format your 'Author's Original Manuscript' (i.e., the unpublished version of the article created by you prior to peer review; formerly a "preprint");
- the right to post on a non-commercial basis your "Author's Original Manuscript" as a digital file on your own website for personal or professional use, or on your institution's network or intranet or website, or in a subject repository that does not offer content for commercial sale or for any systematic external distribution by a third party with the acknowledgment below:

"This is an Author's Original Manuscript of an article submitted for consideration in the [JOURNAL TITLE] [copyright Taylor & Francis]; [JOURNAL TITLE] is available online at http://www.tandfonline.com/[Article DOI]."

- the right to retain on a non-commercial basis your Author's Original Manuscript version of the article as specified above following publication in the journal with the acknowledgment below

"This is an Author's Original Manuscript of an article whose final and definitive form, the Version of Record, has been published in the [JOURNAL TITLE] [date of publication] [copyright Taylor & Francis], available online at: http://www.tandfonline.com/[Article DOI]."

- the right to post on a non-commercial basis your "Author's Accepted Manuscript" (i.e., your manuscript in the form accepted for publication, revised after peer review; formerly a "postprint"), as a digital file on your own website for personal or professional use, or on your institution's network or intranet or website, or in a subject repository that does not offer content for commercial sale or for any systematic

Priloga H3: Odgovor založbe »Taylor & Francis, v zvezi s prošnjo v Prilogi H1.

external distribution by a third party, provided that you do not use the PDF version of the article prepared by us and that you include any amendments or deletions or warnings relating to the article issued or published by us; in compliance with the embargo periods detailed below; and only with this acknowledgement:

"This is an Author's Accepted Manuscript of an article published in [include the complete citation information for the final version of the article as published in the [JOURNAL TITLE] [date of publication] [copyright Taylor & Francis], available online at: http://www.tandfonline.com/[Article DOI]."
As noted above, as part of our Author Services program, Taylor & Francis will deposit the Author's Accepted Manuscript in any designated institutional repository with which Taylor & Francis has a Deposit Agreement, including PubMedCentral (PMC).
Taylor & Francis mandates public access to the final version of your manuscript twelve (12) months after the publication of the Version of Scholarly Record in science, engineering, behavioral science, and medicine; and eighteen (18) months after first publication for arts, social science, and humanities journals, in digital or print form.

- the right to share with colleagues on a non-commercial basis copies of an article in its published form as supplied by Taylor & Francis as a digital eprint or printed reprint;
- the right to make printed copies of all or part of an article on a non-commercial basis for use by you for lecture or classroom purposes provided that such copies are not offered for sale or distributed in any systematic way, and provided that acknowledgment to prior publication in the journal is made explicit;
- the right to facilitate the distribution of the article on a non-commercial basis if the article has been produced within the scope of your employment, so that your employer may use all or part of the article internally within the institution or company provided that acknowledgment to prior publication in the journal is made explicit;
- the right to include an article in a thesis or dissertation that is not to be published commercially, provided that acknowledgment to prior publication in the journal is made explicit
- the right to present an article at a meeting or conference and to distribute printed copies of the article to the delegates attending the meeting provided that this is not for commercial purposes and provided that acknowledgment to prior publication in the journal is made explicit;
- the right to use the article in its published form in whole or in part without revision or modification in personal compilations [in print or digital form] or other publications of your own articles, provided that acknowledgment to prior publication in the journal is made explicit;
- the right to expand an article into book-length form for publication provided that acknowledgment to prior publication in the journal is made explicit.

If you wish to use your article in a way which is not covered by the above license, please contact the Taylor & Francis Permissions Team:
permissionrequest@tandf.co.uk

Such uses might include the reuse of your article for commercial gain by a third party.

3.3 Green open access

You can make your work freely accessible by posting your Author Original Manuscript (AOM) (PDF) or Author Accepted Manuscript (AAM) (PDF) into your institutional or subject repository - this is frequently referred to as "green open access." You may post the AOM at any time, and you may post the AAM after an embargo period, following publication of the Version of Record (PDF) of your paper. Please see our list of journals with applicable embargo periods. (PDF)

3.4 Retrospective coverage

We are happy to extend all these provisions to the many thousands of authors who have signed copyright assignments and licenses to publish in the past with Taylor & Francis or one of its constituent imprints, without the need to seek amendment to the previous agreements.

Priloga H4: Odgovor založbe »Taylor & Francis, v zvezi s prošnjo v Prilogi H1.