Neonicotinoid degradation dynamics in sugar beet plants grown from treated seeds and influence on harmful and beneficial fauna

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DOCTORAL DISSERTATION

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DOCTORAL DISSERTATION

Supervisor:
Prof. Renata Bažok, Ph.D., Full Professor

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Sveučilište u Zagrebu

Agronomski fakultet

Helena Virić Gašparić

DINAMIKA RAZGRADNJE NEONIKOTINOIDA PRIMIJENJENIH TRETIRANJEM SJEMENA ŠEĆERNE REPE I UČINAK NA ŠTETNU I KORISNU FAUNU

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With my signature I confirm that:

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Supervisor biography

Prof. Renata Bažok Ph.D. works at the University of Zagreb as a member of the Faculty of Agriculture. She is a full professor and has been working in higher education for 29 years. She was the coordinator of the undergraduate Plant Protection program and the graduate study program in Plant Medicine. She has been the recipient of three fellowships, including a USDA/ARS, a Cochran, and a Fulbright.

She was involved in two TEMPUS projects and led/coordinated one TEMPUS project. She worked on two USDA/CRO projects and several national scientific projects. She was also the principal investigator in four national scientific projects and one FAO project and two projects funded by the Croatian Science Foundation. She coordinated a structural project jointly funded by the EU and Croatia (IPA 2007/ HR /16IPO/001-040511) and a project on the development of human potential in plant medicine (ESF project). She coordinates an Erasmus capacity building project that is currently developing a PhD study programme in Plant Health. Currently she is participating in two ERASMUS + Strategic Partnership projects focused on entrepreneurial skills development and innovative education methods for organic agriculture. Her research focuses on applied entomology, integrated pest management, crop protection and phytopharmacy. Under her supervision, six students have completed their dissertations and she is currently supervising five PhD students.

She is Editor in-chief of the Journal of Plant Protection and a member of the editorial boards of Agriculture by MDPI, Fragmenta Phytomedica by Croatian Plant Protection Society, and Nature. She received a medal from the Faculty of Agriculture at the University of Zagreb in 2014 for her scientific and professional achievements and the National Science Award of the Croatian Parliament - Annual Award for the Transfer of Scientific Results into practise in 2019. Since 1993, she has conducted research in the field of on integrated control of Colorado potato beetle, wireworms, sugar beet pests, oilseed rape pests, western corn rootworm and other maize pests. Her publications include more than 150 peer-reviewed journal articles and more than 180 miscellaneous (Web of Science Index Expanded - SCI - Expanded, 67 articles, total citations: 564, h-index: 14; Scopus, 74 articles, total citations: 637, h-index: 14). List of her publications is available at: https://www.researchgate.net/profile/Renata_Baok/contributions.

Her current research interests include integrated pest management (IPM) in field crops (maize, sugar beet, potato) and insect resistance development. The general research focus is on the development of safe, effective, and economical methods IPM and the biological/ecological interactions between insect species and their environment. She is a member of the initiative COST TOP-AGRI -Network "Towards zero pesticide agriculture: European network for sustainability".

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Investment in future

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"*In order to have faith in his own path, he does not need to prove that someone else's path is wrong*." ― Paulo Coelho

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„Da bi vjerovao u svoj vlastiti put, nema potrebe da dokazuješ kako je tuđi put pogrešan.“ – Paulo Coelho

Summary

Sugar beet (*Beta vulgaris* var. *saccharifera* L.) is a profitable industrial crop, but also one of the most demanding, considering the production technology and the growing period of almost 180 days. Sugar beet is attacked by numerous pests such as wireworms, sugar beet flea beetles, aphids, and others, which significantly reduce yield, root quality and sugar content. In the last 20 years, sugar beet protection has been successfully carried out using the method of seed treatment with neonicotinoids (imidacloprid and thiamethoxam). Because they are suspected of having negative effects on bee colonies and other beneficial organisms, the EC regulation prohibits their use starting in 2019, except in permanent greenhouses or with special permission. Among the most important beneficial soil fauna of sugar beets are ground beetles and earthworms, which have an indirect positive impact on the crop by increasing soil fertility, regulating the water-air ratio or reducing the number of pests. Due to the ban on neonicotinoids, sugar beet production has halved compared to previous years, amounting to only 10,000 hectares.

The environmental assessment of plant protection products for soil organisms is mainly based on the results of laboratory and extended laboratory studies, while the link from the laboratory to realistic field conditions over several seasons is not well established. Despite the ban, the degradation dynamics of neonicotinoids in plants sown from treated seeds and their effects on these plants and the surrounding soil are still not well understood. Moreover, the residues of neonicotinoids and their bioaccumulation in beneficial soil fauna in Croatia have not yet been determined, nor has their impact on the numbers and composition of these organisms. Therefore, the objectives of this research were: (i) to determine the efficacy of neonicotinoids on the main pests and the degradation dynamics in sugar beet plants grown from seeds treated with imidacloprid and thiamethoxam under different weather conditions; (ii) determine the residues of neonicotinoids in ground beetles, earthworms, and soil of sugar beet fields; and (iii) determine the cenological composition of ground beetles and the possibility of population recovery in sugar beet fields and fields included in a four-year crop rotation.

Monitoring of neonicotinoid efficacy against major pests and degradation dynamics was conducted over two years (2015 and 2016) in Virovitica - Podravina (Lukač) and Vukovar - Syrmium (Tovarnik) counties and under laboratory conditions. Samples were collected using standard protocols, including pitfall traps for ground beetles and ISO - ISO 23611-1:2006 methodology for earthworm sampling. Residue analysis was performed using the LC-MS / MS SPE - QuEChERS method with LOQ of 0.01 mg/kg for plant and soil samples and 0.001 mg/kg for animal samples. A biocenological-synecological analysis of ground beetles was performed to determine the ecological indices of the population, and based on the calculated dominance, the represented species were classified according to Tischler and Haydeman. Collected samples were determined to species using standard identification keys. Results were statistically analyzed using ANOVA.

Results show that insecticide treatment of sugar beet seed leaves minimal residues in plants and is completely degraded by the end of the growing season. Elevated concentrations of residues in the soil indicate that in dry climates or after a dry period, there is a risk to crops that follow sugar beets in the rotation. All neonicotinoid residues detected in beneficial organisms were below levels considered lethal throughout the sampling period, so it can be assumed that insecticides do not accumulate in these organisms. Calculation of the bioconcentration factor using the retrospective analysis method of analytically measured neonicotinoid residues in the samples indicates that there is no risk to earthworms and no potential for secondary poisoning in birds and mammals that feed on them. The composition and abundance of ground beetles in sugar beet fields is strongly influenced by several factors during the growing season, with insecticides having negative effects, while reduced tillage, lower temperatures, and more rainfall lead to higher ground beetle abundance and diversity. Growing sugar beets in a four-year rotation provides a recovery in the ground beetle population. The research also resulted in a comprehensive list of 64 ground beetle determined to species in maize, sugar beets, wheat, and soybeans, and represents a valuable finding that complements previous studies in Croatia. A better understanding of ground beetles in intensive agricultural landscapes is a good starting point for conservation programs that have become standard in the European Union.

Keywords: accumulation, bioconcentration, cenological analysis, degradation, earthworms, efficiency, ground beetles, neonicotinoids, pests, sugar beet, treated seeds.

Prošireni sažetak (Extended summary in Croatian):

Naslov doktorske disertacije na hrvatskom jeziku (title of the doctoral thesis in Croatian):

Dinamika razgradnje neonikotinoida primijenjenih tretiranjem sjemena šećerne repe i učinak na štetnu i korisnu faunu

S obzirom na tehnologiju proizvodnje i duljinu vegetacije od gotovo 180 dana, šećerna repa smatra se najzahtjevnijom poljoprivrednom kulturom (Pospišil, 2013; Kristek, 2015). Tijekom vegetacije izložena je napadu brojnih štetnika koji smanjuju prinos, kvalitetu korijena i sadržaj šećera. Tehnologija proizvodnje šećerne repe obuhvaća velik broj zahvata te intenzivno suzbijanja korova i štetnika, što negativno djeluje na korisne organizme u tlu (Bažok i sur., 2015). Zaštita šećerne repe od štetnih kukaca posljednjih se dvadesetak godina učinkovito provodila tretiranjem sjemena insekticidima iz skupine neonikotinoida u slučaju žičnjaka, buhača i lisnih uši (Dobrinčić, 2002; Bažok, 2010). Folijarna primjena insekticida usmjerena je na suzbijanje repine pipe (Bažok i sur., 2012). Prema Castle i sur. (2005) te Byrne i Toscano (2006), koncentracija imidakloprida između 0,005 i 0,01 mg/kg u biljnom tkivu osigurava učinkovitu zaštitu od štetnika, što se postiže upravo tretmanom sjemena.

Iako su od sredine 1990-ih do danas neonikotinoidi bili najčešće korišteni insekticidi na svijetu, zbog sumnje na negativan utjecaj na pčelinje zajednice, Uredbom Europske komisije (EU) 485/2013 zabranjeno je korištenje tiametoksama, imidakloprida i klotianidina na većini ratarskih kultura. Zabrana nije obuhvaćala šećernu repu jer nije smatrana atraktivnom pčelama. Konačna odluka o zabrani donesena je 27. travnja 2018. (Bažok i Lemić, 2018), a temeljila se na svim relevantnim istraživanjima diljem svijeta (EFSA, 2018a, 2018b, 2018c). Odluka se počela primjenjivati od 2019. u većini država članica EU-a. Iako su neonikotinoidi danas zabranjeni u Europi i Ujedinjenom Kraljevstvu, još se uvijek uz posebne dozvole mogu koristiti za zaštitu pojedinih usjeva (Harrison-Dunn, 2021). Uklanjanjem neonikotinoida iz primjene istodobno se povećala primjena drugih insekticida, najčešće piretroida (Kathage i sur., 2017).

Osim štetnika, u šećernoj repi javljaju se i brojni pripadnici korisne faune s neizravno pozitivnim utjecajem na kultivirane biljke. Najvažnija korisna fauna tla šećerne repe uključuje kukce, osobito red *Carabidae*, odnosno trčke (Kos i Bažok, 2015), i gujavice (Pisa i sur., 2014). Zbog velike brojnosti, poznate taksonomije i osjetljivosti na promjene uzrokovane vanjskim čimbenicima trčci se često koriste u ekološkim istraživanjima (Lövei i Sunderland, 1996). Smanjenje populacije trčaka na nekom području posljedica je većeg unosa agrokemikalija, gubitka travnih pojaseva za ishranu i povećavanja veličine parcela (Fahrig i sur., 2015). Sastav faune trčaka te dinamika njihove pojave u Hrvatskoj nisu poznati iako se često navodi da su insekticidi glavni čimbenik smanjenja njihove brojnosti. Gujavice ili humifikatori važni su članovi faune tla (Luo i sur., 1999). Sudjeluju u fragmentaciji, razgradnji i inkorporaciji organske tvari (Edwards i Bohlen, 1996). U poljoprivrednim tlima čine do 80 % ukupne životinjske biomase (Luo i sur., 1999). Sredstva za zaštitu bilja predstavljaju opasnost za preživljavanje i ponašanje gujavica ometajući razvoj i procese razgradnje tla (Volkov i sur., 2007).

Istraživanje se provodilo tijekom dviju godina (2015. i 2016.) u Virovitičko-podravskoj županiji (Lukač) i Vukovarsko-srijemskoj županiji (Tovarnik) te u laboratorijskim uvjetima na Zavodu za poljoprivrednu zoologiju Agronomskog fakulteta Sveučilišta u Zagrebu. Podaci o vremenskim uvjetima prikupljeni su iz Državnog hidrometeorološkog zavoda. Na oba lokaliteta u obje godine šećerna repa bila je posijana na 3,000 m^2. Varijante su uključivale netretirano (NT) sjeme šećerne repe, sjeme tretirano imidaklopridom (IMI) i tiametoksamom (TMX). Svaka varijanta bilja zasijana je na 1,000 m^2. U laboratorijskim uvjetima sjeme šećerne repe posijano je u supstrat u plastične posude zapremnine 130 l. Osim polja šećerne repe na kojima su zasijani pokusi, na svakom od lokaliteta odabrana su još tri polja na kojima je šećerna repa bila zasijana pred jednu, dvije ili tri godine s ciljem prikupljanja uzoraka faune te kako bi se utvrdili brojnost i sastav faune trčaka i utjecaj intenzivnog uzgoja šećerne repe na navedene organizme.

Štetnici i pripadnici korisne faune tla praćeni su i prikupljani standardnim metodama, a za svakog štetnika na temelju frekvencije biljaka u grupama izračunat je postotak (%) štete (Townsend i Heuberger, 1943). Količina rezidua neonikotinoida analizirana je u: (i) biljkama šećerne repe – 432 uzoraka; (ii) trčcima – 14 uzoraka; (iii) gujavicama – 58 uzoraka; (iv) tlu –18 uzoraka. Određivanje rezidua neonikotinoida provedeno je metodom tekućinske kromatografije / tandemskom spektrometrijom mase (LC-MS/MS) nakon ekstrakcije / razdiobe acetonitrilom i čišćenja disperzivnom SPE – QuEChERS metodom (EN 15662:2008). Na istim lokalitetima i poljima prikupljani su uzorci trčaka za cenološku analizu. Prvi set uzoraka trčaka prikupljen je 2015. U epigejskim lovkama prikupljene su 2,582 jedinke, a u endogejskim lovkama 323 jedinke trčaka. Drugi set uzoraka trčaka prikupljen je 2016. s obje lokacije na četiri polja po lokalitetu (četverogodišnji plodored): polje šećerne repe, polje na kojem je repa bila uzgajana godinu dana prije (2015.), dvije godine prije (2014.) i tri godine prije (2013.). Ukupno je prikupljeno 11,763 jedinki trčaka.

Svi podaci podvrgnuti su analizi varijance (ANOVA).

Rezultati istraživanja učinkovitosti tretiranog sjemena imidaklopridom i tiametoksamom na najvažnije štetnike šećerne repe pokazali su zadovoljavajuću zaštitu od žičnjaka, buhača i repine pipe pri niskom populacijskom pritisku. Gusjenice i lisne uši bile su prisutne u nižoj brojnosti pa se ne može sa sigurnošću utvrditi učinkovitost istraživanih insekticida. Učinkovitost se može očekivati u trajanju od oko sedam tjedana nakon sjetve, što je u skladu s dinamikom razgradnje.

Rezultati istraživanja degradacije neonikotinoida u biljkama šećerne repe pokazali su rezidue IMI ispod tolerance (MRL) koja iznosi 0,5 mg/kg 40 – 50 dana nakon sjetve. Analiza razgradnje TMX u polju utvrdila je rezidue ispod MRL (0,02 mg/kg) 70 – 80 dana nakon sjetve. Razgradnja u kontroliranim uvjetima znatno je sporija te su u 2015. rezidue TMX u korijenu repe bile na razini MRL (0,053 mg/kg), dok je iduće godine koncentracija bila ispod MRL u istom vremenu analiziranja. U vrijeme vađenja korijena šećerne repe (180 dana nakon sadnje) rezidue su bile ispod MRL i uvelike su ovisile o vremenskim uvjetima, posebice o količini oborina. Povišena koncentracija rezidua u tlu pokazuje da postoji rizik u suhim klimatskim uvjetima ili nakon sušnog razdoblja, no potrebna su daljnja istraživanja da bi se procijenio mogući unos neonikotinoida u kulture koje dolaze u plodoredu nakon uzgoja šećerne repe iz tretiranog sjemena.

Istraživanjem rezidua neonikotinoida u korisnim organizmima utvrđena je koncentracija IMI od 0,027 mg/kg u trčcima, dok su rezidue TMX bile ispod limita kvanitfikacije (LOQ) koji u slučaju životinjskih uzoraka iznosi 0,001 mg/kg. Najviša utvrđena koncentracija IMI u gujavicama iznosila je 0,2141 mg/kg, dok rezidue TMX nisu prelazile 0,0008 mg/kg. Sve utvrđeni rezidue neonikotinoida bile su niže od razina navedenih kao letalnih u cijelom razdoblju uzorkovanja, tako da se može pretpostaviti da ne dolazi do akumulacije insekticida u tim organizmima.

Za izračun krivulja razgradnje pesticida u uzorcima gujavica i izmjerene koncentracije u tlu korišteni su parametri razgradnje sredstava za zaštitu bilja (DT_{50}, DT_{90}). Potom su određeni faktori biokoncentracije specifični za spojeve u tlu dijeljenjem analiziranih rezidua pesticida u gujavicama s izračunatim koncentracijama u tlu. Krivulje disipacije na polju (temeljene na EU, EC i EFSA) razumno predviđaju koncentraciju rezidua aktivnih sastojaka u tlu u bilo kojem trenutku nakon primjene. Stoga se analitički utvrđene rezidue u gujavicama s područja Lukača i Tovarika mogu pouzdano koristiti za izračun faktora biokoncentracije. Rezultati su pokazali da primjena istraživanih djelatnih tvari ne predstavlja rizik biokoncentracije za gujavice i nema potencijala sekundarnog trovanja za ptice i sisavce koji se njima hrane.

Praćenjem populacije trčaka na istraživanim lokacijama utvrđeni su čimbenici koji utječu na aktivnost i njihovu brojnost. Tijekom 2015. utvrđen je utjecaj specifičnosti okoliša (tip i struktura tla, klimatski uvjeti) zajedno s mjerama uzgoja (obrada tla i primjena insekticida) na aktivnost i brojnost trčaka. S usjeva pšenice na području u Virovitičko-podravske županije determinirano je 26 vrsta i 15 rodova prikupljenih epigejskim (357) i endogejskim lovkama (59). Na navedenom polju šećerna repa bila je uzgajanja prije tri godine Praćenjem dinamike populacije trčaka utvrđeno je da porast populacije prati pad temperature zraka i tla, dok povećanje količine oborina utječe na smanjenje brojnosti trčaka.

Tijekom istraživanja 2016. prikupljene su i determinirane 64 vrste trčaka koje pripadaju u 33 roda. U Vukovarsko-srijemskoj županiji u razdoblju od 20 tjedana uzorkovanja prikupljene su ukupno 2,382 jedinke trčaka (25 vrsta) od kojih po brojnosti najviše na poljima šećerne repe (1,131), pšenice (656) i kukuruza (342), a najmanje u soji (253). U Virovitičko-podravskoj županiji prikupljena je ukupno 9,381 jedinka trčaka (56 vrsta), od kojih značajno

najviše u kukuruzu (5,656), što još jednom potvrđuje oporavak faune trčaka u višegodišnjem polodoredu. Na istom polju šećerna repa uzgajana je prije četiri godine. U preostalim kulturama utvrđen je broj jedinki kako slijedi: u soji (1,471), šećernoj repi (1,250) i potom pšenici (1,004). Na obje istraživane lokacije karakteristična je eudominantnost pojedinih rodova s velikim brojem pripadnika pojedine vrste. Cenološke analize pokazale su da su sastav i brojnost trčaka u poljima šećerne repe pod jakim utjecajem brojnih čimbenika tijekom vegetacije. Korištenje insekticida nepovoljno utječe na populaciju trčaka, dok smanjena obrada tla, niže temperature i više oborina rezultiraju njihovom većom brojnosti i raznolikosti. Uzgoj šećerne repe u četverogodišnjem plodoredu omogućuje oporavak populacije trčaka. Sveobuhvatan popis od 64 determinirane vrste trčaka iz usjeva kukuruza, šećerne repe, pšenice i soje u Hrvatskoj predstavlja vrijedan nalaz koji nadopunjuje prethodna istraživanja i značajno pridonosi boljem razumijevanju osnovnog stanja populacija trčaka u intenzivnim poljoprivrednim krajobrazima.

Ključne riječi: akumulacija, biokoncentracija, cenološka analiza, degradacija, gujavice, neonikotinoidi, trčci, tretirano sjeme, učinkovitost, šećerna repa, štetnici

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List of abbervation

A.i.	*Active ingredient*
ADI	*Acceptable Daily Intake*
AERU	*Agriculture & Environment Research Unit*
ANOVA	*Analysis of variance*
AOAC	*Association of Official Agricultural Chemists*
APPRRR	*Agencija za plaćanja u poljoprivredi, ribarstvu i ruralnom razvoju*
BBCH	*Biologische Bundesanstalt, Bundessortenamt and Chemical industry - scale for assesment of phenological development stages of plants*
BCF	*Bioconcentration factors*
BCTV	*Beet curly top virus*
BNYVV	*Beet necrotic yellow vein virus*
BYV	*Beet yellows virus*
CABI	*Centre for Agriculture and Bioscience International*
CBS	*Croatian Bureau of Statistics*
CCD	*Colony collapse disorder*
CCPR	*Codex Committee on Pesticide Residues*
CEN	*European Committee for Standardization*
DLLME	*Dispersive Liquid-Liquid Microextraction method*
DM	*Dry matter*
DT_{50}	*Degradation time for 50% of a compound*
DT_{90}	*Degradation time for 90% of a compound*
DZS	*Državnoi zavod za statistiku*
EC	*European Commission*
EFSA	*European Food Safety Authority*
EU	*European Union*
FAO	*Food and Agriculture Organization*
FIS Web Portal	*Website of the Ministry of Agriculture with updated list of registered plant protection products*
GAP	*Good Agricultural Practice*
H	*Shannon index*
HPLC	*High-performance liquid chromatography or high-pressure liquid chromatography*
HSD	*Honestly Significant Difference*
IMI	*Imidacloprid*
IPCS INCHEM	*International Programme on Chemical Safety*

IPM	*Integrated Pest Management*
ISO	*The International Organization for Standardization*
Koc	*Organic carbon-water partition co-efficient*
LAI	*Leaf area index*
LC_{50}	*50% Lethal Concentration*
LC-MS/MS	*Liquid Chromatography-Tandem Mass Spectrometry*
LOD	*Limit of detection*
logPow	*Logarithm of the octanol-water partition*
LOQ	*Limit of Quantification*
MRL	*Maximum Residue Limits*
MS	*Mass spectrometry*
nAChR	*Nicotinic acetylcholine receptors*
NOAEL	*No-Observed-Adverse-Effect-Level*
NOEC	*No-Observed-Effect-Concentrations*
NT	*Untreated seed (hrv. netretirano sjeme šećerne repe)*
PAAFRD	*Paying Agency for Agriculture, Fisheries and Rural Development*
PPDB	*Pesticide Properties Database*
QS	*Sörensen coefficient (hrv. Sörensen koeficijent)*
QuEChERS	*Quick, Easy, Cheap, Effective, Rugged and Safe method*
PPP's	*Plant Protection Products*
Reclict class E	*Eurytopic species*
Relict class A	*Adaptive species*
Relict class R	*Rare and endangered species*
SPE	*Solid Phase Extraction method*
SPE (d-SPE)	*Solid-Liquid Extraction Salting Out and Dispersion method*
SPME	*Solid Phase Microextraction method*
TDI	*Tolerable Daily Intake*
TER	*Toxicity Exposure Ratio*
TMX	*Thiamethoxam*
UNE	*Botanical essence including synthetic, extracts and unrefined oils with unknown or uncertain MoA*
US EPA	*United States Environmental Protection Agency*
USA	*United States of America*
W	*Ecological significance (hrv. indeks ekološke signifikantnosti)*
WHO	*World Health Organization*

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List of publications included in the doctoral dissertation:

Publication No. 1

Viric Gasparic, H., Lemic, D., Drmic, Z., Cacija, M., Bazok, R. (2021). The Efficacy of Seed Treatments on Major Sugar Beet Pests: Possible Consequences of the Recent Neonicotinoid Ban. *Agronomy*, **11** *(7)*, 1277. https://doi.org/10.3390/agronomy11071277

Publication No. 2

Viric Gasparic H., Grubelic, M., Dragovic Uzelac, V., Bazok, R., Cacija, M., Drmic, Z., Lemic, D. (2020). Neonicotinoid residues in Suger Beet plants and Soil under Different Agro-Climatic Conditions. *Agriculture,* **10** *(10)*, 484. https://doi.org/10.3390/agriculture10100484

Publication No. 3

Viric Gasparic, H., Lemic, D., Bazok, R. (2022). Neonicotinoid Residues in Earthworms and Ground Beetles under Intensive Sugar Beet Production: Preliminary Study in Croatia. *Agronomy*, **12** *(9)*, 2102. https://doi.org/10.3390/agronomy12092102

Publication No. 4

Schmidt, T., Kimmel, S., Hoeger S., Lemic D., Bazok, R., **Viric Gasparic, H.** (2022). Plant protection products in agricultural fields - residues in earthworms and assessment of potentially toxic effects to the environment. *Journal of Central European Agriculture,* **23** *(3),* 604 - 614, https://doi.org/10.5513/JCEA01/23.3.3625

Publication No. 5

Virić Gašparić, H., Drmić, Z., Čačija, M., Graša, Ž., Petrak, I., Bažok, R., Lemic, D. (2017). Impact of environmental conditions and agro-technical factors on ground beetle populations in arable crops. *Applied Ecology and Environmental Research*, **15** *(3)*, 697 - 711. http://dx.doi.org/10.15666/aeer/1503_697711

Publication No. 6

Lemic, D., Čačija, M., **Virić Gašparić, H.**, Drmić, Z., Bažok, R., Pajač Živković, I. (2017). The ground beetle (Coleoptera: Carabidae) community in an intensively managed agricultural landscape. *Applied Ecology and Environmental Research*, **15** *(4)*, 661 - 674. http://dx.doi.org/10.15666/aeer/1504_661674

Publication No. 7

Viric Gasparic, H., Göldel, B., Lemic, D., Pajac Zivkovic, I., Bazok, R. (2022). Carabids as indicators of sustainability in arable crops. *Applied Ecology and Environmental Research,* **20** *(6),* 4645 - 4665. http://dx.doi.org/10.15666/aeer/2006_46454665

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Scientific paper Znanstveni rad	**Base** Baza	**Category** Kategorija	**Quartile** Kvartil	**Impact factor** Faktor odjeka
Virić Gašparić, H., Lemic, D., Drmić, Z., Čačija, M., Bažok, R. (2021) The Efficacy of Seed Treatments on Major Sugar Beet Pests: Possible Consequences of the Recent Neonicotinoid Ban. Agronomy, 11 (7), 1277. https://doi.org/10.3390/agronomy11071277	WoS	A1	Q1	3.949
Virić Gašparić, H., Grubelić, M., Dragović-Uzelac, V., Bažok, R., Čačija, M., Drmić, Z., Lemic, D. (2020) Neonicotinoid residues in Suger Beet plants and Soil under Different Agro-Climatic Conditions. Agriculture 10 (10), 484. https://doi.org/10.3390/agriculture10100484	WoS	A1	Q1	3.408
Virić Gašparić, H., Lemic, D., Bažok, R. (2022). Neonicotinoid Residues in Earthworms and Ground Beetles under Intensive Sugar Beet Production: Preliminary Study in Croatia. Agronomy, 12 (9), 2102. https://doi.org/10.3390/agronomy12092102	WoS	A1	Q1	3.949
Schmidt, T., Kimmel, S., Hoeger S., Lemic D., Bazok, R., Viric Gasparic, H. (2022). Plant protection products in agricultural fields – residues in earthworms and assessment of potentially toxic effects to the environment. Journal of Central European Agriculture, 23 (3), 604 – 614, https://doi.org/10.5513/JCEA01/23.3.3625	WoS	A1	Q4	-
Virić Gašparić, H., Drmić, Z., Čačija, M., Graša, Ž., Petrak, I., Bažok, R., Lemic, D. (2017). Impact of environmental conditions and agro-technical factors on ground beetle populations in arable crops. Applied Ecology and Environmental Research. 15 (3), 697–711. http://dx.doi.org/10.15666/aeer/1503_697711	WoS	A1	Q4	0.860
Lemic, D., Čačija, M., Virić Gašparić, H., Drmić, Z., Bažok, R., Pajač Živković, I. (2017). The ground beetle (Coleoptera: Carabidae) community in an intensively managed agricultural landscape. Applied Ecology and Environmental Research. 15 (4), 661–674. http://dx.doi.org/10.15666/aeer/1504_661674	WoS	A1	Q4	0.860
Viric Gasparic, H., Göldel, B., Lemic, D., Pajac Zivkovic, I., Bazok, R. (2022). Carabids as indicators of sustainability in arable crops. Applied Ecology and Environmental Research, 20 (6), 4645–4665. http://dx.doi.org/10.15666/aeer/2006_46454665	WoS	A1	Q4	0.860

Explanation (in Croatian) of the relationship between research hypotheses and published papers and papers in the publication process.

Obrazloženje povezanosti istraživačkih hipoteza i objavljenih radova i radova u postupku objave

Istraživačka hipoteza	Obrazloženje povezanosti hipoteze sa znanstvenim radom
H1. Tretiranjem sjemena šećerne repe s IMI i TMX postići će se, ovisno o vremenskim uvjetima, učinkovita zaštita od štetnika do šest tjedana nakon sjetve za koje se vrijeme ostatci u biljkama razgrade ispod limita detekcije.	Rezultati učinkovitosti tretiranja sjemena imidaklopridom i tiametoksamom šećerne repe prikazani su znanstvenim radom pod rednim brojem **1**. Tijekom istraživanja praćen je napad i štete najvažnijih štetnika na pokusnim poljima u Lukaču i Tovarniku tjedno kroz dvije vegetacijske sezone na unaprijed odabrana 4 reda svake od tri varijante uključene u pokus (površina očitavanja bila je 10 m^2). Na ovim redovima brojanjem sklopa biljaka utvrđene su štete od žičnjaka, očitavane štete od buhača, repine pipe, lisnih uši i gusjenica. Pregledane biljke razvrstane su temeljem utvrđenih oštećenja. U okviru rezultata **potvrđena je** postavljena **hipoteza 1** da su, u standardnim uzgojim uvjetima koji prevladavaju u RH, postignuti zadovoljavajući uvjeti zaštite mladih biljaka šećerne repe. Djelovanje insekticida sukladno je dinamici razgradnje, može se očekivati zadovoljavajuće djelovanje 50-ak dana nakon sjetve na najvažnije štetnike (žičnjake, repine buhače i repine pipe u slučaju slabije zaraze) koji se uobičajeno javljaju, dok je napad gusjenica i lisnih uši bio prenizak za pouzdanu procjenu. Rezultatima prikazanim u znanstvenom radu pod rednim bojem **2** prikazan je tijek degradacije istraživanih djelatnih tvari insekticida u biljkama. U malim biljkama, 25 do 27 dana nakon sjetve, utvrđena je stopa oporavka od 0,028 % za imidakloprid i 0,077 % za tiametoksam tijekom prve godine istraživanja, dok je u drugoj godini istraživanja stopa iznosila 0,003 % za imidakloprid (40 dana nakon sjetve) i 0,022 % za tiametoksam (50 dana nakon sjetve). Nisu utvrđene rezidue neonikotinoida iznad maksimalne razine rezidua u korijenju u vrijeme vađenja korijenja. Propisani MRL za imidakloprid u korijenju šećerne repe iznosi 0,5 mg/kg (EU 491/2014), a za tiametoksam 0,02 mg/kg (EU 2017/671). Na pokusima u polju, u Tovarniku i Lukaču rezidue imidakloprida ispod propisane tolerance utvrđene su 40-50 dana nakon sjetve. Na pokusu u kontroliranim uvjetima imidakloprid se razgrađivao sporije te su se rezidue u listu smanjile ispod tolerance 60-ak dana nakon sjetve. Istovremeno su se insekticidi u korijenu uzgajanih biljaka u kontroliranim uvjetima sporije razgrađivali te su se smanjili ispod tolerance 80-ak dana nakon sjetve. Dinamika razgradnje tiametoksama u uvjetima u polju pokazuje također da su se rezidue tiametoksama smanjile ispod tolerance (0,05 mg/kg) 60-80 dana nakon sjetve. Razgradnja u kontroliranim uvjetima je znatno sporija te su u 2015. rezidue tiametoksama u korijenu repe bile na razini tolerance. Rezultati ovog istraživanja upućuju na zaključak da tretiranje sjemena šećerne repe ostavlja minimalne rezidue u biljkama zbog potpune razgradnje u poljskim uvjetima, zbog čega se **hipoteza 1** prihvaća. Međutim, treba napomenuti da na temelju rezultata laboratorijskog istraživanja, uvjeti suše, nemogućnost ispiranja u dublje slojeve tla mogu rezultirati višim koncentracijama neonikotinoida u tlu što može predstavljati potencijalni rizik za usjeve koji slijede u plodoredu.

H2. Pretpostavlja se da će provedena mjera rezultirati smanjenjem brojnosti trčaka i pojavom rezidua u trčcima, gujavicama i tlu. Sjetvom šećerne repe u četverogodišnjem plodoredu omogućiti će se oporavak faune trčaka.	Znanstvenim radom pod rednim brojem **3** utvrđene su rezidue neonikotinoida u korisnim organizmima. Koncentracija imidakloprida iznosila je 0,027 mg/kg u trčcima, dok su rezidue tiametoksama i klotianidina bile ispod LOQ koji u slučaju životinjskih uzoraka iznosi 0,001mg/kg. Najviša utvrđena koncentracija imidaklooprida u gujavicama iznosila je 0,2141 mg/kg, dok rezidue tiametoksama nisu prelazile 0,0008 mg/kg. Sve utvrđeni rezidue neonikotinoida bile su niže od razina navedenih kao letalnih u cijelom razdoblju uzorkovanja, tako da se može zaključiti da ne postoji akumulacija insekticida u tim organizmima. Znanstvenim radom pod rednim brojem **4** uzorci gujavica prikupljeni su tijekom dvije vegetacijske sezone s osam polja u Hrvatskoj i analizirani na 300 djelatnih tvari. Koncentracije utvrđenih 26 djelatnih tvari bile su prosječno 0,005 mg/kg svježe mase gujavica. Ispod granice detekcije (LOD = ½ LOQ) bilo je 33 % uzoraka, ispod granice kvantifikacije (LOQ = 0,001 mg/kg) bilo je 44 % uzoraka, a iznad LOQ bio je 23 % uzoraka. Na temelju javno dostupnih nacrta izvješća o procjeni EC i EFSA, parametri razgradnje (DT_{50}, DT_{90}) korišteni su za izračun krivulja razgradnje i trenutne koncentracije u tlu na datum uzorkovanja gujavica. Potom su utvrđeni faktori biokoncentracije specifični za tvar u tlu dijeljenjem analiziranih rezidua pesticida u gujavicama s izračunatim koncentracijama u tlu. Primjenjivost i pouzdanost ove metode provjerene su i rezultirale su zaključcima da su faktori biokoncentracije izračunati u ovom istraživanju usporedivi s objavljenim faktorima biokoncentracije; rekonstruirane koncentracije u tlu prikladne su za procjenu rizika potencijalno toksičnih učinaka pojedinačnih aktivnih sastojaka kao i mješavina aktivnih tvari s istim načinom djelovanja. Većina aktivnih tvari ne predstavlja rizik za gujavice i nema potencijal sekundarnog trovanja za ptice i sisavce koji se njima hrane. Znanstveni rad pod rednim brojem **5** daje pregled rezultata vezanih na specifičnosti okoliša (tip i struktura tla, klimatski uvjeti) zajedno s mjerama uzgoja (obrada tla i primjena insekticida) koje utječu na aktivnost i brojnost korisne faune tla odnosno trčaka. Istraživanje je provedeno na istim lokacijama opisanima za H1 (Lukač, Virovitičko-podravska županija i Tovarnik, Vukovarsko-srijemska županija). Uzorci su skupljani jednom tjedno, od svibnja do rujna 2015., epigejskim i endogejskim lovkama na poljima zasijanih tipičnim ratarskim kulturama za ova područja. Epigejskim lovkama sakupljene su ukupno 2,582 jedinke trčaka, a endogejskim lovkama 323 jedinke. Ulovi u Tovarniku su bili znatno niži nego u Lukaču. Iako je u Tovarniku zabilježen veći broj trčaka u usjevu pšenice nije utvrđena statistički značajna razlika između polja. Utvrđena je korelacija između biljnog pokrova i ulova trčaka. Veći ulov trčaka zabilježen je u ozimim usjevima koji su posijani u jesen prethodne godine, u usporedbi sa šećernom repom i kukuruzom koji su posijani u proljeće nakon dugog perioda golog tla. Utvrđeno je da se brojnost trčaka povećala u godinama nakon uzgoja šećerne repe, odnosno u četverogodišnjem plodoredu. Možemo zaključiti da uzgoj šećerne repe zbog intenzivne obrade i učestale primjene insekticida ima najveći negativan utjecaj na populaciju trčaka. Znanstveni rad pod rednim brojem **6** daje detaljan pregled cenološke analize populacije trčaka prikupljenih 2015. u usjevu pšenice na području u Virovitičko Podravske županije. Ukupno je determinirano 1,429 jedinki razvrstanih u 26 vrsta i 15 rodova. Najbrojnije i eudominantne vrste su *Poecilus cupreus* Linnaeus, *Brachinus psophia* Audinet-Serville i

	Pterostichus melas melas Creutzer. Većina ulovljenih vrsta je klasificirana kao euritopna, tj. sposobna nastanjivati krajolike pod jakim antropogenim utjecajem. Ovo istraživanje doprinijelo je detaljnom razumijevanju zajednice trčaka u specifičnom poljoprivrednom području sjeverozapadne Hrvatske. Znanstveni rad pod rednim brojem **7** prikazuje detaljnu cenološke analizu populacija trčaka prikupljenih 2016. s područja Virovitičko-podravske i Vukovsko-srijemske županije koje se razlikuju po klimatskim uvjetima i obradi tla. Uzorci su prikupljeni s polja kukuruza, pšenice, šećerne repe i soje. Prikupljeno je 11,763 jedinki determiniranih do vrste (ukupno determinirano 64 vrste trčaka). Vrste su klasificirane prema Katalogu palearktičkih Coleoptera. Biocenološka analiza po usjevima u Vukovarsko-srijemskoj i Virovitičko-podravskoj županiji utvrdila je da su *H. rufipes, P. melas, P. melanarius melanarius i P. cupreus cuperus* najzastupljenije vrste u proučavanim usjevima. Ulovi u Virovitičko-podravskoj županiji bili su znatno veći od ulova u Vukovarsko-srijemskoj županiji. Kukuruz je u usporedbi s ostalim usjevima ima najveću brojnost trčaka. Najveći ulovi zabilježeni su u rujnu, dok je u srpnju ulov znatno manji uslijed ljetnih vrućina i suše. Na ulove su utjecali mjesto lokacije, usjev i period uzorkovanja, što dokazuje značajno različitu brojnost trčaka na područjima uključenima u istraživanje. U modernoj poljoprivredi EU, konverzacijski programi usmjereni su na očuvanje korisnih vrsta i bioraznolikosti te se promoviraju kao alat za osiguranje održivosti. Rezultati ovog istraživanja značajno su pridonijeli boljem razumijevanju početne situacije o populaciji trčaka, indikatora bioraznolikosti, u intenzivnom poljoprivrednom krajoliku sjeverozapadne i istočne Hrvatske i predstavljaju dobru polaznu točku za buduće konverzacijske programe. Također, ovo istraživanje daje važan doprinos sveukupnom poznavanju faune trčaka s opsežnim popisom vrsta koje se nalaze u usjevima kukuruza, šećerne repe, pšenice i soje u Hrvatskoj. Temeljem gore opisanih znanstvenih radova utvrđeno je da usjev i povijest uzgoja utječu na smanjenje brojnost korisne faune tla modifikacijom okolišnih uvjeta okoliša (karakteristike tla, mikroklimatski čimbenici kao što su temperatura i vlažnost), kao i kroz faktore poremećaja kao što su rasporedi obrade tla i rasporedi žetve/sjetve. Sjetva tretiranog sjemena rezultirala je reziduama u korisnim organizmima, trčcima i gujavicama, ali u koncentracijama manjima od letalnih. Nakon uzgoja šećerne repe u četverogodišnjem plodoredu, utvrđen je oporavak faune trčaka. Na temelju prikazanog provedenog istraživanja i analize rezultata **hipoteza 2** se prihvaća.

1. General introduction

According to the Croatian Bureau of Statistics in 2021 sugar beet was grown in Croatia on about 10 thousand hectares, from which 717 thousand tons of raw material were obtained, corresponding to an average yield of 70 tons per hectare (CBS, 2021). State support was provided to 476 farms for sugar beet cultivation on 10.451 hectares by the Paying Agency for Agriculture, Fisheries and Rural Development (PAAFRD, 2021). Sugar beet production has decreased significantly compared to previous years. Today's production has almost halved since 2017 sugar beet was cultivated on 19.235 thousand hectares (PAAFRD, 2017). Considering the production technology and vegetation period of almost 180 days, it is considered the most demanding agricultural crop (Pospišil, 2013; Kristek, 2015). During the growing season, it is attacked by numerous pests that significantly reduce yield, sugar content, and root quality. The most common pests that attack sugar beet in the early stages of leaf development or youth stage and have been extremely damaging are wireworms (*Agriotes* spp.), flea beetles (*Chaetocnema tibialis* Ill.), sugar beet weevils (*Bothynoderes punctiventris* Germ., *Tanymecus dilaticollis* Gyll., *Psalidium maxillosum* F., *Otiorhynchus ligustici* L. and noctuid moths (*Agrotis segetum* Schiff., *Agrotis ypsilon* Hubn. and *Euxoa temera* Hb.) (Čamprag, 1983; Bažok et al., 2014b; Čačija, 2015; Drmić, 2015; Drmić and Bažok, 2015). According to Gotlin Čuljak (2015) later in vegetation several species of aphid's attack sugar beet. S*mynthurodes phaseoli* West, *Pemphigus fuscicornis* Koch and *Pemphigus betae* Doane attack roots, while *Aphis fabae* Scopoli and *Myzus persicae* Sulzer are pesent on leafs. Caterpillars of the beet moth (*Scrobipalpa ocellatella* Boyd), cabbage moth and bright-line brown-eye moth (*Mamestra brassicae* L. and *Lacanobia oleracea* L.) as well as silver Y (*Autographa gamma* L.) can also attack sugar beet during vegetation, but these pests are rarely suppressed (Lemić, 2015). Beet cyst nematode (*Heterodera schachtii* Schmidt) is widespread in the growing areas and can present a significant problem if the agro-technique of cultivation is not respected (Grubišić, 2015).

Protection of sugar beets has been carried out for twenty years by treating seeds with insecticides from the group of neonicotinoids. Neonicotinoids are agonists of nicotinic acetylcholine receptors (nAChR) in the central nervous system of insects, to which they bind strongly. At low concentrations, they cause stimulation of the nAChR, and at higher concentrations, their blockade, paralysis, and death occur (Tomizawa and Casida, 2005). Seven active ingredients belong to the group of neonicotinoids: imidacloprid, thiamethoxam, clothianidin, thiacloprid, acetamiprid, nitenpyram, and dinotefuran, of which imidacloprid has the largest market share (41%) (Jeschke et al., 2011). Treatment of seeds with neonicotinoids effectively suppressed wireworms, flea beetles and aphids on sugar beet (Dobrinčić, 2002; Bažok, 2010), so foliar application of insecticides during the growing season is mainly aimed at suppression of sugar beet weevils (Bažok et al., 2012). To regulate harmful insects (Aphidae, *Pegomyia betae, Atomaria linearis, Agriotes lineatus,* etc.), the incorporation of insecticides with 15–100 g/ha active ingredient imidacloprid and tefluthrine in the pelleted seed has become a reliable procedure (Wauters, 1997). According to (Castle et al., 2005) and (Byrne and Toscano, 2006), an imidacloprid concentration between 0.005 and 0.01 mg/kg in plant tissue provides effective protection against pests. According to Sur and Stork (2003), 16–20% of the active ingredient in neonicotinoids is taken up by the plant through germination from hulled seeds.

When treating seeds with insecticides, lower doses of the active ingredient are applied per unit area, resulting in less environmental impact, so this method is considered more ecotoxicologically and economically favorable (Dobrinčić, 2002). According to Westwood et (1998), a concentration of IMI of 12.5 mg/kg was detected in leaves of beets grown from treated seed at 21 days and 0.5 mg/kg at 97 days after sowing. Bažok et al. (2014a) found a double concentration (0.959 mg/kg) of IMI in sugar beet leaves 42 days after sowing. At 210 days after sowing, no IMI residues above the detection limit were found in any root sample, while 3.65 mg/kg TMX was found in 2 of 10 root samples. The degradation of IMI and TMX in sugar beet plants during the whole growing season has never been conducted (Bažok et al., 2014a).

However, Krupke et al. (2012) detected residues of thiamethoxam (68 to 13.240 mg/kg) and clothianidin (3.400-15.030 mg/kg) in dust from treated maize seeds. Dust from treated seeds containing less than 2% of the applied insecticides gets onto surrounding flowering plants and is carried by bees into the hive along with pollen (Marzaro et al., 2011; Tapparo et al., 2012). In the European Union, the situation regarding the use of neonicotinoides has changed dramatically due to suspected adverse effects on bee colonies (Vojvodić and Bažok, 2021). European Commission Regulation (EU) 485/2013 of 24/05/2013 temporarily banned the use of thiamethoxam, imidacloprid, and clothianidin on most agricultural crops (European Commission, 2013). The initial ban did not apply to sugar beets, which are not considered attractive to bees. In addition, flowering does not occur until the following year, eliminating the risk of insecticide residues in beet pollen (Bažok et al., 2012). Bees in sugar beets could encounter neonicotinoids via guttation fluid, which is rarely produced in beets (Joachimsmeier et al., 2012), or by feeding on sugar syrup. Eventually. based on all relevant research worldwide European Food Safety Authority (EFSA) made a final decision on the ban (EFSA, 2018a, 2018b, 2018c), on April 27, 2018 (Bažok and Lemić, 2018). The studies analyzed the available scientific work and evaluated the potential risk of using imidacloprid, thiamethoxam, and clothianidin on all crops where they were previously approved. This included analysis of (i) the risk of residues in pollen and nectar for foliar application; (ii) drift to untreated plants; (iii) residues in water sources. For application by seed treatment, risks were analyzed in terms of (i) systemic transmission through the treated plant and possible residues in nectar and pollen (referring to the treated plant and subsequent plants in the cropping sequence); (ii) contamination by dust drift (risk to field margins and adjacent crops); and (iii) use of water with possible insecticide residues. Risks were identified for three types of organisms: Honeybees, Solitary Bees, and Bumblebees.

Therefore, following the recommendations of EFSA, the European Commission adopted a decision to completely ban the use of imidacloprid, thiamethoxam, and clothianidin, except in permanent greenhouses, and the crop obtained in this way remains in a permanent greenhouse throughout its life (EFSA, 2018a, 2018b, 2018c). The decision has been applied in most EU member states since 2019.

However, with special permits, neonicotinoids can still be used for crop protection in EU (Harrison-Dunn, 2021). In a large part of the world, their use is still allowed, and therefore the risk of their negative effects is not eliminated. The ten largest importers of neonicotinoids from the EU, based on the amount of the active ingredient, are Brazil, Russia, Ukraine, Argentina, Iran, South Africa, Singapore, Indonesia, Ghana, and Mali (Dowler, 2021). With the exclusion of neonicotinoids from use, the use of other insecticides (mostly pyrethroids, increased two- to three fold) at the same time, and the result was a significant decrease in yield (up to 15%) (Kathage et al., 2017) and a marked increase in the number of insect pests in the cultivation of, for example, oilseed rape (Kathage et al., 2017). The ban on the use of neonicotinoids in Europe is based on the exclusively harmful impact on pollinators, while the entire evaluation and assessment process does not consider beneficial soil organisms and the possible consequences of neonicotinoids on their abundance and composition.

Numerous members of the beneficial fauna are also found in sugar beet. Beneficial fauna are a set of organisms that indirectly have a positive effect on crops by increasing soil fertility, regulating the water-air ratio, or feeding on and reducing the number of pests (Bažok, 2015). The sugar beet cultivation involves a variety of operations, such as frequent processing, intensive control of weeds and pests, and the fact that part of the vegetation is not covered by plant cover (beet plants are small, and weeds are intensively controlled). Such intensive cultivation can have a negative impact on beneficial insects in the soil. Among the most important beneficial soil fauna of sugar beet are insects, especially the order: Carabidae, i.e., ground beetles (Kos and Bažok, 2015) and earthworms (Pisa et al., 2014).

Ground beetles are important predators of numerous pests, feed on weed seeds, and are also a food source for animals at a higher trophic level. Because of their high abundance, known taxonomy, and sensitivity to change by external factors, they are frequently used in ecological research (Lövei and Sunderland, 1996). Population declines are explained by higher intake of agrochemicals, loss of grasslands for foraging, and increases in plot size (Fahrig et al., 2015). The composition of ground beetle fauna and the dynamics of their occurrence in agricultural crops in Croatia are not known, although it is often claimed that insecticides are the main factor in reducing their numbers. They may encounter insecticides from the group of neonicotinoids by feeding directly on organisms that have fed on the treated crop or through the treated surface on which they move (Albajes et al., 2003; Khani et al., 2012; Moser and Obrycki, 2009; Papachristos and Milonas, 2008; Prabhaker et al., 2011). In a study by Mullin et al. (2010), nearly 100% mortality was observed in 18 ground beetle species exposed to corn treated with IMI, TMX, or clothianidin. According to EC (2006), the marked sensitivity of *Poecilus cupreus* larvae to IMI has been demonstrated. According to the member states, the concentrations tested were too high to reach a conclusion and no further studies were conducted. In modern EU agriculture, conservation programs for beneficial fauna focus on the preservation of species and biodiversity and are promoted as a tool to ensure sustainability. Therefore, it is crucial to define sustainable measures to protect agricultural crops from pests that have less negative impact on the environment and ensure the production of healthy food. From an environmental science perspective, a more accurate knowledge of the composition of beneficial insect populations that can contribute to the natural regulation of pests is important for protection against adverse anthropological impacts.

Earthworms play a key role in the development and maintenance of physical, chemical, and biological soil properties (Lee, 1985). They participate in fragmentation, decomposition, and incorporation of organic matter (Edwards and Bohlen, 1996). In agricultural soils, earthworms account for up to 80% of the total animal biomass (Luo et al., 1999). Pesticides, which are commonly used in agriculture, pose a threat to earthworm survival and behavior by interfering with soil decomposition development and processes. The same neural pathways that allow neonicotinoids to affect invertebrates (Elbert et al., 1991) are also present in earthworms (Volkov et al., 2007). Thus, when neonicotinoids are applied to protect agricultural and horticultural crops, earthworms encounter the applied granules or seeds or with contaminated soil or water through direct contact. The way earthworms feed can cause them to ingest contaminated soil and organic particles (Wang et al., 2012). Residues of active ingredients that remain in plant debris or direct consumption of treated plants pose a risk to earthworms (Kreutzweiser et al., 2009). Toxicological studies show risk of mortality to individual adders of all known species when ingesting soil or organic material containing neonicotinoid residues at concentrations ≥ 1 mg/kg. At a concentration of 3 mg/kg, 50% mortality of earthworms is expected. Detailed studies on the effects of neonicotinoids applied to earthworms under real field conditions have not been conducted.

The environmental risk assessment of plant protection products (PPP's) for soil organisms is mainly based on the results of laboratory and extended laboratory studies while the link from the laboratory to realistic field conditions over several seasons is not well established. The current environmental risk assessment is applied to individual ingredients and does not consider that soil organisms are exposed to varying degrees to a mixture of active ingredients from different pesticides (Ockleford et al., 2017).

Despite the ban, the dynamics of the decomposition of neonicotinoids in plants sown from treated seeds and their impact on that plant and the surrounding soil are still unknown. In addition, neonicotinoid residues in beneficial soil fauna have not been determined so far, nor has their influence on the number and composition of these organisms. Based on this, the hypotheses and objectives of this research were set.

1.1. Hypotheses and the objectives of the research

Research hypotheses

1. Treatment of sugar beet seed with imidacloprid and thiamethoxam provides effective protection against pests for up to six weeks after sowing, depending on weather conditions. During this time, the residues break down in the plants below the detection limit.
2. This measure leads to a reduction in the number of ground beetles and the appearance of residues in ground beetles, earthworms and in the soil. Sowing sugar beet in a four-year rotation allows the recovery of the ground beetle fauna.

Objectives of the research

1. Determination of neonicotinoid efficacy on major pests and degradation dynamics in sugar beet plants grown from seed treated with imidacloprid and thiamethoxam under different weather conditions.
2. Determination of neonicotinoid residues in ground beetles, earthworms, and soil of sugar beet fields
3. Determination of the cenological composition of ground beetles in sugar beet fields and in fields where beets were grown one, two or three years ago.

2. Overview of former research

2.1. Complexity of sugar beet cultivation and current trends

Sugar beets (*B. vulgaris* ssp. *vulgaris* L.) belong to the family Amaranthaceae (formerly Chenopodiaceae) and to the order Caryophylalles, which have a C_3 photosynthetic system. Cultivated beets belong to the subspecies vulgaris and include leaf beets (chard), garden beets (red), fodder beets, and sugar beets (McGrath et al., 2011).

Sugar beet is an economically viable crop produced mainly for white sugar. A 20% of the world’s sugar comes from sugar beet while 80% is produced from sugar cane. The world’s leading sugar beet producers are France, Germany, and Poland (Eurostat, 2021). In Europe sugar beet is grown on about 2,000,000 ha presenting about 70% of the total arable land in the world. In the Republic of Croatia, sugar represents an important export product. Until 2012, it was grown on 23,215 ha with an average yield of 50,95 t/ha (Kristek, 2015). In recent years, sugar beet production has been decreasing not only in Croatia, but in all countries of the EU. For example, in 2018, sugar beet yields per hectare decreased by 15% compared to 2017 (Statistics Netherlands, 2019). During 2021 sugar beet was grown in Croatia on about 10 thousand hectares, from which 717 thousand tons of raw material were obtained, corresponding to an average yield of 70 tons per hectare (CBS, 2021). State support was provided to 476 farms for sugar beet cultivation on 10,451 hectares by the Paying Agency for Agriculture, Fisheries and Rural Development (PAAFRD, 2021). Sugar beet production has decreased significantly compared to previous years. Today's production has almost halved since 2017 sugar beet was cultivated on 19,235 thousand hectares (PAAFRD, 2017). Sugar beet is grown for its thickened roots, which contain 14 - 20% sugar (Pospišil, 2013). Sugar beet production is also important because of its secondary products (beet noodles, molasses), which are valuable components of livestock feed (Kanisek et al., 2008).

The root consists of a head, neck, body, and tail (Rešić, 2017). The head is the uppermost part of the root, located above the ground, where leaves and buds have developed. It contains the least amount of sugar and a lot of non-sugar, so it is better if it is as short as possible. The neck is the thickest part of the root where there are no leaves or lateral roots. It is the part of the root from the petiole of the lowest leaf to the beginning of the lateral furrow on the root body, i.e., the uppermost lateral rhizomes. The neck merges into the root body, which is conical and elongated. The body, as the most important and largest part of the root, begins at the point where the furrow and lateral roots appear, and ends at the point where the root is reduced to about 1 cm in diameter. On the body of the beet there are two opposite furrows, from which lateral roots emerge, supplying the plant with nutrients from the soil layer (Pospišil, 2013). The shape of the beet root is characteristic for certain varieties. The conical shape predominates, which has a higher yield potential than the apple shape with a wider neck. The cross-section shows concentric circles where sugar accumulates. The more circles, the greater the amount of sugar (Rešić, 2017). The tail is the lowest part of the root, about 1 cm thick, which breaks off when the beet is removed and remains in the soil. The tail transforms into a branched system of root vessels that penetrate to a depth of 2-2.5 m. The roots of the beet are the rootlets. These are the roots that supply the plant with water, but also with nutrients, especially if there are none in the arable layer of the soil (Pospišil, 2013).

Regarding the sugar beet cultivation, diverse set of production, harvest, and processing arrangements are possible. Mature plants tolerate modest freezing temperatures, but extended exposure to temperatures below – 5°C results in cell disruption and rotting, requiring harvest and storage before severely freezing temperatures occur. These limits affect the length of the growing season of beets in northern latitudes with cold winters. The farthest northern production regions with sugar industries are in Finland and Sweden (Kaffka and Grantz, 2014).

When in the Northern Hemisphere, usually sugar beets are planted in early spring and harvested 5-9 months later, depending on soil and environmental conditions. In warmer or Mediterranean climates, "winter beets" may be planted in the fall allowing harvest the following spring, summer, or fall (Kaffka and Grantz, 2014). Given the production technology and the length of the growing season of almost 180 days, sugar beet is considered the most intensive agricultural crop (Pospišil, 2013).

Under suitable conditions, such as optimal soil temperature and moisture, the plant develops rapidly from seed, with the seedling sprouting from the soil within 5 - 10 days after planting (Kaffka and Grantz, 2014). Optimal soil temperatures are 6 - 8°C at a depth of 5 cm. Optimal agrotechnical conditions are from March 15 to April 10. Sugar beets are usually sown in a well-prepared soil at a depth of 2 to 3 cm with a row spacing of 45 or 50 cm and in a row at a spacing of 18 to 20 cm (Tot, 2008).

The taproot grows rapidly and can reach 30 cm or more when the first true leaf develops. During the first 30 days, growth is confined mainly to the leaves and fibrous roots. After about 30 days, both crown and storage root growth progress rapidly, with crowns reaching nearly their maximum fresh weight in 60-90 days and crown closure at a leaf area index (LAI) of 3 (Milford, 2006). A fast-growing sugar beet plant is capable of high sucrose accumulation. Dry matter gain (DM) and sugar yield are directly proportional to the amount of solar radiation absorbed by the plant (Jaggard and Qi, 2006). The longer the growing season, the greater the yield potential when all other conditions are equal (Milford, 2006). Growth remains constant at the top, but storage roots continue to grow rapidly for 20-40 weeks (for a 10-month crop). As the plant develops, an increasing amount DM accumulates in the roots. While the number and area of leaves remain relatively constant, in areas with longer growing seasons, the roots consist of larger amounts of crown material, so impurities can also accumulate. These impurities reduce sugar recovery from roots in mills (Harvey and Dutton, 1993).

As root increases in size, there is a constant translocation of sucrose from the leaves into the root, where it is stored mainly in concentric rings of vascular tissues derived from the secondary cambium formed early in root development and in the cells of the root parenchyma, which proliferate and enlarge during growth. Bell et al. (1996) and Milford (2006) summarize a variety of studies and report that the distribution of DM in roots is regulated by cells within the root and is independent of photosynthetic supply. Relative to fresh weight, sucrose content of the root remains relatively constant unless appropriate external factors cause a change in concentration.

The root for industrial processing is extracted at technological maturity, which is determined by analyzing the sugar content in the root and weighing the plants. Then the growth of thickened roots is slowed down, the sugar content is high, and the content of non-sugars is reduced. In our growing area, sugar beets should be harvested at the end of September and finished at the end of November (Pospišil, 2013).

Where successful sugar beet industries have developed, various adaptations to the physiological limits of plant growth have been made, resulting in many different cropping patterns worldwide (Kaffka and Grantz, 2014). Improvements in plant breeding and seed technology have resulted in increasingly rapid seed emergence and establishment. Over time, monogerm seed, improved weed control, improved planters, and seed treatments that reduce losses from pathogens and pests during the sensitive period of crop emergence and establishment have reduced the need for large quantities of seed and hand thinning of seedlings. Planting to a stand and 70 – 80% emergence and establishment have become common in growing areas with advanced agricultural practices (Jaggard and Qi, 2006).

2.2. Sugar beet pests

Controlling pests and diseases is important for profitable crop production. Sugar beet is slow to establish and is susceptible to a weed competition in early stages. Moderate weed infestation is controlled by crop rotation and combination of chemical and mechanical methods (Wisler and Duffus, 2000). The consequences of not following crop rotation are the accumulation of pathogens and pests, the one-sided release of nutrients, especially those that we do not add during regular fertilization, and the fatigue of the soil for growing sugar beets. The best and most common source varieties for sugar beets are small cereals (wheat, barley, oats), potatoes and annual legumes (soybeans). Sugar beets are sown only every 4-5 years on the same area; therefore, the following crop rotation can be recommended: i) sugar beets, ii) wheat, soybeans, or barley, iii) corn, iv) sunflower, v) wheat (Pospišil, 2013). Dumping-off diseases are linked to seedling rots are common during preemergence and postemergence sugar beet stage while other diseases like Cercospora leafspot (*Cercospora beticola* Sacc.), powdery mildew (*Erysiphe polygoni* DC) and rhizomania caused by a virus (beet necrotic yellow vein) occur in later grow stages. Manny diseases are transmitted by insect vectors (curly top transmitted by the sugar beet leafhopper; sugar beet yellows transmitted by aphids) (Wisler and Duffus, 2000) making these insects especially harmful.

The most common pests that attack sugar beet in the early stages of leaf development or youth stage and have been extremely damaging are wireworms (*Agriotes* spp.), flea beetles (*Chaetocnema tibialis* Ill.), sugar beet weevils (*Bothynoderes punctiventris* Germ., *Tanymecus dilaticollis* Gyll., *Psalidium maxillosum* F., *Otiorhynchus ligustici* L. and noctuid moths (*Agrotis segetum* Schiff., *Agrotis ypsilon* Hubn. and *Euxoa temera* Hb.) (Čamprag, 1983; Bažok et al., 2014b; Čačija, 2015; Drmić, 2015; Drmić and Bažok, 2015). According to Gotlin Čuljak (2015) later in vegetation several species of aphid's attack sugar beet. S*mynthurodes phaseoli* West, *Pemphigus fuscicornis* Koch and *Pemphigus betae* Doane attack roots, while *Aphis fabae* Scopoli and *Myzus persicae* Sulzer are pesent on leafs. Caterpillars of the beet moth (*Scrobipalpa ocellatella* Boyd), cabbage moth and bright-line brown-eye moth (*Mamestra brassicae* L. and *Lacanobia oleracea* L.) as well as silver Y (*Autographa gamma* L.) can also attack sugar beet during vegetation, but these pests are rarely suppressed (Lemić, 2015). Beet cyst nematode (*Heterodera schachtii* Schmidt) is widespread in the growing areas and can present a significant problem if the agro-technique of cultivation is not respected (Grubišić, 2015).

2.2.1. Wireworms (*Agriotes* spp.)

Wireworms are the harmful larvae of klick beetles from family Elateridae. They are narrow-bodied and dark-colored beetles up to 15 mm long. The larvae are yellow in color, have a narrow, elongated, and hard body, and resemble a piece of brass wire (Maceljski, 2002). In Croatia, the most important species of the genus *Agriotes* that cause the most damage are *Agriotes brevis* Candèze, *Agriotes lineatus* Linnaeus, *Agriotes obscurus* Linnaeus, *Agriotes sputator* Linnaeus and *Agriotes ustulatus* Schäller (Kozina and Bažok, 2013).

All harmful species of wireworms have perennial development. Depending on external conditions, especially temperature and humidity, the same species may undergo a two- or three-year development or a three- or four-year development (3-5 calendar years). Because of the perennial development, the larvae cause damage for many years. Larvae feed more intensively the older they are, so the damage is greater. Therefore, the threat to an individual crop depends not so much on the crop grown the previous year, but on the crops grown in the previous two or three years, because the larvae of some species cause the greatest damage only in the second, third or fourth year after the crop on which they laid their eggs (Maceljski, 2002).

They are economically important soil pests for agricultural crops, especially corn, sugar beets, potatoes, and sunflowers. They live in the soil and feed on the roots of young plants and seeds. The adult insects (imago) feed on vegetative and generative plant parts (flowers, pollen, nectar) and their diet has no economic importance (Čamprag, 1997). They cause the greatest damage in spring at the time of plant emergence. Plants are susceptible to wireworm infestation at the time of germination and emergence when wireworms burrow into the seed or soil of the germinated plant. The main damage in spring manifests itself in thinning of the plant and reduction of yield and plants with damaged roots lag in growth and development. Infested plants are very easy to pull out of the soil because the root is destroyed, or the stem is bitten into the soil so that it can be easily torn off the root (Maceljski, 2002). According to Hauer et al. (2017) and Furlan and Kreutzweiser (2015) there is less than 10% occurrence of wireworms in sugar beet fields in north Europe and very low occurrence in the Netherlands, Belgium, Germany, Sweden, Denmark and Italy. Furlan et al. (2017) reported that wireworm infestation was less than 15% in 70% of the fields observed over a period of 29 years. However, in more than 10% of the fields, the damage exceeded 40%. Poggi et al. (2018) reported damage above 15% in about half of the fields observed in northern France.

2.2.2. Flea beetles (*Chaetocnema tibialis* Ill.)

In the past, when soil insecticides were used, the sugar beet flee beetle (*Chaetocnema tibialis* Ill.) was one of the most important pests of sugar beet. It is a small insect of 1.5-2 mm long, greenish black in color, and metallic sheen. It moves around by jumping (Maceljski, 2002).

It overwinters as adult in the soil near last year's beets. The emergence starts with the emergence of sugar beets, when the air temperature rises to 12°C and the soil temperature (5 cm deep) warms by more than 5°C (Maceljski, 1999). After copulation in May, the female lays up to 40 eggs in the soil near the plants. After two weeks, a larva appears in the soil and feeds on various roots without causing much damage to the plants. In early August, the imago hatches, feeds on leaves, and in the fall crawls into the soil to overwinter.

They infest new fields from the edges toward the center. Their activity depends on weather conditions. The warmer it is, the more active they are and vice versa. In addition to beets, it also damages the cotyledons of beets, chard, and weeds from the Chenopodiaceae family. It causes damage by biting into the leaves in the form of small round holes 1 mm in diameter which spread as the leaf grows. Sometimes it bites not only the leaf but also the stem. At the cotyledon stage, one flea beetle damages 33%, three flee beetles 62%, and five flee beetle 90% of the plant per day. As plants grow, to a stage of 4 or more true leaves, damage is less. The number of holes on the leaves indicates the number and occurrence of the infestation next year (Maceljski, 2002).

2.2.3. Sugar beet weevil (*Bothynoderes punctiventris* Germ.)

The body length of this weevil varies from 5 to 15 mm and is usually gray, brown to black in color (Bažok, 2010). It has one generation per year overwintering on the old sugar beet field at depth of 20 to 65 cm. Sometimes it also overwinters on the fields of another crop, located on the edges of the places where sugar beets were deposited after harvest. Emergence of adult from the soil occurs gradually at the end of March and/or beginning of April and depends on temperatures. The first individuals appear on plots prepared for sowing of spring crops. On plots where winter crops have been sown, the surface is cooler, and weevils appear later. When the air temperature reaches 20°C and above, adult flight begins, and when the temperature exceeds 23°C, mass flight begins. Weevils fly intensively at the time of sexual maturity and egg laying on the new tails, which is different from the first migratory flight in search of food. Mating takes place in May. The larvae develop in or directly next to the root. After 6-7 weeks, the larvae pupate and develop into an adult that comes to the surface. Shortly thereafter, it returns to the soil to overwinter.

In addition to sugar beets, the weevils also eat beet, spinach, and weeds of the Amaranthaceae and Polygonaceae families. It feeds on stems, cotyledons, true leaves, plants on the surface and below the soil surface. The sugar beet weevil causes the most damage from the budding stage of sugar beet to the appearance of the first pair of true leaves. Decades ago, weevils occurred only occasionally in masses, and only recently have their mass occurrence each spring become common (Drmić, 2016). Young sugar beet crops, located near the old beet field, are the most frequently and rapidly affected. One weevil can eat off 5 to 16 sugar beet plants at the cotyledon stage, i.e., more than 140 mm^2 of leaf area, on a warm day (Maceljski, 2002). The greatest damage is manifested in the destruction of entire plants at the cotyledon stage and thinning of the crop. The weevil also causes damage later when it bites the beet leaves and destroys the leaf surface. As the plant grows, the damage decreases. Infestation of young plants starts from the edges towards the middle of the field, and later, after the beginning of summer, the damage also occurs in the middle of the field, so the inspection should be carried out on the whole area (Drmić and Bažok, 2015).

2.2.4. Noctuid moths

Noctuid moths are species whose caterpillars remain in the soil and feed on underground organs of plants. They most commonly infest broadleaf crops, including sugar beets. The moths are nocturnal butterflies that are unsightly brown-black in color. The caterpillars are earthy gray in color, naked and hairless. The body of the adult caterpillars acquires a greasy sheen in the later stages of development. At the end of development, the caterpillars reach a length of 35-45 mm. The greatest damage to sugar beets is caused by infestations of the first generation of the turnip moth *Agrotis segetum* and dark sword-grass or ipsilon dart *Agrotis ypsilon*. Caterpillars feed on emerged plants, their underground and soil parts. It is common for the caterpillars to completely gnaw off the plant. The result of the infestation is thinning of the stands, which is later reflected in lower yield. The damage caused by these species is greater in late-sown crops. Due to large damage, it may sometimes be necessary to reseed part or entire area (Maceljski, 2002). The (*Agrotis ypsilon* Hubn) is a periodic pest. It overwinters as an adult caterpillar in the soil. The pupa forms in April, and by the end of May the butterflies fly out and lay their eggs in fields under debris or in young alfalfa fields. The caterpillars of this species appear in May and develop in 30-35 days. During the day they remain in the soil, and at night they feed on the surface of the soil and nibble on sprouting plants. Later in August they develop the second generation. The first generation of caterpillars causes more economic damage. A mass appearance of adults in our region can be expected in early May, and the caterpillar from the second half of May (Lemić, 2015). The *Euxoa temera* Hb. is a periodic pest. It is the earliest of all species to appear in the spring. It overwinters as a caterpillar in an egg case. The caterpillars hatch from the eggs in early spring and feed on the underground and soil organs of plants for 2 months. During the summer (June, July) they go into diapause. After diapause, butterflies emerge in August, which lay eggs after copulation. For oviposition, they choose alfalfa fields, weedy fallows, or winter crops.

2.2.5. Aphids

Aphids cause two types of damage to aboveground organs: direct damage by sucking on plants and indirect damage by transmitting viruses. Direct damage is manifested by curling of leaves, plants are weak and have poorly developed roots. The green peach aphid (*M. persicae*) and black bean aphid (*A. fabae*) are very serious pests of sugar beet, transmitting many plant mosaic diseases. One individual *M. persicae* causes same damage as 20 individuals of *A. fabae* which was a major problem in technology production until sugar beet seeds were treated with insecticides (Gotlin Čuljak, 2015). *M. persice* is pale yellow-green with three dark lines on the back. The life cycle involves two hosts. The female reproduces parthenogenetically during summer and produces sexual males and females in autumn (Encyclopedia Britannica, 2022). The species is polyphagous and damages more than 400 plant species. Direct damage consists of curling of leaves, deformation of shoots, and unfertilized flowers. Indirect damage is important because the species transmits more than 100 viral diseases of which sugar beet yellows virus is the most dangerous (Gotlin Čuljak, 2015). *A. fabae* is dull black sometimes with a distinct greenish hue and highly variable in size. White wax markings appear on old aphid colonies (Influential Points, 2022). It is a highly polyphagous, migratory pest with many wild species potentially serving as reservoirs for crop infestations. However, *A. fabae* has a heteroecious and holocyclic life cycle and requires the presence of the overwintering host *Euonymous europaeus* L. to establish in new areas. Holocyclic populations have a greater potential to become invasive, especially in subtropical and tropical regions. The aphid can also be transmitted in the trade of planting materials and some vegetable products (CABI, 2022a).

Aphids that occur on the roots are less common. Their infestation is more severe during the dry season, when infestation can lead to desiccation and decay of plants. Symptoms of the presence of aphids on sugar beets appear in the form of bare soil within crop, and more often in the first half of the growing season. The plant responds to infestation by changing the biochemical composition of the sap and physiological processes. Cell decomposition occurs, the content of raw sugar and dry matter increases, while the raw sugar content decreases sharply (Maceljski, 2002). In addition to the decrease in sugar content, other parameters that determine the technological value of the roots also deteriorate (Gotlin Čuljak, 2015).

2.2.6. Leaf moths

The most common are three species of leaf moths (Maceljski, 2002), the cabbage moth (*Mamestra brassicae* L.), the bright-line brown-eye moth (*Lacanobia oleracea* L.) and the silver Y (*Autographa gamma* L.). According to Čamprag et al. (2003), the most common species affecting sugar beets is the cabbage moth. The cabbage moth is gray to dark brown in color and has a body length of about 20 mm. The caterpillars go through six stages of development and grow up to 40-45 mm in size. Young caterpillars have 3 pairs of thoracic and 5 pairs of abdominal legs, they are green at first and later take on a gray-green, dark green or dark brown color.

Cabbage and vegetable moths have two generations per year. They overwinter in the form of a pupa in the soil in the fields where the caterpillars lived. The flight of butterflies begins in late May and early June. The second generation of butterflies' flies in late July and early August. During the flight period, the butterflies prefer areas where many plants are in bloom (Čamprag, 1983). The females lay their eggs on sugar beets, but also on cabbage and other cultivated and weedy species. The first generation of caterpillars appears in late June and July, the second generation in late August. The caterpillars are hygrophilous, i.e. they like moisture in the plant. The maximum population is found in the second half of June to the end of September. The silver butterfly is a migratory species that develops part of its population in our country, but the greater part comes from the southern regions. The timing of the appearance of butterflies and caterpillars is similar to the other two species, although this species develops several generations (3-4), and it is possible that the generations overlap (Lemić, 2015).

2.2.7. Beet moth (*Scrobipalpa ocellatella*, Boyd)

The beet moth as a pest of sugar beets was first recorded in our region in 1947 in Slavonia, and as early as 1950 it was mentioned as a pest of industrial beets, occurring in almost all beet fields (Fajt, 1951). The moth is light brown in color, 8 mm long, and has a wingspan of 12-15 mm. The fully developed caterpillars of the moth are dark red in color and up to 12 mm long (Maceljski, 2002).

The beet moth is native to the Mediterranean region, and it reproduces under dry conditions. It develops 4-5 generations in one growing season and overwinters as an adult caterpillar or pupa (Čamprag, 2000; Maceljski, 2002). Because the moth has several generations and overwinters in different forms, mixing of generations and simultaneous occurrence of all developmental stages in sugar beet crops occurs. The development of one generation of the moth lasts 40-60 days, depending on climatic conditions. The caterpillars pupate in the soil at a depth of 1-5 cm. The butterfly of the new generation flies after 10-20 days, and they can fly several kilometers during their life. Beet moth reproduction is favored by dry and warm weather, early spring, and long autumn.

Beet moth caterpillars are oligophagous and feed on all plants of the genus *Beta*. They do the most damage to sugar beets, but they also occur on fodder beets and turnips (Čamprag, 2000) during root and seed production. In addition to the genus *Beta*, the host plants of the beet moth are plants from the genera *Amaranthus*, *Chenopodium*, and *Suaeda* (CABI, 2022b). In sugar beet fields, Fajt (1951) recorded the first attacks at the edges and only later in the middle of the plots. In infested plants, the caterpillars connect the youngest leaves with cobweb threads, gnaw them, infest them with black faeces, so that in the end they turn the entire middle part into a completely black mass of dead and dried leaf parts (Sekulić and Kereši, 2003). In a mass infestation, the caterpillars attack not only the central leaves but also the stems of older leaves, the head and neck of the roots, and even the parts of the roots that lie underground. In warm and dry years, especially when beet root aphid (*P. fuscicornis*) and leaf spot disease (*C. beticola*) occur simultaneously on beet tails, such damage can cause the entire plant to wither, because the outer leaves are dry, and the young plants cannot be formed due to the gnawed rosette (Maceljski, 2002). Such condition ultimately affects the yield and sugar content of the roots (Sekulić and Kereši, 2003). The caterpillars can also damage the beet root in the trap while waiting for processing. The first damage can be observed as early as the beginning of May, and as the weather warms, the number of caterpillars increases and so does the proportion of damaged plants (Sekulić and Kereši, 2003). The greatest damage is caused at the end of summer. When an infestation of 10-20 caterpillars per plant is observed, there is a 19% decrease in root yield and a 48% loss of sugar (Čamprag, 2000).

2.2.8. Beet cyst nematode (*Heterodera schachtii*, Schmidt)

The females are worm-shaped at the beginning of their development, later becoming lemon-shaped cysts of milky white to pale yellow color. In the body of the female there are 200-600 eggs in an egg sac. The size of the cyst is about 1 mm. Males have a typical worm-like shape and are up to 1.6 mm long. In the bucal cavity there is a stylet up to 35 μm long. The larvae are up to 500 μm long, have a strong stylet and a conical tail part (Oštrec, 1998).

The species *H. schachtii* develops and reproduces on more than two hundred cultivated species, but also on weeds from 23 families, mainly Chenopodiaceae, Cruciferae, Polygonaceae, etc. Significant damage and yield losses of up to 50%, except on sugar beet, have also been observed on cabbage, cauliflower, broccoli, mustard, chard, spinach, etc. (Chen et al., 2004). The secondary roots of sugar beet develop more due to infestation by second-stage infectious larvae, so that tertiary roots develop on them. In this way, the sugar beet root acquires a short, bearded appearance. Main root fails in development, the leaf mass of the aerial part also turns yellow and dries up in high heat. Young plants are noticeable for their elongated petioles, which are often yellow in color.

Initially, the parasitized plants are observed in places, and as the infection spreads, they appear in the form of bare soil within crop, that is, where plants dry out and rot. Infection on surfaces may be localized but may also cover the entire surface (Grubišić, 2015).

2.3. Pest management and influence on beneficial organisms

2.3.1. Most common pest management practices

Curative control of most pests occurs after infestation is detected, but some of the pests can only be successfully controlled preventively, i.e., before seeding or simultaneously with seeding (Bažok, 2015). To do so, it is necessary to implement integrated pest management (IPM). IPM carefully considers all available pest control techniques and then integrate appropriate measures that prevent the development of pest populations and limit the use of pesticides and other measures to levels that are economically justified and reduce or minimize risks to human and animal health and the environment. IPM emphasizes the growth of a healthy crop with minimal disruption to agroecosystems and promotes natural pest control mechanisms (Vetek et al., 2017). Table 1. shows the integrated pest management approach for controlling most important sugar beet pests.

Forecasting methods that determine the infestation and sets the decision threshold are shown in table 2. Pesticides should only be applied when the pest population is above an economic threshold, i.e., above a certain number of pest populations that result in yield losses. Economic decision thresholds have been used for entomological purposes since the 1970s (Stern, 1973).

Table 1. Integrated pest management approach for controlling most important sugar beet pests (Bažok, 2015).

	IPM												
	Agrotehnical									**Physical**	**Mechanical**	**Biological**	
Pest	CR (a)	SI (b)	S (c)	T (d)	DPR (e)	I (f)	F (g)	W (h)	RPGM (i)	AWT (j)	CC (k)	EP (l)	BT (m)
Agriotes spp.				d^1			g^1						
Agrotis segetum, *Euxoa temera*, *Agrotis ypsilon*								w^1					
Bothinoderes punctiventris			c^3	d^3						j^3	k^3		
Chaetocnema tibialis			c^4										
Aphis fabae			c^5				g^5						
Scrobipalpa ocellatella				d^6									
Noctuidae, Mamestra brassicae, Autographa gamma								w^7				l^7	m^7
Heterodera schachtii			c^8				f^8	w^8					

Agrotechnical mesaures: CR – crop rotation; SI – spatial isolation; S – sowing; T – tillage; DPR - destruction of plant remains; I – irrigation; F – fertilization; W – weeds; RPGM – rapid plant growth mesaures; **Physical mesaures:** AWT – area wide trapping; **Mechanical mesaures:** CC – catching channels; **Biological mesaures:** EP – application of endoparasites, BT – applicatipn of *Bacillus thurinhiensis* insecticides.

Additional explanation:
d^1 – any type, g^1 - mineral fertilizers that develop ammonia
w^2 - weeds provide feed and oviposition, the damage is less on culltivated crop
c^3- earlier sowing time, d^3- less inter-row spacing and headland sowing in a slightly denser structure, j^3 - mass trapping by pheromones of aggregation when using 30 pheromones/ha, k^3 - digging catching channels around the old sugar beet fields
c^4- earlier sowing time
c^5- avoid sowing near forests and on warm, lighter soils; g^5- moderate fertilization
d^6 - deep plowing
w^7 - weeds provide feed and oviposition, the damage is less on culltivated crop, l^7 - *Trichogramma* endoparasit insect eggs, m^7 - application of insecticides based on *Bacillus thuringiensis* var. *kurstaki*
a^8 – 3 to 5 years including potatoes, soybeans, grains, onions, alfalfa, corn, peas, beans, buckwheat, c^8-sowing of trapping crops at the end of August which include cabbage, oil radish or mustard, sowing of tolerant varieties, earlier sugar beet sowing time, f^8 - balanced fertilization, w8 – weed control necessary

Table 2. Threshold limits for pests that occur during sugar beet production

Species	Forecast method	Trashold	References
Agriotes spp.	By examining the soil after harvest or in the spring before sowing: burying wet maize feeding baits under foil.	8-10 larvae/m^2 or 1 larvae/ maize feeding baits	Bažok (2015)
Agrotis segetum, Euxoa temera, Agrotis ypsilon	Adults can be tracked with pheromones to determine the start of flight and when to inspect the crop. Caterpillar infestation is determined by inspecting the soil and plants over an area of 1 m^2 starting in mid-May.	1-2 caterpillars/m^2	Bažok (2015)
Bothinoderes punctiventris	Number of weevils is determined by visual inspection of the crop in at least 4 randomly selected places by a wooden frame of 1 m^2.	1 adult/m^2 in old sugar beet field 0,1-0,3 adults/m^2 in young crop	Čamprag et al. (2003)
Chaetocnema tibialis	Determine the number of flea beetles and the number of holes (bites) per plant by visual inspection. All plants must be inspected at a minimum of 4 places per 10 m row.	2 holes (bites) on the leaf or 0,2-0,3 adults/plant on young plants after emergence. 3-5 holes (bites) on the leaf or 0,5 adults/plant in the phase of the first pair of leaves	Drmić (2015)
Aphis spp.	The percentage of infected plants is determined by visual inspection of the plants at least 4 places per 10 m row	If 20-30% of the plants are infested with aphids, treat only the edges	Bažok (2015)
Scrobipalpa ocellatella	The flight of can be tracked with pheromones to determine the start of the flight and the number. The percentage of infected plants and the average number of caterpillars are determined by visual inspection of the plants in at least 4 places in per 10 m row.	4-5 caterpillars/plant on 50-70 % of the examined plants	Lemić (2015)
Mamestra brassicae, Lacanobia oleracea, Autographa gamma		0,5-1 caterpillars/plant	Bažok (2015)
Heterodera schachtii	Taking soil samples and analysis for the presence of cysts nematodes in nematological laboratories.	3-8 eggs/g soil in cooler soils 1 egg/g soil in warmer soils 50 cist/100 ml soil avoid planting	Cooke (1987)

With chemical control, one treatment can suppress several pests at the same time. Most used methods for sugar beet pest management are seed treatment, soil treatment and foliar treatment (Bažok, 2015).

Seed treatment with insecticides to control soil pests is considered one of the most ecologically and economically sound measures (Igrc Barčić and Maceljski, 2001). In this method, significantly lower doses of the active ingredient are applied. Lower amounts per unit area provide lower costs while allowing for lower uptake, sometimes very persistent insecticides in the soil, and lower environmental impact. Sugar beet seed treatment is applied during pelleting process. This is most safe for the user, but at the same time makes it impossible to adjust the dose used to actual needs. Insecticides used to treat seeds against insects act mainly through the digestive tract, that is, only after the insects have eaten the contaminated food of the germinated plant. For this reason, the effect of these insecticides is not sufficient in the case of heavy infestations of soil pests (Bažok, 2015). However, they are effective only at low to moderate levels of infestation (Igrc Barčić et al., 2000). This method reduces insecticide use and environmental pollution. According to Ministry of Agriculture (Croatian Ministry of Agriculture, 2022) today, all seed treatments with neonicotinoids are banned (more on this in the next section). Only seed treatment with the insecticide tefluthrin from the pyrethroid group is still used. Teflutrin is contact insecticide for treating cereal, sugar, and fodder beet seeds. It has contact and gastric action. It is applied in an amount of 60 ml per 100.000 seeds. The maximum permissible amount of the product used is 66 ml/ha, which corresponds to a maximum amount of 110,000 sugar beet seeds/ha. The product is applied undiluted. It is applied directly to the seed once a year, using conventional seed treatment machinery during the grafting process. It is used for suppression of Elateridae, *Agrotis* spp., Melolontinae larvae, *Gryllotalpa gryllotalpa* L., *Atomaria linearis* Stephens, *Blaniulus guttulatus* Fabricius, *Scutigerella immaculata* Newport *and C. tibialis.*

Soil application of granular and liquid insecticides was a common measure in the 1980s to protect sugar beets from soil pests on plots where there was a risk of infestation (Bažok et al., 2012). Most often, granular formulations were applied in strips during seeding using granular depositors. Somewhat less frequently, the entire area was treated before seeding, and incorporation with a harrow, disc harrow, or seeder was mandatory. At that time, insecticides with high toxicity, poor selectivity, and less ecotoxicologically favorable were mainly on the market, for example, lindane (Bažok et al., 2012). Phorate, phoxim, and chlorpyrifos were also used. Today, only teflutrin granular insecticide with contact action has permission for application (Croatian Ministry of Agriculture, 2022).

Foliar treatment suppresses above-ground pests. Before starting the treatment, it must be determined if thresholds have been exceeded by means of a forecast carried out according to the described method decisions for each pest. In recent years, the number of pesticides approved for sugar beet, including insecticides, has been significantly reduced (Bažok, 2015). According to FIS database of Croatian Ministry of Agriculture (FIS, 2022) list of insecticides currently allowed for pest management in sugar beet is presented in Table 3.

Table 3. List of registered PPPs on 13.08.2022. for foliar susspresion of sugar beet pests according to Insecticide Resistance Action Committee (IRAC) mode of action classification (FIS, 2022).

IRAC grouop	A.i.	No. of allowed treatments	Application rate	Time of application	Target pest
1A Carbamates	Pirimikarb	1	40-60 g/100 l	at the beginning of the attack	Aphididae
3A Piretroids	Deltamethrin	1	0.3-0.5 l/ha	When pests appear, in the early phase of the attack	*Aphis fabae, Chaetocnema tibialis, Mamestra brassicae, Agrotis segetum, Agrotis ypsilon, Atomaria linearis, Pegomyia betae* Curtis, *Cassida vittata* Villers, *Lixus juncii* Boheman, *Conorrhynchus mendicus* Gyllenhal
		1	0,075-0,125 l/ha		Above mentioned including *Spodoptera* spp.
	lambda-Cyhalothrin	2	1,5 ml/100m^2	when pests appear within 10 days	Aphididae, Halticinae and harmful-sucking or biting insects
		2	0,15 l/ha	From emergence to 9 or more developed leaves	*C. tibialis, P. betae* and *A. segetum*
	Cypermethrin	2	100 ml/ha	When pests appear	Aphis spp., Myzus spp., L. juncii, C. mendicus, Trialeurodes vaporariorum Westwood, C. vittate and Noctuidae
	tau-Fluvalinate	2	0.2 l/ha	Before the appearance of symptoms of leaf curl	*A. fabae*
UNE*	Fatty acid potassium salts	5	3-10 l/ha	From the stage of visible leaves (BBCH 10) to the stage when 80% of quivers are open (BBCH 89)	above-mentioned aphids and *Bemisia tabaci* Bellows & Perring, *T. vaporariorum*, *Tetranychus urticae* C. L. Koch and *Frankliniella occidentalis* Pergande

** UNE - Botanical essence including synthetic, extracts and unrefined oils with unknown or uncertain MoA*

2.3.2. Pesticides and pesticide residues

A **pesticide** is any substance or mixture of substances used for/as **(i)** preventing, destroying, repelling, or mitigating pest; **(ii)** plant regulator, defoliant, or desiccant; **(iii)** nitrogen stabilizer. The term refers to various pesticides such as insecticides, fungicides, herbicides, and nematicides (USEPA, 2022a). Pesticide products contain both "active" and "inert" ingredients, with an **"active ingredient"** responsible for suspresion or repeletion of pests or acting as a plant regulator, defoliant, desiccant, or nitrogen stabilizer. All other ingredients are designated as **"inert ingredients"** are important to the performance and usability of the product (USEPA, 2022b).

Pesticides are potentially toxic to humans and especially to those who work with them or come into close contact with them. But pesticides also play an important role in food production: they protect or increase yields and the number of times per year a crop can be grown on the same land. This is particularly important in countries facing food shortages (FAO/WHO, 2020). The application of pesticides to crops and animals can leave residues in or on food when consumed, and these specified derivatives are considered toxicologically relevant. A **pesticide residue** is any substance or mixture of substances in food for humans or animals resulting from the use of a pesticide and includes all specified derivatives such as degradation and transformation products, metabolites, reaction products, and impurities that are considered toxicologically significant. The term "pesticide residues" includes both residues from unknown sources (i.e., background residues) and residues from known uses of the chemical of concern (IPCS INCHEM, 1975). The general population is most exposed to these residues through the consumption of treated foods or through close contact with pesticide-treated areas, such as farms or lawns (USEPA, 2022c).

Most chemical residues, especially derivatives of chlorinated pesticides, exhibit b**ioaccumulation** that can reach harmful levels in the body and the environment (Crinnion, 2009). Persistent chemicals can be magnified through the food chain and have been detected in products ranging from meat, poultry, and fish to vegetable oils, nuts, and a variety of fruits and vegetables (Chung and Chen, 2011). Persistent chemicals have been found to be present in a wide variety of foods and vegetables. All matters related to legal limits for pesticide residues in food and feed are covered by Regulation EU (EC) No 396/2005.

To protect consumer health, most countries have legal maximum limits for pesticide residues in food. If the limits vary from country to country, trade difficulties may arise. The Codex Committee on Pesticide Residues (CCPR) is responsible for setting Codex **Maximum Residue Limits (MRLs)** for pesticide residues in specific foods or groups of foods or feeds that move in international trade (Food and Agriculture Organization and World Health Organization, 2020). According to Regulation (EC) No. 396/2005, MRLs are the upper limits of pesticide residues legally permitted in or on food or feed based on good agricultural practice (GAP) and the lowest exposure necessary to protect vulnerable consumers. They are derived after a comprehensive evaluation of the properties of the active ingredient and the intended use of the pesticide (EFSA, 2022).

Before a Codex MRL can be set, human health risk assessments must be conducted to ensure the safety of the food supply trade (Food and Agriculture Organization and World

Health Organization, 2020). Humans may be exposed to various chemical substances by oral route (i.e., ingestion of food, drinking of groundwater, hand-to-mouth transmission). Therefore, it is necessary to determine the maximum amount of a chemical that can be ingested daily over a lifetime with no appreciable health risk by calculateing the **Acceptable Daily Intake (ADI)**. For pesticide residues and food contaminants, the ADI can also be referred to as the **Tolerable Daily Intake (TDI).** The ADI value is usually derived from the lowest **no observed adverse effect level (NOAEL)** determined from long-term animal studies (*in vivo*). The ADI is calculated by applying a safety or uncertainty factor, usually 100, to the NOAEL of the most sensitive animal species. The 100-fold safety factor is based on the need to account for differences between animal species as well as differences in toxicokinetics and toxicodynamic. The ADI is expressed in milligrams of the chemical, as it appears in the food, per kilogram of body weight per day (mg/kg/day) (Chilakapati and Mehendale, 2014).

The measurement of pesticide residues in food or feed is of crucial importance since high pesticide levels accumulated in organism lead to development of adverse effects to human or animal health. In this context, the use of mass spectrometry, with its high information content and unambiguous confirmation, is recommended worldwide for pesticide residue monitoring (Hajšlová and Zrostlíková, 2003; Libin et al., 2006; Liu et al., 2006; Nguyen et al., 2007; Liu et al., 2008). **Mass spectrometry (MS)** is an important analytical technique that allows the identification of various groups of chemical compounds, including pesticides. Regardless of its design or intended use, a mass spectrometer measures the mass-to-charge ratio of charged molecules. Nowadays, **liquid chromatography-tandem mass spectrometry (LC-MS/MS)** is one of te most widely used techniques for pesticide multiresidue analysis in food due to their high sensitivity and selectivity and their ability to screen many pesticides from different chemical classes in a very complex matrix in a single run. LC-MS /MS is suitable for both more polar pesticides and pesticide metabolites, which are often more polar and less volatile than the pesticide itself (Stachniuk and Fornal, 2016). LC-MS /MS is still a challenging analysis due to the often-low concentrations of chemicals and the complexity of the different matrices (Romero-González et al., 2014; Parrilla Vázquez et al., 2016; Valverde et al., 2018; Abbaspour et al., 2019), so the pretreatment of the samples is still one of the most important steps to consider when optimising the method.

For the **extraction of pesticides** from food, several methods have been described in recent years: (i) solid phase extraction (SPE) (Huo et al., 2016; Shamsipur et al., 2016); (ii) solid phase microextraction (SPME) (Pelit et al., 2015; Liang et al., 2017; Choi et al., 2020; Kasperkiewicz and Pawliszyn, 2021) and (iii) dispersive liquid-liquid microextraction (DLLME) (Chu et al., 2015; Farajzadeh et al., 2017; Ghoraba et al., 2018). However, compared to these extractive methods, the so called (iv) "**QuEChERS"** (**Quick, Easy, Cheap, Effective,**

Rugged and Safe) method has become the accepted pre-treatment method for most laboratories worldwide (Barchanska et al., 2018). QuEChERS is a two-step procedure consisting of solid-liquid extraction salting out and dispersion SPE (d-SPE) purification. It was developed in 2003 for the determination of multiclass pesticide residues in fruits and vegetables (Anastassiades et al., 2003). Two international standards organisations have in fact established two different versions of the original QuEChERS method as official methods for the determination of pesticides in food by CEN method 15662:2018 (European Committee for Standardization, 2018) and the AOAC International (Lehotay et al., 2007). This accounts for the broad applicability of the method to a wide range of organic compounds and food matrices. In addition, QuEChERS extraction in combination with chromatographic techniques coupled to mass spectrometry (MS) allows the multiresidue analysis without compromising the sensitivity and selectivity of the method. This approach to sample clean-up, with simplified and streamlined sample preparation, has been recognised for a variety of media, including fruits and vegetables, and is now widely established (Dušek et al., 2018).

2.3.3. Concern for beneficial organisms

Although pest control is as old as agricultural production, Bažok et al. (2020) point out that it was not until the 20th century, with the advent of the first pesticides, that the revolutionary development of the chemical method of crop protection began. They also point out that as the facts about the toxicity of pesticides and their harmful effects on humans, the environment, and non-target organisms became known, concern about the consequences grew. All this has led to an evolution of chemical pest management, i.e., a reduction in the dosage of pesticides used, more ecological studies in the approval process, and the introduction of pesticides with lower toxicity and better biodegradability. In a review of scientific studies, Müller (2018) points out that pest control also exposes non-target organisms to insecticides that, although not lethal to them, can affect their development, physiology, behavior, and communication.

Intensification and modernization of agricultural production has led to a decline in the number of individuals or species due to the negative effects of several factors (Bažok et al., 2015). Special concern is put on **beneficial fauna**. Beneficial fauna is a group of organisms that indirectly have a positive effect on crops by increasing soil fertility, regulating the water-air ratio, or feeding on pests and reducing their numbers. Beneficial soil fauna of sugar beet crops includes insects, earthworms, nematodes, mites, and spiders. Insects that belong to the beneficial soil fauna and are important as indicators of the biological stability of habitats include springtails (order: Collembola) and ground beetles (order: Carabidae) (Kos et al., 2014). Earthworms play a key role in the development and maintenance of physical, chemical, and biological soil properties (Lee, 1985). All species contribute to some degree to the fragmentation and mixing of organic and inorganic matter, forming aggregates that are important for soil drainage and wettability (Edwards, 1998).

The **ground beetles** are important **predators** of numerous pests, they also feed on weed seeds, and are a food source for animals at a higher trophic level. Because of their high abundance, known taxonomy, and sensitivity to change by external factors, they are often used in ecological research (Lövei and Sunderland, 1996). The decline in ground beetle populations has been explained by higher intake of agrochemicals, loss of grasslands for foraging, and increased plot size (Fahrig et al., 2015). The composition of ground beetle fauna and the dynamics of their occurrence in agricultural crops in Croatia is not known, although it is often claimed that insecticides are the main factor in reducing their numbers.

With more than 40,000 described species, ground beetles are the largest family of beetles (Coleoptera) (Lövei and Sunderland, 1996) inhabiting agricultural crops worldwide (Kromp, 1999). Edaphic organisms inhabit different soil layers (Thiele, 1977). They usually move by running on the soil surface, and they can quickly penetrate through soil layers into pre-existing tunnels dug in the soil by some other animals (Stork, 1987). Ground beetles are important in the food web within existing ecosystems (Thiele, 1977, Holland, 2002), especially in agricultural areas or forests where they feed on various economically damaging species (Sunderland, 2022). Depending on their size and macromorphological adaptations for hunting, they may feed on snails, larvae of other insects, springtails, etc. Prey is sought mainly by the sense of smell and, in a smaller number of species, by the sense of sight (for example, species of the genera *Cicindela* Linné and *Notiophilus* Duméril) (Thiele, 1977; Holland, 2002). Only a few genera are herbivorous, such as species of the genera Amara Bonelli and Zabrus Clairville (Thiele, 1977; Toft and Bilde, 2002). Although they are mostly carnivorous, ground beetles can also feed on fungi, weed seeds, fruits, and other plant parts at certain stages of development (Thiele, 1977). At the same time, they constitute a significant part of the diet of animals at a higher trophic level (mammals and birds) (Thiele, 1977; Holland, 2002). Because of their large abundance, known taxonomy, and sensitivity to changes caused by external factors, they are frequently used in ecological research (Lövei and Sunderland, 1996). Declines in ground beetle populations in an area are a result of increased use of agrochemicals, loss of grass belts for feeding, and increased plot size (Fahrig et al., 2015). The composition of ground beetle fauna and the dynamics of their occurrence in Croatia are not known, although it is often claimed that insecticides are the main factor in reducing their numbers. They may encounter insecticides from the group of neonicotinoids by feeding directly on organisms that have fed on the treated crop or through the treated surface on which they move (Albajes et al., 2003; Papachristos and Milonas, 2008; Moser and Obrycki, 2009; Prabhaker et al., 2011; Khani et al., 2012). In a study by Mullin et al. (2010), nearly 100% mortality was observed in 18 ground beetle species exposed to maize treated with IMI, TMX, or clothianidin. According to EC (2006), the marked sensitivity of larvae of the species Poecilus cupreus Linnaeus to IMI has been demonstrated. Member States considered that the concentrations tested were too high to reach a conclusion and no further studies were carried out.

Earthworms as **humifiers** are important members of the soil fauna (Luo et al., 1999). They are classified into three ecological categories depending on the functional adaptation of earthworms to soil conditions and morphological and physiological characteristics (Edwards and Bohlen, 1996). According to Bouché,(1977), the ecological categories include: **(i) epigeic species**, which live on the surface, under plant debris, are smaller, and have distinctive pigmentation. They feed detritivorily, that is, they feed on organic material of plant and animal origin. Their role is the most important in building humus. Their reproduction rate is high. The most important representatives are *Dendrobaena octaedra* Savigny, *Lumbricus rubellus* Hoffmeister, *Eisenia andrei* Bouché, *Eisenia fetida* Savigny. They are followed by **(ii) endogeic species** living in the mineral layer up to 15 cm below the soil surface. They are weakly pigmented or pigmentless. They are geophagous, that is, they feed on soils rich in organic matter. They are often referred to as "humus eaters." They build non-permanent horizontal burrows. The main representatives are: *Allolobophora rosea* Savigny, *Aporrectodea caliginosa* Savigny and *Octolasion lacteum* Örley. There are also **(iii) anecic species** that live at depths up to 3 m. They build permanent and semi-permanent vertical burrows that lead into the mineral layer of the soil and open at the soil surface, where they feed. Pigmentation is present on the dorsal side of the body, while they are unpigmented on the ventral side. They are the largest in size and feed mostly detritally, but there are also species that feed on soil. The most important representatives are *Lumbricus terrestris* Linnaeus and *Aporrectodea longa* Ude.

Earthworms play a key role in the development and maintenance of physical, chemical, and biological soil properties (Lee, 1985). They participate in fragmentation, decomposition, and incorporation of organic matter (Edwards and Bohlen, 1996). All species contribute to some degree to the fragmentation and mixing of organic and inorganic matter, forming aggregates that are important for soil drainage and wettability. In feeding, they stimulate microbiological activity in the soil, which accelerates the decomposition and stabilization of the humus fraction of organic matter; they increase the rate of mineralization of organic matter, which affects nutrient cycling and easier availability to plants; they decrease the rate of carbon and nitrogen in organic matter with food; they convert nitrogen to ammonium or nitrate compounds, while phosphorus and potassium are plant-available (Edwards, 1998).

In agricultural soils, earthworms account for up to 80% of the total animal biomass (Luo et al., 1999). Most common species present in soils under sugar beet production include two engogeic species: *Allolobophora caliginosa* Savigny and *Allolobophora rosea* Savigny and one anecic specie of *Lumbricus terrestris* Linnaeus (Poier and Richter, 1992). Plant protection products, which are commonly used in agriculture pose a threat to earthworm survival and behavior by hindering soil development and decomposition processes. The same neural pathways that allow neonicotinoids to affect invertebrates (Elbert et al., 1991) are also present in earthworms (Volkov et al., 2007). Thus, when neonicotinoids are applied to protect agricultural crops, earthworms encounter the applied granules or treated seeds (as in case of sugare beet) or with contaminated soil or water through direct contact. The way earthworms feed can lead them to ingest contaminated soil and organic particles (Wang et al., 2012).

The seriousness of the problem of contamination of soils and living organisms with pesticides is shown by the results of a study conducted in France (Pelosi et al., 2021). In this study, at least one pesticide was detected in all soil samples and in 92% of the earthworms studied, both in treated crops and in untreated habitats. The vulnerability of earthworms is reflected in the fact that a mixture of at least one insecticide, one herbicide, and one fungicide was found in 90% of soil samples and 54% of earthworm samples. Earthworms are important members of the fauna of agricultural soils, where they account for up to 80% of the total animal biomass (Luo et al., 1999). They play a key role in the development and maintenance of physical, chemical, and biological soil properties (Lee, 1985).

2.4. Neonicotinoids – forbidden yet highly efficient active substances

2.4.1. Neonicotinoid properties and application

With the discovery and market introduction of neonicotinoids, the share of the other group of insecticides began to decline. Neonicotinoids are a group of seven insecticidal active ingredients: imidacloprid, thiamethoxam, clothianidin, thiacloprid, acetamiprid, nitenpyram and dinotefuran. This makes them the most widely used insecticides in the world with a global market value of two to six billion dollars, of which imidacloprid accounts for the largest share at 41% (Jeschke et al., 2011). Imidacloprid was the first to be registered in 1991 and was followed by registrations for nitenpyram and acetamiprid in 1995, thiamethoxam in 1998, thiacloprid and clothianidin in 2001, and dinotefuran in 2002 (Hladik et al., 2018). Until then, during the 1990s, organophosphorus insecticides (43%), pyrethroids (18%), and carbamates (16%) were the most represented in the insecticide market (Jeschke et al., 2011).

Applied to the surface of the plant they act systemically and are transmitted through the xylem to all parts of the plant, providing long-term protection against harmful insects (Magalhaes et al. 2009) cited in Mirjanić and Mitrić (2012). Systemic insecticides act mainly on sucking insects and less on biting insects (Maceljski et al., 2004).

Neonicotinoids were applied mainly by foliar treatment, seed treatment, and soil application (in the form of granules). About 60% of the neonicotinoids produced were used for seed treatment in the form of granules (Jeschke et al., 2011). Seed treatment with neonicotinoids has led to an increase in the frequency of seed treatment as a method of pest control. In the United States, seed treatments have increased from an initial 30% to nearly 100%, and these seeds are sown on approximately 90 million acres (Douglas and Tooker, 2015) cited in Gurian-Sherman (2017). Where permitted, neonicotinoids are also used to treat canola and sunflower seed and for foliar treatment of fruit crops (Valavanidis, 2018).

Because neonicotinoids are used worldwide, their residues can be found in foods, including fruits, vegetables, meat, dairy products, cereals, honey, and baby food, and remain in the environment. Analysis of samples shows that low levels of neonicotinoids are present in fruits and vegetables commonly consumed in the United States (Craddock et al., 2019).

2.4.2. Mode of action and toxicity to non-target organisms

Neonicotinoids have agonistic effects on nicotinic acetylcholine receptors in the central nervous system of insects and mammals (Tan et al., 2007). They mimic acetylcholine, which transmits nerve impulses. The enzyme acetylcholinesterase degrades acetylcholine, leading to the interruption of impulse transmission. Neonicotinoids bind to nicotinic acetylcholine receptors found in the insect synapses (Figure 1). Since acetylcholinesterase cannot degrade neonicotinoids, the transmission of impulses in the cell is not stopped, binding is irreversible resulting in stimulation and paralysis and death of insects (Janjić, 2005). Symptoms of poisoning include hyperactivity, uncoordinated abdominal tremors, wing flexion, tremors, and severe shaking of the whole body, leading to languor and death of the insect (Laurino et al., 2011).

Neonicotinoids are highly toxic to most arthropods and have been widely used for pest control in agriculture and horticulture (Goulson, 2013). particularly for control of insects in the order Hemiptera, the suborder Heteroptera, the order Coleoptera, and the order Lepidoptera (Iwasa et al., 2004).

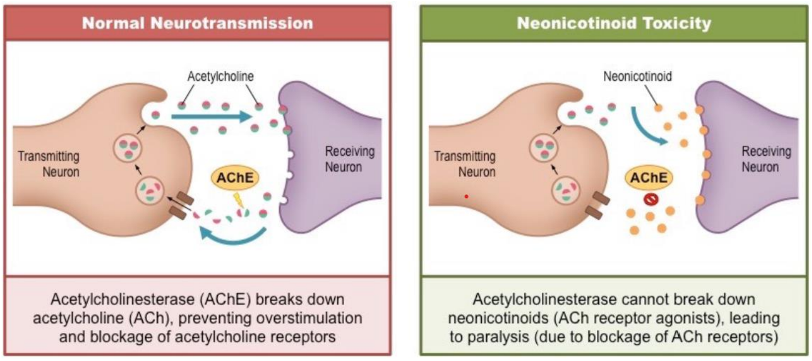

Figure 1. Comparison of normal neurotransmission (left) with neonicotinoid mode of action (right). Source: Iowa State University (2022)

Neonicotinoids have low acute toxicity to mammals, birds, and fish (Tomizawa and Casida, 2005). The active ingredients imidacloprid, clothianidin, dinotefuran, and thiamethoxam are very toxic to bees, thiacloprid and acetamiprid are moderately toxic to bees, and bees exposed to sublethal doses of neonicotinoids may have problems with flight and navigation, loss of sense of taste, and problems learning new tasks, which may impair their ability to forage (Hopwood et al., 2012). In addition, bees exposed to sublethal doses of neonicotinoids overwinter less frequently (Lu et al., 2014).

2.4.3. Causes and consequences of legal prohibition

From 2013 to the present, the situation regarding the use of neonicotinoids has changed drastically in some parts of the world (especially in the European Union). In 2013, the European Commission banned the use of three active ingredients from this group of insecticides (imidacloprid, thiamethoxam, and clothianidin) and placed a two-year moratorium on seed and soil treatments for crops that attract bees and for spring cereals. The authorization remained in effect for the treatment of seeds of winter cereals and sugar beets and for use in protected areas, as well as for foliar treatment of all crops for which insecticides were authorized, but only after flowering. The ban was issued for a period of two years, with the explanation that additional scientific studies are needed to make a final decision. EFSA (European Food Safety Authority) asked all scientific institutions to participate in further research on the effects of neonicotinoids on bees. The ban was then extended for another two years (until 2017). Although it was supposed to expire in 2017, EFSA postponed its lifting until further notice, and the final decision was made on April 27, 2018 (Bažok and Lemić, 2018). The decision was based on three studies prepared by experts based on a detailed review of all relevant research on the above insecticides worldwide (EFSA, 2018a, 2018b, 2018c). The studies analyze the available scientific work and assess the potential risks of using imidacloprid, thiamethoxam, and clothianidin on all crops where they were previously registered. At the same time, the following risks were analyzed for foliar applications: (i) the risk of residues in pollen and nectar; (ii) carryover onto untreated plants; (iii) residues in water sources.

For application by seed treatment, the risks were related to: (i) systemic transmission by the treated plant and possible residues in nectar and pollen (refers to the treated plant and the following plants in the rotation); (ii) contamination by dust drift (risk for field margins and neighboring crops); and (iii) using water with possible insecticide residues. Risks were identified for three organism species, honeybees, solitary bees, and bumblebees. Following EFSA's recommendations, the European Commission adopted a decision to completely ban the use of imidacloprid, thiamethoxam, and clothianidin, except in permanent greenhouses, and the crop thus obtained remains in a permanent greenhouse throughout vegetation (EFSA, 2018a, 2018b, 2018c). The decision was applied in most EU member states from 2019. Already during the decision-making process, many stakeholders expressed their concern about the possible consequences that the ban of these active substances will have on agricultural production. Even then, there were studies (based on the temporary ban of neonicotinoids) that showed that after the ban of neonicotinoids, for example in rapeseed cultivation, there was a significant increase in the number of harmful insects. With the exclusion of neonicotinoids from use, the use of other insecticides (mostly pyrethroids, and by a factor of two to three) increased, resulting in a significant decrease in yields (up to 15%) (Kathage et al., 2017). In addition, in January 2020, on the recommendation of Abdourahime et al. (2019), the European Commission adopted a decision on the temporary extension of the authorization for the use of thiacloprid until April 30, 2020, with a decision on the withdrawal of the authorization for thiacloprid to be taken by the Member States no later than August 3, 2020. Any potential delays must expire no later than February 3, 2021 (FAO/WHO, 2020). This decision was made due to concerns about adverse effects of the active ingredient thiacloprid and its metabolites on the environment, particularly groundwater, and on human health due to reproductive toxicity. This means that the fourth neonicotinoid, thiacloprid, is banned in the EU.

As one of the most important factors for the ban of neonicotinoids is the use of treated seeds and the use of pneumatic seeders for sowing (Elbert et al., 2008) cited by Tapparo et al. (2012). In foliar application, insecticides can cause undesirable damage, killing beneficial insects (pollinators and natural enemies of pests) on treated areas and outside treated areas. Therefore, seed treatment has been considered an environmentally sound alternative with significantly less exposure to bees than foliar application (Cresswell, 2011). However, after sowing treated seeds, neonicotinoids are distributed throughout the plant and reach all plant parts, including pollen, nectar, and swallowing fluid, increasing pollinator exposure to insecticides. Neonicotinoids are believed to be major cause of colony collapse disorder (CCD) (Girolami et al., 2009). In the example of the state of Indiana, USA, 94% of bees are exposed to varying levels of neonicotinoids when sowing treated corn seed (Krupke et al., 2017).

In case of earthworms' toxicological studies show the risk of mortality of individual of all known species when they ingest soil or organic material containing neonicotinoid residues at a concentration ≥ 1 mg/kg. At a concentration of 3 mg/kg, 50% mortality of sparrows is expected. Detailed studies on the effects of neonicotinoids applied to sparrows under real field conditions have not been conducted (Pisa et al., 2014).

In a study by Mullin et al. (2010), nearly 100% mortality was observed in 18 ground beetle species exposed to corn treated with imidacloprid, thiamethoxam or clothianidin. According to (EC, 2006), the marked sensitivity of *Poecilus cupreus* L., larvae to imidacloprid has been demonstrated. According to the member states, the concentrations tested were too high to reach a conclusion and no further studies were conducted. In addition to the active ingredients of neonicotinoids, their metabolites are also harmful to bees and other pollinators. The most common metabolites of imidacloprid are 5-hydroxy-imidacloprid, 4-hydroxy-imidacloprid, dihydroxy-metabolite, olefin, guanidine, and 6-chloronicotinic acid ((Broznić et al., 2008). Two metabolites of imidacloprid, 5-hydroxy-imidacloprid and olefin, have similar toxicity to imidacloprid due to similar chemical structures (Suchail et al., 2001).

Faced with the ban, farmers had to switch to alternative insecticides that are currently approved. And these insecticides have their disadvantages (weaker effect, higher price, more complex application, unique mechanism of action, etc.) (Bažok and Lemić, 2018). Due to the broad spectrum of action of other insecticides and their frequent use, many problems have arisen for agricultural producers in the European Union. Since the ban took effect in December 2013, the United Kingdom has experienced severe crop losses in 2014, 2015, and 2016 due to cabbage stem flea beetles (*Psylliodes chrysocephala*) and aphids (*Myzus persicae*) that have developed resistance to the alternative pyrethroid sprays used to control them. This has led to increased crop losses, lower yields, and a significant reduction in acreage, resulting in fewer flowering crops available in the spring. This is likely to have a negative impact on local bees as well (Dewar, 2017). As expected, the ban on the use of the active ingredients imidacloprid, thiamethoxam, clothianidin and finally thiacloprid in the countries of the European Union has led to major changes in agricultural production. On the one hand, there was an increased occurrence of pests as well as the emergence of pest resistance to older insecticides previously used, a decrease in yields and crop quality, and foliar applications of insecticides also increased significantly. Treating seed with alternative insecticides approved for use can achieve expected yields and protection from pests. The positive sides of the ban, which do not include an analysis of the condition of bee colonies, are certainly the introduction and implementation of alternative methods of pest control and scientific evidence showing that despite the reduced possibility of selecting and using insecticides, it is possible to maintain a stable crop with reduced use of insecticides (Vojvodić and Bažok, 2021).

3. Results and discussion

3.1. Overview of published scientific papers

3.1.1. Publication No. 1

Virić Gašparić, H., Lemic, D., Drmić, Z., Čačija, M., Bažok, R. (2021). The Efficacy of Seed Treatments on Major Sugar Beet Pests: Possible Consequences of the Recent Neonicotinoid Ban. *Agronomy*, **11** *(7)*, 1277. https://doi.org/10.3390/agronomy11071277

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Article

The Efficacy of Seed Treatments on Major Sugar Beet Pests: Possible Consequences of the Recent Neonicotinoid Ban

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Abstract: Sugar beet production remains unprotected after the ban on neonicotinoids, while pest pressure is increasing. Although the organic approach to agriculture is highly welcomed, the question remains whether it will be possible to grow sugar beet without pesticides. The aim of this study is to determine the efficacy of seed treatments with neonicotinoids on the main sugar beet pests, to determine the susceptibility of the pests under the specific climatic conditions and to discuss possible consequences of the ban of neonicotinoids on the future of sugar beet production in southeast Europe. The study was conducted in two different climatic regions in Croatia in two consecutive years. The tested variants were: seed coated with imidacloprid, seed coated with a combination of thiamethoxam and teflutrin and untreated control. Our results showed that seed coatings with imidacloprid and thiamethoxam provided satisfactory protection against wireworms, flea beetles and sugar beet weevils at low population pressure. These pests are regular pests of sugar beet in southern and eastern Europe and therefore need to be controlled. Caterpillars and aphids were present in low populations, so the efficacy of the insecticides tested cannot be determined with certainty. A further research program is needed to find alternative solutions and develop easily implementable strategies for all sugar beet pests. We would propose an authorization of neonicotinoids for seed treatment of sugar beet in the regions with high infestation pressure of the main sugar beet pests.

Keywords: efficacy; imidacloprid; insect pests; thiametoxam; teflutrin; seed

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1. Introduction

According to Kristek et al. [1], sugar beet is a profitable industrial crop grown commercially for sugar production. It is grown in Europe over approximately 2,000,000 ha, which is about 70% of the total arable land in the world. In the Republic of Croatia, sugar represents an important export product. Until 2012, it was grown on 23,215 ha with an average yield of 50.95 t/ha [1]. In recent years, sugar beet production has decreased not only in Croatia, but in all countries of the EU. For example, in 2018, sugar beet yield per hectare decreased by 15% compared to 2017 [2]. Croatian sugar beet production in 2018 was reduced by 40%, which means up to 524 thousand tons compared to the production of the previous year [3].

Sugar beet has a long growing season of up to 200 days, during which it can be exposed to many diseases, insect pests and fungal diseases [4]. According to Meier et al. [5], phenological growth stages are defined as follows: Germination from 00-dry seed to 09-emergence: shoot emerges through the soil surface; leaf development or youth stage from 10-first leaf visible: cotyledons unfolded horizontally to 19-nine and more leaves unfolded; rosette growth (crop cover) from 31-beginning of crop cover: leaves cover 10% of the ground to 39-crop cover complete: Leaves cover 90% of the ground; development of harvestable vegetative plant parts is defined by code 49-Beet root has reached harvestable size. Other stages represent the appearance of inflorescences in the 2nd year of growth.

BBCH identification codes are shown in Figure 1. The decrease in production is due to economic reasons and changing climatic conditions, which led to major problems related to the inability to effectively control pests. In Croatia, pests are a limiting factor in sugar beet production [6].

Figure 1. Phenological development of sugar beet plants is shown by using BBCH codes as follows: 00 sowing (dry seed), 01–09 germination, 10–19 leaf development, 31–39 rosette growth, and 49–development of harvestable vegetative plant parts (root), Meier et al., (1993).

The most common pests that attack sugar beet in the early stages of leaf development or the youth stage (BBCH 10–19) and cause major damage are wireworms (Agriotes spp., Coleoptera: Elateridae), which live in the soil and feed on roots. The main damage occurs in spring and is manifested by thinning of the crop stand and reduction in yield [7]. Flea beetles (*Chaetocnema tibialis* Ill., Coleoptera: Chrysomelidae) cause damage by feeding on leaves and forming small round holes (1 mm in diameter) that enlarge as leaves grow. Sometimes they feed on the stem in addition to the leaves [7]. When the plant is at the cotyledon stage, one flea can cause 33% damage per day, three fleas up to 62% and five fleas can cause as much as 90% damage to the plant. Their activity increases with higher temperatures, i.e., warmer climate-more damage [8]. Sugar beet weevils (*Bothynoderes punctiventris* Germ., Coleoptera: Curculionidae) emerge from the soil in early spring when the upper layer reaches a temperature of 6–10 °C [9]. Normally, sugar beets are at the cotyledon stage at this time, so the damage can be extensive. In one day, an adult weevil can consume up to 50% of the emerged plants in m^2. Again, the insect's feeding rate increases with temperature. At 20 °C, an adult weevil eats 34 mm^2 of leaf area, while at 32 °C the area increases up to 145 mm^2 [8]. The caterpillars of noctuid moths (*Agrotis segetum* Schiff., *Agrotis ypsilon* Hubn. and *Euxoa temera* Hb., Lepidoptera: Noctuidae) can cause damage to more than 150 host plants. The first generation of caterpillars is the most damaging, feeding on underground and aboveground parts of newly emerged plants. Infestation can lead to thinning of the crop stand and reduced yields. Often a caterpillar can bite off the plant haze. According to Čamprag [10], 5–10 caterpillars of the species *A. ipsilon* can damage 90% of plants up to 8 cm high. Later in vegetation, sugar beet can be attacked by several species of aphids (Hemiptera: Aphididae) such as *Smynthurodes phaseoli* West, *Pemphigus fuscicornis* Koch and *Pemphigus betae* Doane on roots and *Aphis fabae* Scopoli and *Myzus persicae* Sulzer as the most common species on aboveground organs [11]. In addition to aphids, caterpillars of the rapeseed moth (*Scrobipalpa ocellatella* Boyd, Lepidoptera: Gelechiidae), the cabbage moth and bright-line brown-eye moth (*Mamestra brassicae* L. and *Lacanobia oleracea* L.) as well as silver Y (*Autographa gamma* L., Lepidoptera: Noctuidae) can also attack sugar beet during vegetation, but these pests are rarely controlled [12].

According to Bažok et al. [13], soil pests (mainly wireworms) were regularly controlled in Croatia by the application of lindane, terbufos, forat, chlormephos, chlorpyrifos, phoxim and carbofuran. For flea beetle control, a wide range of active ingredients such as diazinon, phosalone, monocrotophos, thiometon, carbaryl, alphamethrin, cypermethrin and deltamethrin were used. Aphids were mostly controlled with systemic active ingredients tiometon, dimethoate, methyl demeton, carbamil etc. or permethrin. The average amount of active ingredient/ha of sugar beet grown was 1.64 kg during 1981–1989. During the 1990s to 2018, the pests were controlled with 0.05 to 0.1 kg active ingredient of neonicotinoids as seed treatment/ha of grown sugar beet. Foliar application of insecticides was made only when necessary to control sugar beet weevil. Therefore, neonicotinoids contributed to a large reduction in the amount of insecticide used in sugar beet cultivation [13]. It has been confirmed that treatment of sugar beet seeds with imidacloprid provides satisfactory protection of young plants against low to moderate infestation by wireworms, flea beetles and aphids [14]. Hauer et al. [15] analyzed the possible consequences of the ban of neonicotinoids on pest incidence on sugar beet under production conditions in north and central Europe. They concluded that seed treatment with neonicotinoids provides sufficient protection against aphids, the vectors of sugar beet virus. Since aphids do not occur annually in every field, they predicted that the ban would not have serious consequences for sugar beet production. In addition, they suggested developing monitoring systems and models to identify regions (and years) with high pest risk and allowing the use of insecticides for seed treatment only when aphid pressure is expected to be high. In 2018, the EU Commission completely banned the use of the active substances imidacloprid, clothianidin and thiamethoxam in the field [16–18], and only their use in permanent greenhouses remains possible because of the risk to bees. Now, a large proportion of arable and industrial crops, including sugar beet, remain unprotected while pest pressure increases. In their work, Hauer et al. [15] analyzed sugar beet production in the countries of northwestern Europe and did not consider the different climatic conditions and pest occurrence in eastern and southeastern Europe. Considering climate change, the global economic and health crisis and the FAO Sustainable Development Goals (SDGs) (e.g., zero hunger by 2030 [19]) the question remains whether it will be possible to grow food, in this case sugar beet, without pesticides.

The aim of this study was to determine: (1) the efficacy of seed treatment with neonicotinoids on the main sugar beet pests during two growing seasons and different climatic conditions at two locations; (2) the actual vulnerability to individual pests under the specific agro climatic conditions and the extent to which neonicotinoid seed treatment is effective in preventing damage; and (3) the possible consequences of the ban of neonicotinoids on the future of sugar beet production in southeastern Europe.

2. Materials and Methods

2.1. Experimental Fields and Trial Design

The efficacy of neonicotinoids applied by seed treatment was investigated by two-year experiments in 2015 and 2016 on the territory of two dissimilar counties in Croatia, Virovitica-Podravina County at location Lukač (45°52′26″ N 17°25′09″ E) and Vukovar-Sirmium County at location Tovarnik (45°09′54″ N 19°09′08″ E).

Treatment 1 was sown with the untreated seeds, treatment 2 was treated with imidacloprid at 0.91 mg a.i./seed and treatment 3 was treated with a combination of thiamethoxam and teflutrin at 0.36 + 0.036 mg a.i./seed. Seeds were sown between 29 March and 9 April at both locations in both years. Seeding was done with a six-row harrow at a depth of 3 cm. Distance between rows was 45 cm, while the inter-row spacing was 18 cm (i.e., 123,321 seeds/ha). Each experiment included three treatments in three replicates, each sown in four rows on a total area of 1000 m^2.

2.2. Trial Assessments

Climatic conditions (air and soil temperature as well as amount of precipitation) were monitored for the period from April to September in both years by Croatian Meteorological and Hydrological Service. Data on mean air and ground temperatures and total precipitation were collected and analyzed for the meteorological stations Virovitica and Gradište, located no further than 20 km from the experimental sites.

For each of the three treatments in the experimental fields in Lukač and Tovarnik assessment of the attack and damage of the most important pests was carried out through two growing seasons at selected internal two rows (to avoid edge effect) of 10 m^2 length. The readings were conducted once per week. At each reading the plant development stage according BBCH scale [5] was recorded.

Damages by wireworms were evaluated by counting all emerged plants within 10 m^2 in order to establish crop stand. Flea beetle damages were assessed by visual inspection. The examined plants were classified into six categories, 0 to 5 according to Čamprag [10]. Damages from sugar beet weevil were identified using a plastic square tool covering 1 sqm thrown randomly four times across the surface of each treatment. All plants within the square were examined and classified based on the percentage of damage into five categories [10]. The intensity of aphids' infestation of the examined plants was determined according to Banks 1–5 scale [20]. The percentage of plants infected by caterpillars was determined by visual inspection of the plants.

According to Townsend and Heuberger [21] percentage of damage (%) of flea beetles, sugar beet weevil and aphids was calculated based on the frequency of plants in the groups for each particular pest:

$$D(\%) = \left(\frac{\Sigma(f \times n)}{(a \times N)}\right) \times 100 \quad (1)$$

where D (%) = percentage of damage; f = number of plants in particular class; n = class value; a = number of classes; N = number of assessed plants.

2.3. Statistical Analysis

The data on the crop stand/10 m^2, percent of damage caused by flea beetles, sugar beet weevil and aphids and percent of infected plants by caterpillars were analyzed by analysis of variance (ANOVA) using the AOV factorial method with three factors using ARM 9 software [22]. Where appropriate, data were logx + 1 or arc. sin $\sqrt{x}$ transformed. The first factor was location which was considered as a fixed factor due to a limited production area of sugar beet and characteristic weather conditions. The second factor was insecticide treatment and the third factor was year. A Tukey Post-Hoc test was used to determine which mean values of the variants were significantly different after a significant test result ($p < 0.05$).

3. Results

Figure 2A–C show the climatic analysis between Lukač and Tovarnik during two growing seasons. There were no significant differences between climatic conditions at both sites between the two years studied. Compared to Tovarnik, average air and soil temperatures were lower in Lukač in both years of study. The amount of precipitation was higher in Lukač in 2015, while in 2016 the differences were not significant.

During the two growing seasons, infestations of wireworms, flea beetles, sugar beet weevils, aphids and caterpillars were recorded depending on the location. No pests were detected before BBCH 12, which corresponds to youth stage of leaf development (first pair of leaves unfolded). Table 1 shows the results on crop stand on the experimental plots and indicates wireworm damage on sugar beet plants. In 2015, a heavy wireworm infestation was detected at the Tovarnik site. Plant density on the untreated plots was significantly reduced during BBCH 19 and 31 compared to the treated plots. In Lukač, no significant difference was found between the variants. In 2016, the evaluation showed differences in the number of plants on treated and untreated plots at almost all stages of development, leading to the conclusion that both sites were infested with wireworms (Table 1).

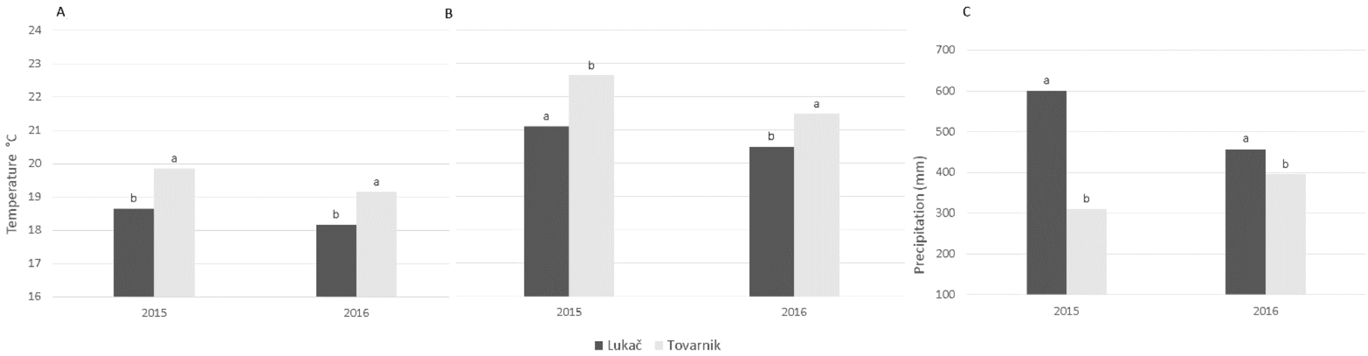

Figure 2. Climate conditions of (**A**) average air temperature, (**B**) average soil temperature, and (**C**) precipitation) in Lukač and Tovarnik monitored at nearest climate stations and results of statistical analysis. Values followed by the same letters are not significantly different ($p > 0.05$; HSD test) between locations. Significant differences between years were not established.

Table 1. Crop stand (number of plants/10 m^2) established on different treatments in different plant developmental stages (BBCH).

Treatment	Locality	BBCH 12	BBCH 16	BBCH 19	BBCH 31	BBCH 34
Wireworm damages, 2015						
Untreated	Lukač	96.75 ± 1.71 ^{a}	99.25 ± 6.55 ^{a}	93.75 ± 2.63 ^{ab}	94.00 ± 1.83 ^{ab}	n/a
	Tovarnik	54.75 ± 6.40 ^{c}	57.75 ± 6.02 ^{c}	33.50 ± 2.38 ^{c}	31.50 ± 2.89 ^{d}	n/a
Imidacloprid	Lukač	97.75 ± 10.37 ^{a}	106.00 ± 4.83 ^{a}	104.25 ± 4.86 ^{a}	104.50 ± 1.91 ^{a}	n/a
	Tovarnik	92.25 ± 4.03 ^{a}	93.75 ± 2.75 ^{a}	92.00 ± 1.41 ^{ab}	90.50 ± 0.58 ^{b}	n/a
Thiamethoxam ± teflutrin	Lukač	90.50 ± 19.35 ^{a}	93.75 ± 19.94 ^{a}	86.25 ± 22.13 ^{b}	90.75 ± 17.00 ^{b}	n/a
	Tovarnik	77.25 ± 3.50 ^{b}	79.25 ± 2.06 ^{b}	79.00 ± 1.83 ^{b}	78.00 ± 2.16 ^{c}	n/a
LSD $p = 0.05$		12.51	12.86	14.08	10.90	n/a
Wireworm damage, 2016						
Untreated	Lukač	66.75 ± 6.18 ^{ab}	70.50 ± 9.15 ^{ab}	70.25 ± 8 ^{bc}	69.75 ± 9.43 ^{bc}	69.50 ± 9.00 ^{bc}
	Tovarnik	58.00 ± 3.74 ^{b}	56.50 ± 3.11 ^{b}	55.75 ± 3.59 ^{c}	55.00 ± 2.71 ^{c}	54.25 ± 3.40 ^{b}
Imidacloprid	Lukač	82.25 ± 29.24 ^{a}	83.50 ± 26.96 ^{a}	85.75 ± 27.55 ^{ab}	86.00 ± 28.24 ^{ab}	84.75 ± 28.91 ^{a}
	Tovarnik	77.25 ± 6.34 ^{ab}	76.50 ± 6.18 ^{ab}	75.50 ± 6.03 ^{abc}	74.75 ± 7.04 ^{abc}	74.25 ± 6.70 ^{ab}
Thiamethoxam ± teflutrin	Lukač	86.50 ± 12.71 ^{a}	90.75 ± 13.12 ^{a}	92.75 ± 14.52 ^{a}	93.00 ± 15.23 ^{a}	92.50 ± 15.29 ^{a}
	Tovarnik	87.25 ± 8.54 ^{a}	87.00 ± 8.37 ^{a}	86.50 ± 9.33 ^{ab}	85.25 ± 8.66 ^{ab}	85.00 ± 9.13 ^{a}
LSD $p = 0.05$		21.06	19.93	20.18	20.83	21.17

Means followed by same letter within the column do not significantly differ ($p = 0.05$, Duncan's New MRT). Means descriptions are reported in transformed data units and are not de-transformed. Analyses were performed on arcsine square root percent transformed data.

Crop stand as a result of wireworm damage was significantly affected by location, insecticide treatment and their combination at all stages of plant development (from BBCH 12 to BBCH 34), proving that wireworm infestation was significantly different at two locations and also that neonicotinoid seed treatments are able to protect plants from wireworm infestation under different environmental conditions (Table 2). The third factor, year, significantly affected plant density in the first two observations (BBCH 12 and 16), while later in plant development plant density was not significantly affected by year. However, plant densities were significantly ($p > 0.05\%$) influenced by the combination of location and year and by the combination of insecticide treatment and year throughout the course of plant development from BBCH 12 to BBCH 34. The combination of all three factors significantly influenced plant densities over the period of plant development from BBCH 19 to BBCH 34.

Table 2. Factorial analysis (ANOVA) of the plant density in different developmental stages of the beets.

Source of Variation	df	BBCH				
		12	16	19	31	34
Total	47					
Replication	3					
Location (A)	1	0.0018 **	0.0001 **	0.0001 **	0.0001 **	0.0001 **
Insecticide application (B)	2	0.0003 **	0.0001 **	0.0001 **	0.0001 **	0.0001 **
A × B	2	0.05 *	0.0564	0.0027 **	0.0002 **	0.0001 **
Year (C)	1	0.0241 *	0.0036 **	0.3071	0.4013	0.9953
A × C	1	0.0347 *	0.0437 *	0.0297 *	0.0034 *	0.0008 **
B × C	2	0.0915	0.0323 *	0.0257 *	0.0190 *	0.0032 **
A × B × C	2	0.1931	0.4022	0.0226 *	0.0039 **	0.0005 **
Error	33					

* significant at p = 0.05, ** significant at p = 0.01.

Table 3 shows the percentage of flea beetle damage on sugar beet plants. In 2015, flea damage was higher at the very beginning of vegetation in Tovarnik than in Lukač and significantly higher on untreated varieties in both locations. In Lukač, the plants developed well until the end of BBCH 16 (about day 45), as the infestation stopped. All treated varieties in both trials and both years of the study significantly reduced pest infestation. Significant differences in flea beetle numbers on treated versus untreated variants were observed during sugar beet rosette growth and plant cover development. In 2016, a significant difference in flea damage between treated and untreated variants was observed in the Lukač trial throughout the development stage. At the same time, the damage in the Tovarnik trial was much lower and the differences between the treated and untreated variants were smaller (Table 3).

Table 3. Sugar beet flea beetle damage (according to Towsend-Heuberger) on sugar beet plants in different developmental stages (BBCH).

Treatment	Locality	BBCH 12	BBCH 16	BBCH 19	BBCH 31	BBCH 34
Flea beetle damages, 2015						
Untreated	Lukač	19.89 ± 0.21 b	42.45 ± 0.51 b	51.60 ± 1.38 b	n/a	n/a
	Tovarnik	69.54 ± 0.29 a	74.99 ± 4.32 a	60.84 ± 0.16 a	82.27 ± 1.65 a	83.33 ± 0.00 a
Imidacloprid	Lukač	0.61 ± 0.12 c	3.66 ± 0.32 cd	6.10 ± 3.26 c	n/a	n/a
	Tovarnik	1.43 ± 0.36 d	1.59 ± 0.98 c	1.86 ± 0.17 b	2.40 ± 0.85 b	2.98 ± 1.25 c
Thiamethoxam ± teflutrin	Lukač	1.20 ± 0.15 c	7.23 ± 0.44 c	5.88 ± 0.50 c	n/a	n/a
	Tovarnik	3.63 ± 0.06 cd	3.54 ± 0.19 c	5.71 ± 0.34 b	4.25 ± 0.44 b	5.62 ± 0.44 b
HSD p = 0.05		3.01	5.50	6.87	2.27	1.86
Flea beetle damages, 2016						
Untreated	Lukač	43.80 ± 2.70 a	50.42 ± 0.79 a	65.78 ± 1.46 a	73.83 ± 1.70 a	79.00 ± 2.05 a
	Tovarnik	13.16 ± 2.36 bc	14.31 ± 3.17 b	15.36 ± 2.76 b	15.60 ± 1.49 c	19.84 ± 3.12 b
Imidacloprid	Lukač	14.13 ± 3.86 b	15.76 ± 1.46 b	17.60 ± 1.86 b	19.66 ± 0.68 b	21.32 ± 1.92 b
	Tovarnik	7.10 ± 2.32 c	7.88 ± 2.23 c	8.34 ± 2.04 c	9.26 ± 1.72 d	12.65 ± 2.24 c
Thiamethoxam ± teflutrin	Lukač	9.94 ± 1.30 bc	13.05 ± 2.70 b	17.41 ± 1.38 b	18.60 ± 1.24 bc	20.11 ± 2.24 b
	Tovarnik	7.12 ± 2.86 c	7.70 ± 2.82 c	8.26 ± 3.14 c	8.88 ± 3.32 d	11.14 ± 4.40 c
HSD p = 0.05		6.34	4.67	3.91	3.77	5.65

Means followed by the same letter do not significantly differ (p = 0.05, Tukey's HSD). Means descriptions are reported in transformed data units and are not de-transformed. Analyses were performed on arcsine square root percent transformed data.

Plant damage caused by flea beetles was significantly influenced by location and year at two plant development stages (BBCH 16 and 19) (Table 4). The average percentage of damage at the Lukač site was significantly higher and amounted to 22.1 and 27.4% compared to the damage observed at the Tovarnik site, where it amounted to 17.4% and 18.6% in BBCH 16 and BBCH 19, respectively. At the same time, plant damage was significantly affected by insecticide treatments at all three stages of plant development (BBCH 12–19), proving that neonicotinoid seed treatments protect plants against flea beetle infestation. The significant ($p > 0.05\%$) interaction between all three factors (location × insecticide treatment × year) for flea beetle damage was present at all three plant developmental stages. A significant insecticide treatment × location interaction for flea beetle damage was observed at the first and last observed plant development stages (BBCH 12 and 19). Significant interactions between "location × year" and "insecticide application × year" for flea beetle damage existed at all three observed plant development stages (from BBCH 12–19). As no flea beetle damage was observed at the Lukač site in 2015 at BBCH 31 and BBCH 34, factorial analysis was not performed for these two samplings.

Table 4. Factorial analysis (ANOVA) of the percent of damages (according to Towsend-Heuberger) caused by sugar beet flea beetle in different developmental stages of sugar beet plants.

Source of Variation	df	BBCH		
		12	16	19
Total	47			
Replication	3			
Location (A)	1	0.5112	0.0001 **	0.0001 **
Insecticide application (B)	2	0.0001 **	0.0001 **	0.0001 **
A × B	2	0.0048 **	0.9624	0.0001 **
Year (C)	1	0.7003	0.0003 **	0.0101 **
A × C	1	0.0001 **	0.0001 **	0.0001 **
B × C	2	0.0001 **	0.0001 **	0.0001 **
A × B × C	2	0.0001 **	0.0001 **	0.0001 **
Error	33			

** significant at $p = 0.01$.

Table 5 shows the damage caused by the sugar beet weevil damages on sugar beet plants. The attack of sugar beet weevils was relatively weak in 2015 at both locations and treatments. As expected, some efficacy of insecticides in reducing the level of damage was observed in the Lukač trial. No more sugar beet weevils were observed during BBCH 31–34. In 2016, the infestation was significantly higher, especially in the trial in Tovarnik. Damage on untreated plots was significantly higher than on treated ones. Under these conditions, seed treatment achieved satisfactory results in protecting sugar beet at the most sensitive stages of development.

Table 5. Sugar beet weevil damage (according to Towsend-Heuberger) on sugar beet plants in different developmental stages (BBCH).

Treatment	Locality	BBCH 12	BBCH 16	BBCH 19	BBCH 31	BBCH 34
Sugar beet weevil damages, 2015						
Untreated	Lukač	0.51 ± 0.59 ns	0.21 ± 0.16 ns	5.46 ± 4.94 ^{a}	n/a	n/a
	Tovarnik	0.00 ± 0.00 ns	0.32 ± 0.41 ns	3.07 ± 7.26 ^{ab}	n/a	n/a
Imidacloprid	Lukač	0.31 ± 0.63 ns	0.00 ± 0.00 ns	0.59 ± 5.28 ^{ab}	n/a	n/a
	Tovarnik	0.00 ± 0.00 ns	2.46 ± 0.37 ns	1.85 ± 7.20 ^{ab}	n/a	n/a
Thimatetoxam ± teflitrin	Lukač	0.28 ± 0.56 ns	0.00 ± 0.00 ns	0.00 ± 0.00 ^{b}	n/a	n/a
	Tovarnik	0.00 ± 0.00 ns	0.87 ± 0.32 ns	1.78 ± 5.46 ^{ab}	n/a	n/a
HSD $p = 0.05$		0.81	2.56	5.31	n/a	n/a
Sugar beet weevil damages, 2016						
Untreated	Lukač	4.76 ± 1.38 ^{b}	2.59 ± 0.14 ^{cd}	6.35 ± 4.52 ^{b}	11.57 ± 6.13 ^{ab}	11.87 ± 5.32 ^{bc}
	Tovarnik	20.05 ± 0.24 ^{a}	17.01 ± 0.42 ^{a}	17.03 ± 2.70 ^{a}	19.32 ± 3.42 ^{a}	19.98 ± 1.91 ^{a}
Imidacloprid	Lukač	0.00 ± 0.00 ^{c}	0.86 ± 0.60 ^{d}	5.17 ± 5.21 ^{b}	17.17 ± 2.53 ^{a}	17.15 ± 2.26 ^{ab}
	Tovarnik	0.00 ± 0.00 ^{c}	7.63 ± 0.28 ^{b}	7.63 ± 1.75 ^{b}	6.30 ± 2.17 ^{b}	7.48 ± 1.73 ^{cd}
Thimatetoxam ± teflitrin	Lukač	0.00 ± 0.00 ^{c}	1.21 ± 0.47 ^{d}	2.33 ± 1.16 ^{b}	3.88 ± 2.03 ^{b}	3.16 ± 4.00 ^{d}
	Tovarnik	0.00 ± 0.00 ^{c}	6.43 ± 0.05 ^{bc}	6.43 ± 0.32 ^{b}	6.87 ± 1.04 ^{b}	6.78 ± 1.32 ^{cd}
HSD $p = 0.05$		8.59	3.11	6.24	7.82	7.75

Means followed by the same letter do not significantly differ ($p = 0.05$, Tukey's HSD). Means descriptions are reported in transformed data units and are not de-transformed. Analyses were performed on arcsine square root percent transformed data.

Plant damage caused by sugar beet weevil was significantly ($p > 0.05\%$) influenced by location and insecticide treatment at all three plant development stages (from BBCH 12 to BBCH 19) and significantly influenced by year at BBCH 16 and BBCH 19 (Table 6).

The average percentage of damage at the Lukač site was significantly lower. It was 0.63, 0.67 and 2.49% compared to the damage observed at the Tovarnik locality, where it was 3.42, 5.36 and 5.41% at BBCH 12, BBCH 16 and BBCH 19, respectively. At the same time, plant damage was significantly affected by the insecticide treatments at all three stages of plant development (BBCH 12–19), proving that the neonicotinoid seed treatments protect plants from sugar beet weevil attack at the early stages of development. The significant interaction ($p > 0.05\%$) between all the three factors (location × insecticide treatment × year) for sugar beet weevil damages was present only at BBCH 19. No significant insecticide "treatment × location" interaction for sugar beet weevil damage was observed at any stage of plant development. Significant interactions ($p > 0.05\%$) between "location × year" and "insecticide application × year" for sugar beet weevil damage existed at two of three observed plant developmental stages (BBCH 12 and BBCH 16). The factorial analysis was not performed for BBCH 31 and BBCH 34, as no sugar beet weevil damages were recorded at either location.

Table 6. Factorial analysis (ANOVA) of the percent of damages (according to Towsend-Heuberger) caused by sugar beet weevil in different developmental stages of sugar beet plants.

Source of Variation	df	BBCH		
		12	16	19
Total	**47**			
Replication	3			
Location (A)	1	0.0001 **	0.0001 **	0.0018 **
Insecticide application (B)	2	0.0001 **	0.0009 **	0.0001 **
A × B	2	0.9085	0.0862	0.4230
Year (C)	1	0.7003	0.0001 **	0.0001 **
A × C	1	0.0001 **	0.0017 **	0.1660
B × C	2	0.0001 **	0.0361 *	0.9670
A × B × C	2	0.6905	0.4048	0.0430 *
Error	33			

* significant at $p = 0.05$, ** significant at $p = 0.01$.

Table 7 shows the percentage of sugar beet plants infested with caterpillars at different stages of development. In 2015, no caterpillars were observed in the youth stage in the field trials in Tovarnik. In Lukač, caterpillars appeared 20 days earlier than expected, at the leaf development stage at BBCH 19. In 2016, no caterpillar damage was observed in Tovarnik, while in Lukač, caterpillars appeared from BBCH 31. The maximum caterpillar infestation was lower than in the previous year.

Table 7. Sugar beet plants infected (in %) by caterpillars in different developmental stages (BBCH).

Treatment	Locality	BBCH 19	BBCH 31	BBCH 34	BBCH 36	BBCH 38
Caterpillar damages, 2015						
Untreated	Lukač	38.52 ± 3.68 [a]	19.99 ± 0.61 [a]	53.91 ± 7.20 [a]	17.94 ± 5.38 [a]	18.26 ± 0.08 [a]
	Tovarnik	0.00 ± 0.00 [c]	0 ± 0 [c]	0.47 ± 4.64 [b]	0.47 ± 4.66 [c]	1.33 ± 0.26 [c]
Imidacloprid	Lukač	5.48 ± 1.66 [b]	8.69 ± 0.51 [ab]	8.98 ± 12.21 [b]	11.43 ± 3.89 [ab]	9.31 ± 0.15 [ab]
	Tovarnik	0.00 ± 0.00 [c]	0 ± 0 [c]	1.64 ± 8.59 [b]	1.63 ± 8.55 [bc]	2.01 ± 0.55 [bc]
Thiamethoxam ± teflutrin	Lukač	0.06 ± 2.78 [c]	7.32 ± 1.53 [b]	9.56 ± 3.51 [b]	10.23 ± 2.65 [ab]	6.61 ± 0.2 [abc]
	Tovarnik	0.00 ± 0.00 [c]	0.00 ± 0.00 [c]	0.28 ± 3.48 [b]	0.41 ± 4.36 [c]	0.97 ± 0.22 [c]
HSD $p = 0.05$		1.09	10.83	12.03	7.52	6.16
Caterpillar damages, 2016						
Untreated	Lukač	0.00 ± 0.00 ns	88.15 ± 16.88 [a]	99.60 ± 7.24 [a]	100 ± 0.00 [a]	100.00 ± 0.00 [a]
	Tovarnik	0.00 ± 0.00 ns	0.00 ± 0.00 [c]	0.00 ± 0.00 [c]	0.00 ± 0.00 [c]	0.00 ± 0.00 [c]
Imidacloprid	Lukač	0.00 ± 0.00 ns	17.23 ± 13.03 [ab]	80.33 ± 9.58 [b]	74.75 ± 4.53 [b]	61.58 ± 12.31 [b]
	Tovarnik	0.00 ± 0.00 ns	0.00 ± 0.00 [c]	0.00 ± 0.00 [c]	0.00 ± 0.00 [c]	0.00 ± 0.00 [c]
Thimatetoxam ± teflitrin	Lukač	0.00 ± 0.00 ns	53.16 ± 9.95 [b]	75.27 ± 16.68 [b]	70.69 ± 8.17 [b]	63.34 ± 15.69 [b]
	Tovarnik	0.00 ± 0.00 ns	0.00 ± 0.00 [c]	0.00 ± 0.00 [c]	0.00 ± 0.00 [c]	0.00 ± 0.00 [c]
HSD $p = 0.05$		ns	21.28	11.89	1.99	18.87

Means followed by the same letter do not significantly differ ($p = 0.05$, Tukey's HSD). Means descriptions are reported in transformed data units and are not de-transformed. Analyses were performed on arcsine square root percent transformed data.

The percentage of plants damaged by caterpillars was significantly influenced by location on all sampling dates (Table 8). The percentage of damaged plants was significantly higher in Lukač than in Tovarnik. At the same time, the percentage of damaged plants was significantly influenced by insecticide treatments at BBCH 19, 31, 34 and 36, proving that insecticide treatments significantly protected young sugar beet plants from caterpillars until BBCH 38. No significant differences were observed between two insecticide treatments in percentage of damaged plants except in Lukac at BBCH 19. The percentage of damaged plants was also significantly influenced by the year. Damage was higher in 2016 compared to 2015 at all observed plant development stages. The significant interaction ($p > 0.05\%$) between all three factors (location × insecticide treatment × year) for damage caused by caterpillars was present only at BBCH 36. Significant interaction between insecticide "treatment × location" and "location × year" for damage caused by caterpillars was observed at all stages of crop development. Significant interactions between "insecticide application × year" for damage caused by caterpillars were only present at one observed plant development stage (BBCH 36).

Table 8. Factorial analysis (ANOVA) of the percent of plants with damages caused by caterpillars in different developmental stages of sugar beet plants (** significant at $p = 0.01$.).

Source of Variation	**df**	**BBCH**				
		19	**31**	**34**	**36**	**38**
Total	**47**					
Replication	3					
Location (A)	1	0.0001 **	0.0001 **	0.0001 **	0.0001 **	0.0001 **
Insecticide application (B)	2	0.0001 **	0.0017 **	0.0001 **	0.0001 **	0.0715
A × B	2	0.0001 **	0.0017 **	0.0001 **	0.0001 **	0.0871
Year (C)	1	0.0001 **	0.0001 **	0.0001 **	0.0001 **	0.0009 **
A × C	1	0.0001 **	0.0001 **	0.0001 **	0.0001 **	0.0001 **
B × C	2	0.5607	0.8614	0.8940	0.0001 **	0.4821
A × B × C	2	0.5607	0.8614	0.6514	0.0002 **	0.8316
Error	33					

** significant at $p = 0.01$.

No significant aphid damage was observed in the 2015 trials (Table 9). Some minor damage occurred at the later stage, during rosette growth (BBCH 31), but according to the Townsend-Heuberger formula the percentage of infested plants did not exceed 3.5%. In 2016, during the whole vegetation, the aphid infestation on the trial in Lukač was below 2%. In Tovarnik, damage occurred during BBCH 31 and ranged from 4% in the control to 12% in the imidacloprid treatment, while no significant damage was observed in the thiamtetoksam treatment.

Table 9. Aphid damages on sugar beet plants in different developmental stages.

Treatment	**Locality**	**BBCH 19**	**BBCH 31**	**BBCH 34**	**BBCH 36**	**BBCH 38**
Aphid damages, 2015						
Untreated	Lukač	0.05 ± 1.53 ns	0.00 ± 0.00 ^b	0.10 ± 2.25 ^b	1.65 ± 0.23 ns	0.11 ± 0.12 ns
	Tovarnik	0.13 ± 2.42 ns	0.19 ± 0.18 ^ab	0.33 ± 2.50 ^b	1.43 ± 0.13 ns	0.51 ± 0.31 ns
Imidacloprid	Lukač	0.00 ± 0.00 ns	0.00 ± 0.00 ^ab	0.05 ± 1.44 ^b	0.95 ± 0.20 ns	0.15 ± 0100 ns
	Tovarnik	0.23 ± 3.38 ns	0.75 ± 0.62 ^a	2.87 ± 3.79 ^a	1.77 ± 0.38 ns	3.27 ± 3.68 ns
Thiamethoxam ± teflutrin	Lukač	0.41 ± 2.58 ns	0.00 ± 0.00 ^b	0.55 ± 1.51 ^ab	2.82 ± 0.18 ns	0.21 ± 0.16 ns
	Tovarnik	0.16 ± 2.65 ns	0.28 ± 0.24 ^ab	0.29 ± 3.70 ^b	2.03 ± 0.14 ns	0.48 ± 0.24 ns
HSD $p = 0.05$		1.31	0.66	1.35	2.77	3.48
Aphid damages, 2016						
Untreated	Lukač	0.00 ± 0.00 ^c	0.00 ± 0.00 ^c	0.00 ± 0.00 ^b	0.86 ± 0.12 ^bc	0.0 ± 0.01 ^b
	Tovarnik	1.76 ± 0.20 ^b	1.76 ± 0.20 ^b	4.52 ± 0.30 ^a	3.65 ± 0.30 ^ab	4.52 ± 0.30 ^a
Imidacloprid	Lukač	0.00 ± 0.00 ^c	0.00 ± 0.00 ^c	0.20 ± 0.16 ^b	0.28 ± 0.22 ^c	0.20 ± 0.16 ^b
	Tovarnik	5.52 ± 0.18 ^a	5.52 ± 0.18 ^a	11.69 ± 0.24 ^a	9.91 ± 0.25 ^a	11.69 ± 0.24 ^a
Thiamethoxam ± teflutrin	Lukač	0.00 ± 0.00 ^c	0.00 ± 0.00 ^c	0.17 ± 0.08 ^b	0.35 ± 0.14 ^c	0.17 ± 0.08 ^b
	Tovarnik	0.00 ± 0.00 ^c	0.00 ± 0.00 ^c	1.05 ± 0.25 ^b	1.20 ± 0.19 ^bc	1.05 ± 0.24 ^b
HSD $p = 0.05$		2.00	2.00	1.84	2.23	1.84

Means followed by the same letter do not significantly differ ($p = 0.05$, Tukey's HSD). Means descriptions are reported in transformed data units and are not de-transformed. Analyses were performed on arcsine square root percent transformed data.

Aphid infestation was very low in both study years and was significantly ($p > 0.05$) influenced by location on four of five sampling dates (from BBCH 31 to 38) (Table 10). Percentages of damage were significantly higher in Tovarnik (0.66, 1.19, 2.36 and 4.02% at BBCH 31, 34, 36 and 38, respectively) than in Lukač (0.03, 0.05, 0.77 and 0.15% at BBCH 31, 34, 36 and 38, respectively). The percentage of damage was significantly affected by insecticide treatments at the three observed plant development stages (BBCH 31–36). However, damage was significantly higher in imidacloprid treated plots compared to thiamethoxam and untreated plots. Percent damage was significantly influenced by year at two observed plant developmental stages. The significant interaction ($p > 0.05\%$) between all three factors (location × insecticide treatment × year) for aphid damage does not exist in any observed plant development stage. Significant ($p > 0.05\%$) interaction between insecticide "treatment × location" for aphid damage was observed in all observed plant developmental stages. A significant interaction between "location × year" and "insecticide application × year" for aphid damage exists in three out of three observed plant development stages (from BBCH 34 to BBCH 38).

Table 10. Factorial analysis (ANOVA) of the percent of damages caused by aphids in different developmental stages of sugar beet plants (* significant at $p = 0.05$, ** significant at $p = 0.01$.).

Source of Variation	df	BBCH				
		19	31	34	36	38
Total	**47**					
Replication	3					
Location (A)	1	0.4094	0.0001 **	0.0001 **	0.0001 **	0.0001 **
Insecticide application (B)	2	0.6401	0.0102 *	0.0001 **	0.2186	0.0007 **
A × B	2	0.0423 *	0.0412 *	0.0001 **	0.0064 **	0.0008 **
Year (C)	1	0.0114 *	0.1394	0.6362	0.1289	0.0009 **
A × C	1	0.6372	0.0593	0.0040 **	0.0001 **	0.0009 **
B × C	2	0.2380	0.4219	0.0061 **	0.0012 **	0.0462 *
A × B × C	2	0.6846	0.2182	0.2918	0.1922	0.0552
Error	33					

* significant at $p = 0.05$, ** significant at $p = 0.01$.

4. Discussion

EFSA is requested to evaluate the justifications submitted by Member States that authorisations of neonicotinoids (imidacloprid, thiamethoxam and clothianidin) for seed coating of sugar beet are necessary due to a risk from certain pests that cannot be controlled by any reasonable means. EFSA is expected to report the results of its assessment by 2 October 2021. The results of our study provide important input and additional arguments for this assessment.

With the main objective of determining the efficacy of seed treatments with neonicotinoids on the main sugar beet pests, this study led to five main findings: (i) neonicotinoid treatments maintain crop stand and successfully suppress wireworms; (ii) neonicotinoid seed coating significantly reduces flea beetle damage; (iii) neonicotinoid seed coating can provide adequate control against weevils under low population pressure; (iv) neonicotinoid seed coating cannot reduce damage by noctuids at later growth stages of sugar beet; (v) at low population pressure of aphids, a solid conclusion on the effectiveness of neonicotinoid seed coating is not possible.

The experimental site in Tovarnik is located in the eastern part of Croatia, while Lukač is located in the northwestern part. No significant differences were found between years for all three observed climatic factors and at both sites. When comparing the sites, the Tovarnik site has higher average annual air and soil temperatures and lower precipitation, although the amount of precipitation in 2016 did not differ significantly between the sites. Therefore, we can conclude that our study was conducted in two regions with different climatic conditions. Similar results for these regions are reported by other authors [23–26].

4.1. Wireworms

Before the introduction of neonicotinoids in Croatia (between 1980 and 1990), wireworms were controlled on 50 to 95% of all sugar beet fields in the region of east Croatia. The average consumption of insecticides for wireworm control ranged from 0.8 to 1.4 kg active ingredient/ha of sown sugar beet, depending on the year. The most commonly used active ingredients were lindane (organochlorine), terbufos, forate, chlormephos, chlorpyrifos, phoxim (organophosphates) and carbofuran (carbamates) [13]. After the introduction of neonicotinoids in the mid-1990s, all fields were sown with coated seed and 100% of the fields were treated. However, additional treatments against wireworms were not applied and the amount of insecticides used was much lower compared to the previous period. It was 0.073 kg active ingredient/ha of sown sugar beet [13]. Routine prophylactic use of neonicotinoid seed coatings as comfort insecticides is no longer allowed in EU countries. Based on the present results as well as the reports of other authors [27,28], neonicotinoid seed coatings should only be applied when the wireworm population reaches a threshold level. For this purpose, different decision strategies are studied [29] and proposed [27,30]. As outlined by Barcsics et al. [31], rational IPM strategies exist and appropriate treatment options or monitoring tools are under development. However, it remains unclear whether the same tools are applicable to sugar beet. Based on the fact that other tools exist for wireworm management in other crops, further research will be conducted to determine if neonicotinoids can be fully substituted for wireworm management in sugar beet production. However, it would make sense to use neonicotinoids only as a very last resort for wireworm control when there is a real risk from infestation (based on forecasts), as also suggested by Hauer at al. [15].

In Croatia, the economic thresholds for wireworms in sugar beet and maize fields are 1–3 larvae/m^2 in dry areas and 3–5 larvae/m^2 in areas with more rainfall, suggesting that these larval densities can cause the same economic damage in both maize and sugar beet. According to Furlan et al. [27], no yield reduction is expected in maize when wireworm plant damage is less than 15% of the crop. In contrast, in France, an infestation of 10% of maize plants in a field corresponds to a loss of 500 to 1000 kg/ha [32]. The occurrence of wireworms in the studied fields as well as the data presented by Čamprag et al. [33] show that in Croatia and in the neighbouring countries the occurrence of wireworms could be significantly higher compared to north Europe, as presented by Hauer et al. [15]. According to Hauer et al. [15] and Furlan and Kreutzweisser [34] there is less than 10% occurrence of wireworms in sugar beet fields in north Europe and very low occurrence in the Netherlands, Belgium, Germany, Sweden, Denmark and Italy. Furlan et al. [27] reported that wireworm infestation was less than 15% in 70% of the fields observed over a period of 29 years. However, in more than 10% of the fields, the damage exceeded 40%. Poggi et al. [28] reported damage above 15% in about half of the fields observed in northern France.

In our experiments, wireworm damage differed in terms of number of plant stands between sites and years, demonstrating that wireworms are serious pests at some sites and in some years. Plant stand on untreated plots was reduced by 43% at the Tovarnik site in 2015 and by 13% at the Lukač site in 2016. The application of insecticides in 2015 resulted in an increase in plant stand of about 11% in Lukač and 69% in Tovarnik. The increase in plant population in 2016 ranged from 22% to 32% in Lukač and from 37% to 55% in Tovarnik. Therefore, insecticide treatments significantly maintained plant stand at both locations and in both years. The obtained results are very similar to those of Kereši et al. [35,36] who showed that neonicotinoid seed treatment can ensure plant stand in sugar beet fields.

4.2. Beet Flea Beetle

Before the introduction of neonicotinoids, the beet flea beetle was controlled on 10 to 65% of all sugar beet fields in eastern Croatia. The average consumption of insecticides for beet flea beetle control ranged from 0.1 to 0.59 kg active ingredient/ha of sown sugar beet, depending on the year [13]. After the introduction of neonicotinoids, all fields were sown with treated seeds, additional treatments against the beet flea beetle were not applied and the amount of insecticides used was significantly lower than in the period before neonicotinoids.

Kereši et al. [36] reported very severe damage by flea beetles in the experiment under extremely hot and dry weather conditions in Vojvodina, where seed dressing with thiamethoxam resulted in a fourfold increase in seedling weight. However, due to the other factors affecting yield, the increase in yield in the plots treated with thiamethoxam was only 13%. Satisfactory protection of seedlings against beet flea beetle was achieved with thiamethoxam alone or in mixture with tefluthrin and a mixture of imidacloprid + tefluthrin [35]. These treatments yielded significantly lower percentages of damaged plants than the untreated, while significantly increasing yield. Non-chemical alternatives for beet flea beetle control in sugar beet are not available and the only alternative is foliar spraying with pyrethroids. Therefore, the need to control the pests by spraying with pyrethroids has increased after the ban of neonicotinoids in 2018. In Croatia, we have already observed resistance of the sugar beet flea beetles to pyrethroids (Bažok, unpublished data). This could be one of the reasons why ten EU countries have requested an Emergency Authorisation of imidacloprid, thiamethoxam and clothianidin for seed treatment of sugar beet [37].

Flea beetle damage observed in both years and locations averaged 44% on untreated plots in BBCH 16 and 52% in BBCH 19. The observed level of damage proves that flea beetles are a serious pest in Croatia, as in other neighbouring countries [35]. At the same time, [15] did not report beet flea beetle as a serious pest in north Europe. Although damage by beet flea beetle occurred regularly in our experiments, their intensity varied at different locations and in different years. Both location and year significantly influenced flea beetle infestation.

Seed coating with neonicotinoids resulted in significant damage reduction. In 2015, seed coating with imidacloprid reduced damage by 88 to 97% on Lukač and from 96 to 98% on Tovarnik compared to the untreated control. Slightly lower efficacy was observed on plots treated with thiamethoxam and tefluthrin (from 83 to 94% in Lukač and from 91 to 95% in Tovarnik, respectively). Insecticide efficacy was lower in 2016. Seed coating with imidacloprid reduced damage by 68 to 73% in Lukač and by 36 to 46% in Tovarnik. At the same time, the effectiveness of the combination of thiamethoxam and tefluthrin ranged from 74 to 77% in Lukač and from 43 to 46% in Tovarnik, respectively.

4.3. Sugar Beet Weevil

From 1965 until the early 2000s, the sugar beet weevil was not an important pest in Croatia. It was important in Serbia, in the region of Vojvodina, which borders eastern Croatia [33]. As Čamprag [9] stated, this species is the most important pest of sugar beet in Vojvodina. In the last 60 years, it has destroyed a total of more than 250,000 hectares of young sugar beet and caused reseeding of stands. Between 1975 and 2004, an average of 3.3 individuals per square meter was counted. In eastern Croatia (on the border with Vojvo-dina), the population of the pest was below the economic threshold until 2008 [13]. After that, the population of the sugar beet weevil increased significantly and was regularly very high, causing severe damage [6]. Increased occurrence of sugar beet weevil in Croatia, Ukraine and Vojvodina is associated with global climate change and increased temperatures [13,38,39]. In Poland, Austria, Hungary and some eastern European countries, the sugar beet weevil is in a stage of downgrading and causes economically significant damage [40]. The reason for the increase in abundance can also be found in the combination of favorable climatic conditions (hot and dry spring) with the prohibition of effective insecticides [40].

In the eastern part of Croatia, the sugar beet weevil occurs regularly [41]. At the same time, we did not expect its occurrence at the Lukač site. Weather conditions contributed to the low abundance of the pest in 2015 at both locations. However, the abundance of the pest in 2016 was high in Tovarnik, with plant damage on untreated plots of about 20%

and significantly higher than in Lukač, where plant damage on untreated plots was up to 12%. Under these conditions, insecticide treatments significantly reduced plant damage. Seed treatments achieved satisfactory results in protecting sugar beet at the most sensitive stages of development under the condition of low weevil infestation.

4.4. Caterpillars

The surface-feeding species *Mamestra brassicae* L, *Lacanobia oleracea* L. and *Autographa gamma* L. are among the most damaging Noctuidae pests in sugar beet in Croatia. They have the potential to remove much (or all) of the aboveground foliage from young plants and dramatically affect plant growth and development [42]. The first appearance of the caterpillars is usually in June, two to three months after sowing. Due to the long period between sowing and the appearance of the pest, these pests are usually not controlled by seed dressing with neonicotinoids. In our experiments, significantly higher infestation was recorded in both years on Lukač, which is characterized by higher precipitation, confirming the results of Bažok et al. [43] on the influence of weather conditions on moth occurrence and damage. They reported the decrease in caterpillar damage caused by a very warm and dry growing season. As expected and reported by other authors [15], neonicotinoid seed coating did not significantly reduce damage. Due to their occurrence in the middle of the growing season, noctuid and moth caterpillars should be controlled by foliar application of insecticides.

4.5. Aphids

Aphids damage the crop mainly by sucking, resulting in reduced assimilate availability for plant growth and leaf area production [15]. They can also transmit Virus Yellows [44], which can cause significant damage in some countries of southern and eastern Europe [10,14], while in northern Europe, according to Kozlowska-Makulska et al. [45], transmission of the virus does not play an important role in the spread of Virus Yellows in sugar beet.

Significant infestation of aphids was not detected in the experiment. Based on the results of other authors [14,46], we expected a high efficacy of seed coatings with neonicotinoids against aphids. Although the percentage of damage was very low, better efficacy of imidacloprid was observed in 2016 compared to thiamethoxam and untreated variants (Table 9). However, our results do not provide a solid basis for conclusions on the efficacy of neonicotinoid seed coating against aphids.

5. Conclusions

In our trials, imidacloprid and thiamethoxam seed coatings provided satisfactory protection of young sugar beet plants against wireworms, sugar beet flea beetle, and, at low infestations, sugar beet weevil. These pests occur regularly in southern and eastern Europe and therefore require control measures.

Although there are many reports of high efficacy of neonicotinoid seed treatments against aphids, we could not draw any conclusions due to the low infestation of aphids in both trials. There are alternatives for the control of wireworms, sugar beet weevils, caterpillars and aphids. However, they should be further investigated as the application rate is not very high.

Further research program is needed to find alternative solutions and develop easily implementable strategies for all sugar beet pests. Based on the results obtained, we would propose an authorization of neonicotinoids for seed treatment of sugar beet in the regions with high infestation of the main sugar beet pests.

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Article

Neonicotinoid Residues in Sugar Beet Plants and Soil under Different Agro-Climatic Conditions

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Abstract: European sugar beet was mostly grown from seeds treated by neonicotinoids which provided efficient control of some important sugar beet pests (aphids and flea beetles). The EU commission regulation from 2018 to ultimately restrict the outdoor application of imidacloprid, thiamethoxam, and clothianidin could significantly affect European sugar beet production. Although alternative insecticides (spinosad, chlorantraniliprole, neem) are shown to have certain effects on particular pests when applied as seed treatment, it is not likely that in near future any insecticide will be identified as a good candidate for neonicotinoids' substitution. The aim of this research is to evaluate residue levels (LC-MS/MS method) of imidacloprid and thiamethoxam applied as seed dressing in sugar beet plants during two growing seasons in fields located in different agro-climatic regions and in greenhouse trials. In 2015, 25 to 27 days post planting (PP) maximum of 0.028% of imidacloprid and 0.077% of thiamethoxam were recovered from the emerged plants, respectively. In 2016, the recovery rate from the emerged plants 40 days PP was 0.003% for imidacloprid and 50 days PP was up to 0.022% for thiamethoxam. There were no neonicotinoid residues above the maximum residue level in roots at the time of harvesting, except in case of samples from thiamethoxam variant collected from greenhouse trials in 2016 (0.053 mg/kg). The results of this research lead to the conclusion that the seed treatment of sugar beet leaves minimal trace in plants because of the complete degradation while different behavior has been observed in the two fields and a glasshouse trial regarding the residues in soil. Dry conditions, leaching incapacity, or irregular flushing can result in higher concentrations in soil which can present potential risk for the succeeding crops. The results of our study could provide additional arguments about possible risk assessment for seed treatment in sugar beet.

Keywords: sugar beet; degradation; residues; neonicotinoids; imidacloprid; thiamethoxam

1. Introduction

Sugar beet (*Beta vulgaris* var. *saccharifera* L.) is an economically viable crop produced mainly for white sugar. The world's leading sugar beet producers (France, Germany and Poland) account for almost 50% of total world production (111.7 million tons in 2016). However, only 20% of the world's sugar comes from sugar beet; 80% is produced from sugar cane [1]. Given the production technology and the length of the growing season of almost 180 days, sugar beet is considered the most intensive agricultural crop [2].

The economically important pests of South East Europe sugar beet include wireworms, pigmy mangold beetle, sugar beet and corn weevil, black beet weevil, alfalfa snout beetle, several species of noctuid moths, sugar beet flea beetle, aphids, and beet cyst nematode [3–11]. Their appearance depends on the region and the year.

Since the introduction of neonicotinoid seed treatment in the 1990s, there has been a strong decrease in insecticide use in Croatia [12]. Wireworms, aphids, and flea beetles were successfully controlled by neonicotinoid seed treatments [7,13–15] so additional treatment was only required in the case of severe infestation of some foliar pests that cannot be successfully controlled with neonicotinoids (e.g., sugar beet weevil) [16]. In north-western Europe, only aphids require occasional control with foliar insecticides [17].

Seed treatment is a method that has brought many advantages to modern agriculture [18–24], although there are some negative effects as well. In heavy infestations the efficacy against wireworms and sugar beet weevil is weak, so additional protection measures are necessary [7]. It is often applied at higher rates [24] or when control is not even necessary.

The use of neonicotinoids has become a major controversy because of their negative effects on bees, other pollinators, and possibly other non-target organisms [25–27]. According to the available evidence and a risk assessment carried out by EFSA, the use of neonicotinoid pesticides (clothianidin, imidacloprid, and thiamethoxam) was severely restricted by European Commission (EC) in 2013 by the implementation of Directive 485/2013 [28]. The restriction applied to bee-friendly crops such as maize, oilseed rape, and sunflower, with the exception of greenhouse crops and the post-flowering treatment of certain crops, and to winter cereals. Based on the EFSA peer review of the pesticide risk assessment carried out for clothianidin [25], imidacloprid [26], and thiamethoxam [27], the Commission adopted on 30 May 2018, regulations banning completely the outdoor use of imidacloprid, clothianidin, and thiamethoxam to protect domestic honey bees and wild pollinators [29]. The only risk identified by EFSA for the treatment of sugar beet seeds with neonicotinoids was the risk of succeeding crop scenario [25–27].

In the succeeding crop scenario, the residues of neonicotinoids are expected to remain in the soil and be absorbed by the succeeding crop or weeds in the same field. Thus, if the significant concentrations of neonicotinoids were to remain in the soil after the growing season, they could be adsorbed by the succeeding crop (or weeds) from the soil and then the neonicotinoids could be found in pollen or excreted in guttation fluid.

The Commission has not considered the possibility of proposing further options in addition to the total ban on the treatment of sugar beet seed with neonicotinoids. This decision could endanger sugar beet production. The ban was justified by the fact that some ecologically more acceptable substitute chemicals (diamides) are effective in controlling the most serious pests and that tools to control most pests are available under integrated pest management (IPM). However, the arguments do not fully apply to all economically important pests that damage sugar beet production in all production areas in the EU.

Hauer et al. [17] discussed neonicotinoid seed treatments in European sugar beet cultivation with regard to their effectiveness against target pests and their impact on the environment. They proposed to develop monitoring systems and models to identify regions (and years) with a higher risk of occurrence of pests and to allow the use of insecticide seed treatments only when high pest pressure is likely. In their analysis, Hauer et al. [17] only looked at sugar beet production in northwestern European countries and did not consider the different climatic conditions and the occurrence of pests in eastern and southeastern Europe, where problems in production are mainly caused by flea beetles and sugar beet weevils. This fact makes their proposal even more important.

The aim of this research was to determine the residue levels of imidacloprid and thiamethoxam used as a seed treatment in sugar beet plants in different agroclimatic regions in order to estimate environmental risk and possible transfer to other crops. Greenhouse trials have been established in order to provide insight to neonicotinoid behavior in controlled conditions.

2. Materials and Methods

2.1. Field Site and Experimental Design

2.1.1. Field Site

The two-year study was conducted in 2015 and 2016 on three different locations. Field trials were located in two distinct counties of Croatia, Virovitica-Podravina County in Lukač (45°52′26″ N 17°25′09″ E) and Vukovar-Sirmium County in Tovarnik (45°09′54″ N 19°09′08″ E), while greenhouse trial was set up in Zagreb at the Faculty of Agriculture, Department of Agricultural Zoology (45°82′77′′ N, 16°03′09′′ E).

2.1.2. Characteristics of the Soil

To determine the physical and chemical soil properties in Lukač and Tovarnik, soil samples were taken in 2016 according to an internal protocol for annual crops provided by the Department of Plant Nutrition (University of Zagreb Faculty of Agriculture). At each site, 15 individual soil samples were taken on the same date from a depth of 0–30 cm, evenly distributed over the entire plot. A homogenized sample was prepared and 1.000 g were extracted for analysis. Chemical soil properties and texture analyses were carried out according to standard methods (ISO 11277 2004) in the pedological laboratory of the Department of Soil Science University of Zagreb Faculty of Agriculture.

2.1.3. Climatic Data

The data on climatic conditions were collected by Croatian Meteorological and Hydrological Service. The climatic conditions were monitored by the nearest climate stations (Virovitica for Lukač and Gradište for Tovarnik). The distance between the meteorological stations and the experimental sites was not more than 20 km. For the period from April to September, data on mean air and soil temperatures and total precipitation were collected and analyzed for Virovitica and Gradište in both years under investigation.

2.1.4. Design of Experiments

At each site, sugar beet seed was sown in three treatments, one of which was untreated seed (0 mg a.i./seed), the second treatment was sugar beet seed treated with imidacloprid (0.91 mg a.i./seed) and the third treatment was seed treated with thiamethoxam and teflutrin (0.36 + 0.036 mg a.i./seed). In both years sowing was done in regular spring terms (2015: 9 April—Lukac, 10 April—Zagreb, 11 April—Tovarnik; 2016: 26 March—Tovarnik, 1 April—Lukac, 7 April—Zagreb). In field trials, each treatment was sown on 1.000 m^2 in three repetitions. Each repetition was 123 m long and was sown with a six-row sowing harrow (i.e., 333 m^2) at a depth of 3 cm, the distance between rows was 45 cm and the distance in one row was 18 cm (i.e., 123,321 seeds/ha). In the greenhouse research the sowing conditions in the arable layer (30 cm) were simulated. The same treatments were sown in plastic containers of 90 cm × 50 cm × 38 cm (length × width × height) filled with 100 L Klasmann-Deilmann GmbH Supstrat 1 (EN Standard). The substrate used was a mixture of white peat (H_2–H_5) and black peat (H_6–H_8) with a pH value (H_2O) of 5.5–6.5 and 14:10:18 NPK fertilizer. The amount of heavy metal was significantly below the maximum permissible concentration. The sowing was done by hand at a depth of 3 cm and the distance between the seeds was 5 cm with an approximate quantity of 45 seeds per container. A total of six containers were sown per treatment (2 per repetition).

2.2. Sampling

2.2.1. Sampling of Sugar Beet Plants

Starting four weeks after sowing, sugar beet plant samples were collected every two weeks at all three locations during the two growing seasons (2015 and 2016). In the first four sampling periods, whole plants were collected. From the fifth sampling until the end of the experiment, the collected plants were divided into leaves and roots, which were analyzed separately. The last sampling concerned only the roots. Three samples were taken for each treatment. A total of 432 sugar beet samples were collected and analyzed for neonicotinoid residues. Each sample contained five plants with a minimum weight of 20 g. The collected samples were carefully labeled and transported in portable coolers to an accredited laboratory for analysis.

2.2.2. Sampling of Soil

In order to determine neonicotinoid residues in the soil, two samples were taken once at each site from the depth of a plow layer (30 cm). In 2016, 15 sub-samples (each weighing 1.000 g, depending on field size) were taken, pooled and homogenized at each site, and a subset of the pooled soil samples (20 g) from each treatment area was taken and stored in a freezer until analyzed.

2.3. Sample Analysis

2.3.1. Neonicotinoid Residues Analysis in Sugar Beet Plants and Soil

The determination of neonicotinoid residues in sugar beet plants and soil was performed by an accredited laboratory by liquid chromatography/tandem mass spectrometry (LC -MS/MS) using acetonitrile extraction and the QuEChERS method (EN 15662: 2008). The limit of quantification (LOQ) for this method is 0.01 mg/kg. The neonicotinoids were extracted from the homogenized sample with acetonitrile. Neonicotinoids, imidacloprid, thiamethoxam, and clothianidin were determined using the LC -MS/MS technique applied to the filtered extract with the Agilent Technologies 6460 Triple Quad LC/MS apparatus. Thiamethoxam is converted to clothianidin in soil and plant tissues, therefore the thiamethoxam residues were determined as the sum of thiamethoxam and clothianidin [30].

2.3.2. Statistical Analysis

The data on neonicotinoid residues were analyzed by analysis of variance (ANOVA) using the AOV factorial method with two or three factors [31]. The first factor was location which was considered as a fixed factor because of a limited production area of sugar beet and characteristic weather conditions. The second factor was insecticide treatment and the third factor was the plant part. This factor was analyzed for sampling during the growing season, where leaves and roots were sampled separately. A Tukey post-hoc test was used to determine which mean values of the variants were significantly different after a significant test result ($p < 0.05$).

3. Results

3.1. Climatic and Edaphic Conditions

Our analyses confirmed earlier data published by other authors [32–35] that the average annual temperatures in Tovarnik (Table 1) are higher than in Lukač. Precipitation varied from place to place in one of the two years of investigation and confirmed earlier published data [32–35] that when comparing Lukač (west) and Tovarnik (east), temperatures increased while precipitation decreased in the eastern part.

In both years the mean air and soil temperatures in the area of Lukač were significantly lower compared to Tovarnik, and the precipitation was significantly higher in 2015 in the same place, while in 2016 the differences were not significant. Between the years studied (2015 vs. 2016) there were no significant differences between the climatic conditions at both locations.

Table 1. Characteristics of the weather conditions prevailing at the two locations where the field investigations were carried out and the corresponding ANOVA results.

Climatic Factor	Location	Year 2015	Year 2016	HSD [2] (p = 5%)
Mean air temperature (°C) (April–September)	Lukač	18.65 ± 0.72 b *	18.16 ± 0.59 b	ns
	Tovarnik	19.85 ± 0.75 a	19.15 ± 0.56 a	ns
	HSD (p = 5%)	0.338	0.325	
Mean soil temperature (°C) (April–September)	Lukač	21.1 ± 0.88 b	20.5 ± 0.75 b	ns
	Tovarnik	22.63 ± 0.97 a	21.47 ± 0.7 a	ns
	HSD (p = 5%)	0.676	0.517	
Total amount of precipitation (mm) (April–September)	Lukač	600.03 ± 68.02 a	457.80 ± 34.99	ns
	Tovarnik	309.72 ± 40.05 b	395.25 ± 30.62	ns
	HSD [1] (p = 5%)	236.82	ns	

* Values followed by the same lowercase letters are not significantly different (p > 0.05; HSD test), [1], small letters refer to no differences among locations; [2], small letters refer to no differences among years within same location; ns, letters refer to no differences.

The edaphic conditions differed between the locations. The soil in Tovarnik has a higher content of soil organic matter than the soil in Lukač (Table 2). In addition, both soils are classified as silty clay according to the soil particle size fractions. A detailed description of the regional physical and chemical soil properties is given in Table 2.

Table 2. Physical and chemical soil properties in Lukač and Tovarnik, 2016.

	Particle Size Distribution (%) in mm					Chemical Soil Properties						
						pH		%		Al-mg/100 g		$CaCO_3$
	Fine Sand 0.2–0.063	Coarse Silt 0.063–0.02	Fine Silt 0.02–0.002	Clay < 0.002	Texture Mark	H_2O	nKCl	Soil Organic Matter	N	P_2O_5	K_2O	%
Lukač	25.50	31.60	24.60	14.00	Silty clay	6.38	5.17	1.54	0.10	12.90	10.20	0.00
Tovarnik	1.90	40.60	31.90	25.00	Silty clay	8.42	7.24	2.70	0.14	29.70	26.50	10.20

3.2. Degradation in Soil

Table 3 shows that there were no residues of neonicotinoids above LOQ in Lukač. Tovarnik showed concentrations of imidacloprid residues above LOQ and slightly increased thiamethoxam, while higher residues were found in the greenhouse.

Table 3. Residues of neonicotinoids (mg/kg) in soil samples taken from field sites at the end of the growing season 2016 (i.e., 180 days' post planting), Croatia.

Locality	Untreated	Imidacloprid (mg/kg)	Thiamethoxam (mg/kg) (Including Chlothianidin)
Lukač	<0.01	<0.01	<0.01
Tovarnik	<0.01	0.17	0.04
Zagreb	<0.01	5.34	2.65

3.3. Degradation Dynamics in Plants

Figure 1 shows a degradation dynamic of imidacloprid in sugar beet plants.

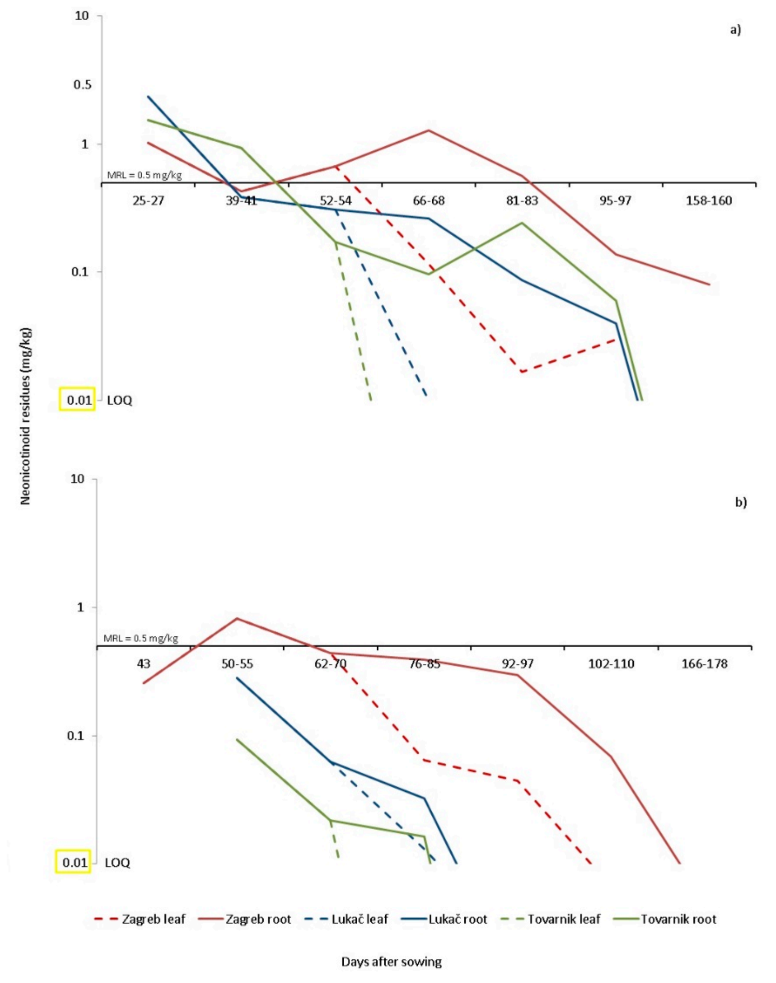

Figure 1. Degradation dynamics of imidacloprid during the growing seasons 2015 (**a**) and 2016 (**b**) in sugar beet plants in Lukac, Tovarnik and in greenhouse trials, in compliance with the maximum permitted residue level of 0.5 mg/kg; LOQ— limit of quantification; MRL—maximum residue level.

The maximum residue level (MRL) for imidacloprid in sugar beet roots is 0.5 mg/kg (EU No 491/2014) [36]. Concentrations of imidacloprid in whole plants collected in field trials (Lukač and Tovarnik) fell below the MRL of 0.5 mg/kg (EU No. 491/2014) 40–55 days after sowing in both years under investigation [36] (Figure 1). After that, residues in the leaves of sugar beets grown under field conditions were almost no longer detectable. Root samples were taken 60 days after sowing, and from the first sample onwards the residue level in the roots was below the MRL. At the time of harvesting the roots (180 days after planting), no residues above LOQ were detected. In the greenhouse trial (Zagreb), degradation was much slower because no regular water rinsing was possible. Residues of imidacloprid in leaves from greenhouse trials fell below the MRLs ten days later compared to field conditions (i.e., 60 days after sowing). A slightly faster degradation of imidacloprid residues in roots of sugar beet grown in greenhouse trials was observed in 2016 compared to 2015. In general, the residue level of imidacloprid in roots was below the MRL 80 days after sowing. At the time of harvest, the residue level in roots was quite low, 0.08 mg/kg in 2015 and <0.01 mg/kg in 2016.

The results of the statistical analysis are presented in Tables 4–7. Residue levels were significantly affected by treatment with imidacloprid at almost all sampling times, except for two final samples where degradation was completed in both years of the study. Residue levels in plants from treated seeds were significantly higher compared to those in untreated plants throughout the vegetation until harvest where degradation was completed. In 2015, residues of imidacloprid were significantly influenced by location (i.e., agroclimatic conditions) in almost all but two of the last samples taken (Tables 4 and 5). In 2016, residues were significantly site-dependent (i.e., agroclimatic conditions) in only one sampling (76–85 days after sowing) when residues were significantly higher under greenhouse conditions in Zagreb (Tables 6 and 7). The third factor (plant part) was observed in three samples. In 2015, residues of imidacloprid were significantly affected in two out of three samples (Table 5), while in 2016 residues of plant parts were not affected at all (Table 7), confirming the good systemic translocation of imidacloprid.

Table 4. Imidacloprid residues in the whole sugar beet plants during the first three observing periods and for roots at harvesting in 2015.

Source of Variation	df	Days after Sowing			
		Whole Plant			Root
		25–27	39–41	52–54	158–160
Total	17				
Rep	2				
Location (A)	2	0.0079 **	0.0004 **	0.0048 **	0.0620
Insecticide application (B)	1	0.0001 **	0.0001 **	0.0001 **	0.0826
A × B	2	0.0901	0.0006 **	0.0063 **	0.0620
Error	10				

Analysis of variance for imidacloprid residues in the whole sugar beet plants and root. ** significant at $p = 0.01$.

Table 5. Imidacloprid residues in different plant parts during the vegetation period in 2015.

Source of Variation	df	Days after Sowing		
		66–68	81–83	95–97
Total	35			
Rep	2			
Location (A)	2	0.0001 **	0.1882	0.2633
Insecticide application (B)	1	0.0001 **	0.0087 **	0.1669
A × B	2	0.0001 **	0.1882	0.4588
Plant part (C)	1	0.0001 **	0.0127 *	0.1964
A × C	2	0.0011 **	0.2117	0.3212
B × C	1	0.0001 **	0.0127 *	0.1964
A × B × C	2	0.0015 **	0.2117	0.3212
Error	22			

Analysis of variance for imidacloprid residues in different plant parts. * significant at $p = 0.05$, ** significant at $p = 0.01$.

Table 6. Imidacloprid residues in the whole plants during the first two observing periods and for roots at harvesting in 2016.

Source of Variation	df	Days after Sowing		
		Whole Plant		Root
		50–55	62–70	166–178
Total	17			
Rep	2			
Location (A)	2	0.1380	0.1822	1.000
Insecticide application (B)	1	0.0001 **	0.0135 *	1.000
A × B	2	0.1380	0.1822	1.000
Error	10			

Analysis of variance for imidacloprid residues in the whole sugar beet plants and root. * significant at $p = 0.05$, ** significant at $p = 0.01$.

Table 7. Imidacloprid residues in the different plant parts during the vegetation period in 2016.

Source of Variation	df	Days after Sowing		
		76–85	92–97	102–110
Total	35			
Rep	2			
Location (A)	2	0.0001 **	0.1041	0.1346
Insecticide application (B)	1	0.0001 **	0.0106 *	0.0087 **
A × B	2	0.0001 **	0.1041	0.1346
Plant part (C)	1	0.7046	0.1628	0.0517
A × C	2	0.0234 *	0.5097	0.3246
B × C	1	0.7046	0.1628	0.0517
A × B × C	2	0.0234 *	0.5097	0.3246
Error	22			

Analysis of variance for imidacloprid residues in different plant parts. * significant at $p = 0.05$, ** significant at $p = 0.01$.

The significant interaction between all three factors (location × insecticide treatment × plant part) for the imidacloprid residue level was present at the first sampling when plant parts were sampled separately (i.e., 66–68 days after sowing in 2015 and 76–85 days after sowing in 2016). A significant insecticide "treatment × location" interaction for imidacloprid residues was not observed in the first and the last two samples in 2015 (Tables 4 and 5), while in 2016 the significant interaction was only observed when samples were taken 76 to 85 days after sowing (Tables 6 and 7). For all other sampling data, the significant interaction "insecticide treatment × location" did not exist for imidacloprid residues. Significant interactions between "location × plant part" and "insecticide application × plant part" for imidacloprid residues existed only occasionally in both years of the study.

Figure 2 shows a degradation dynamic of thiamethoxam (expressed as sum of thiamethoxam and clothianidin) in sugar beet plants.

The maximum residue level (MRL) for thiamethoxam and clothianidin has been reduced in Europe from 0.05 mg/kg to 0.02 mg/kg in 2017 (EU 2017/671) [37]. For sugar beets grown under field conditions, the residue content of thiamethoxam in the leaves and roots of sugar beets dropped below the MRL between 70 and 80 days after sowing, depending on the year and location (Figure 2). No residues were found in sugar beet roots in open field cultivation at the time of harvest.

Similar to imidacloprid, the degradation of thiamethoxam was much slower in greenhouse trials. The residues of thiamethoxam in sugar beet roots in greenhouse cultivation were above the MRL (i.e., 0.053 mg/kg) at harvest time in 2015 (Figure 2), while in 2016, 100 days after sowing, the residues fell below the MRL of 0.02 mg/kg in 2016.

The results of the statistical analysis are presented in Tables 8–11. Residue levels were significantly affected by thiamethoxam treatment at all sampling dates including the last sampling in 2015, indicating that degradation at harvest is not complete in all trials. At the time of harvest in 2015, residues (0.053 mg/kg) were confirmed in beet roots grown in greenhouses (see Figure 2).

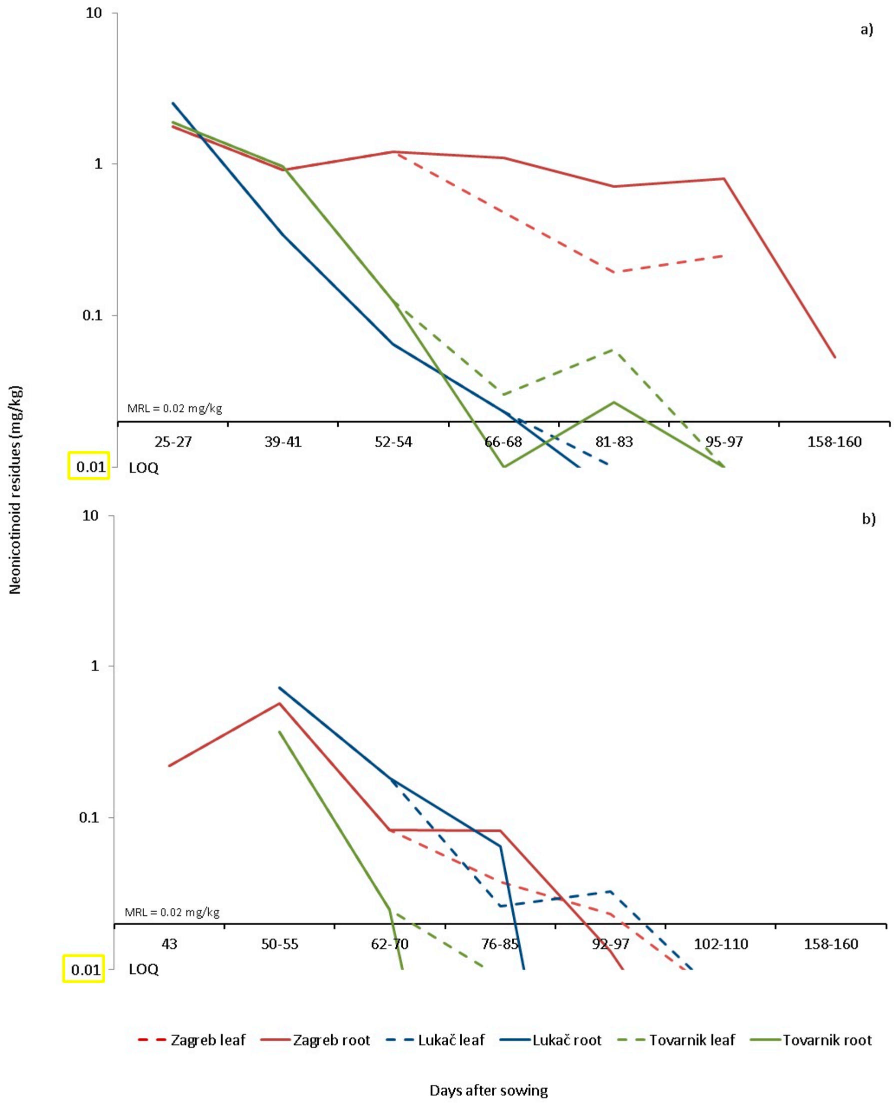

Figure 2. Degradation dynamics of thiamethoxam (expressed as sum of thiamethoxam and clothianidin) during the growing seasons 2015 (**a**) and 2016 (**b**) in sugar beet plants in Lukac, Tovarnik and in greenhouse trials, in compliance with the maximum permitted residue level of 0.02 mg/kg; LOQ— limit of quantification; MRL—maximum residue level.

Table 8. Thiamethoxam (including chlothianidin) residues in the whole plants during the first three observing periods and for roots at harvesting in 2015.

Source of Variation	df	Days after Sowing			
		Whole Plant			Root
		25–27	39–41	52–54	158–160
Total	17				
Rep	2				
Location (A)	2	0.1246	0.0025 **	0.0001 **	0.0003 **
Insecticide application (B)	1	0.0001 **	0.0001 **	0.0001 **	0.0011 **
A × B	2	0.0452 *	0.0025 **	0.0001 **	0.0003 **
Error	10				

Analysis of variance for thiamethoxam residues in the whole sugar beet plants and root. * significant at $p = 0.05$, ** significant at $p = 0.01$.

Table 9. Thiamethoxam (including chlothianidin) residues in different plant parts during the vegetation period in 2015.

Source of Variation	df	Days after Sowing		
		66–68	**81–83**	**95–97**
Total	35			
Rep	2			
Location (A)	2	0.0001 **	0.0001 **	0.0001 **
Insecticide application (B)	1	0.0001 **	0.0001 **	0.0001 **
A × B	2	0.0001 **	0.0001 **	0.0001 **
Plant part (C)	1	0.0049 **	0.0262 *	0.0263 *
A × C	2	0.0006 **	0.0002 **	0.0103 *
B × C	1	0.0062 **	0.0262 *	0.0263 *
A × B × C	2	0.0006 **	0.0002 **	0.0103 *
Error	22			

Analysis of variance for thiamethoxam residues in different plant parts * significant at $p = 0.05$, ** significant at $p = 0.01$.

Table 10. Thiamethoxam (including chlothianidin) residues in the whole plants during the first three observing periods and for roots at harvesting in 2016.

Source of Variation	df	Days after Sowing		
		Whole Plant		Root
		50–55	**62–70**	**166–178**
Total	17			
Rep	2			
Location (A)	2	0.1380	0.1822	1.0000
Insecticide application (B)	1	0.0001 **	0.0135 *	1.0000
A × B	2	0.1380	0.1822	1.0000
Error	10			

Analysis of variance for thiamethoxam residues in the whole sugar beet plants and root. * significant at $p = 0.05$, ** significant at $p = 0.01$.

Table 11. Thiamethoxam (including chlothianidin) residues in the different plant parts during the vegetation period in 2016.

Source of Variation	df	Days after Sowing		
		76–85	**92–97**	**102–110**
Total	35			
Rep	2			
Location (A)	2	0.0001 **	0.0255 *	0.1346
Insecticide application (B)	1	0.0001 **	0.0007 **	0.0087 **
A × B	2	0.0001 **	0.0255 *	0.1346
Plant part (C)	1	0.7046	0.0672	0.0517
A × C	2	0.0234 *	0.1438	0.3246
B × C	1	0.7046	0.0672	0.0517
A × B × C	2	0.0234 *	0.1438	0.3246
Error	22			

Analysis of variance for thiamethoxam residues in different plant parts. * significant at $p = 0.05$, ** significant at $p = 0.01$.

In 2016, residue levels were significantly affected by thiamethoxam treatment on all but the last sampling dates, indicating that degradation at harvest was complete under all conditions studied, including greenhouse trials. In 2015, residues of thiamethoxam were significantly influenced by location (i.e., agroclimatic conditions) at almost all sampling dates except the first sampling (Table 8). In 2016, residues were significantly influenced by the location (Tables 10 and 11) on only two samples (76–85 and 92–97 days after sowing), when residues were significantly higher under greenhouse conditions in Zagreb (Figure 2). The third factor (plant part) was observed in three samples. In 2015 the residues of thiacloprid were significantly influenced by plant parts in three samples (Table 9), whereas in 2016 the residues were not influenced by plant parts at all (Table 11). A significant insecticide "treatment × location" interaction for thiamethoxam residues was observed in 2015 in all samples (Tables 8 and 9), while in 2016 the significant interaction was observed in only two samples taken after 76–85 days and 92–97 days after sowing (Table 11). Significant interactions between "location × plant part" and "insecticide application × plant part" for thiacloprid residues were complete in 2015. In 2016, these interactions only existed on a single sampling date for the "location × plant part" interaction. The significant interaction between all three factors (location × insecticide treatment × plant part) for thiacloprid residue level existed in 2015 for all three samples and in 2016 for only one sample when plant parts were sampled separately (i.e., 76–85 days after sowing in 2016).

4. Discussion

When the neonicotinoids were introduced to the market, they were considered safe to use because they are stable in soil and have low toxicity to mammals [38]. However, recent studies have shown that neonicotinoids have adverse effects on bees, other pollinators, and possibly other non-target organisms [25–27]. A complete EU Commission Regulation ban on the outdoor use of imidacloprid, thiamethoxam, and clothianidin could have a significant impact on the practice of sugar beet production in Europe, as 100% of all commercial sugar beet seeds have been treated with neonicotinoids. According to Ester et al. and Lanka et al. [39,40] spinosad and chlorantraniliprole applied as seed treatment were ineffective at controlling flea beetles and cabbage aphid [39] as well as adult stages of rice water weevil [40]. It is unlikely that they will become a good substitute of neonicotinoid seed treatment. Hauer et al. [17] have pointed out the lack of effective alternatives for the control of *M. persicae* on sugar beet in Central and North Europe. Moreover, Bažok et al. [16] achieved the same conclusions for substituting control of sugar beet flea beetle in South and Eastern Europe. Therefore, the problems related to the control of the above mentioned pests could become a serious problem in the future if no alternatives are developed.

In our study, at the end of sugar beet cultivation (180 days after planting), imidacloprid residues at a concentration of 0.17 mg/kg and thiamethoxam residues at a concentration of 0.04 mg/kg were found in the soil of Tovarnik, while in Lukač all residues were below LOQ levels (Table 4). Such a result is partially consistent with that of [41] who randomly sampled 74 soils after the cultivation of maize, wheat, and barley grown from treated seeds. Imidacloprid was found in all samples, so the authors concluded that imidacloprid is always present in the soils after cultivation and is easily detectable if sampling is carried out in the year of treatment.

Alford and Krupke [42] concluded that high water solubility of neonicotinoid seed treatment applications makes it unlikely that they will remain near the relatively confined rhizosphere of the target plant long enough to be absorbed by the plant when not on the seed. The loss of neonicotinoids from agricultural soils is thought to occur through degradation or leaching in soil water [43]. EFSA's risk assessment [25–27] did not take into account the results of [42] on the low probability of residues of neonicotinoids remaining in soil for a longer period of time. Their findings, together with those of [44] on the recycling of neonicotinoid insecticides from contaminated groundwater back to crops, point to the possible risk scenario of irrigation, which will be further investigated. In our laboratory study, the sugar beet plants were sown at five times higher density than in the field, which means that the concentration of neonicotinoids is also significantly higher (40.95 mg imidacloprid and 32.76 + 1.62 mg thiamethoxam + teflutrin per container 100 l soil). Soil from greenhouse trials treated with imidacloprid contained the average value of 5.34 mg/kg a.i., while the thiamethoxam-treated variant of the sample form contained 2.65 mg/kg a.i. (Table 4). This is much higher if we consider that in open field the application rate as seed coating is 112.2 g imidacloprid or 44.4 + 4.44 g thiamethoxam + tefluthrin to one ha, while one ha contains on average three million liters of soil (calculation of the average soil layer of 30 cm). This is the average concentration of 0.04 mg/kg a.i. imidacloprid or

0.015 + 0.0015 mg/kg thiamethoxam + tefluthrin. Our result confirms that high concentrations of neonicotinoids in soil are to be expected in case of dry conditions, leaching incapacity, or irregular flushing (bottom of the container) into ground water meaning that they can present potential risk for the succeeding crops. Concerning field trials, there is no systematic monitoring of the presence of pesticides in water in Croatia and no data on concentrations of neonicotinoids in the area of our study are available.

Studies on the degradation of neonicotinoids in soil depend on temperature, moisture, and soil type, in particular on texture and organic matter content, pH and UV radiation [41]. According to Bonmatin [41], persistence is highest under cool, dry conditions and in soils with high organic matter content. On average, Lukač has more precipitation (more humid soil), lower soil and air temperatures, while Tovarnik is drier with low precipitation and slightly higher air and soil temperatures (Table 2). Table 3 shows that in our investigations the pH of the soil at both locations was between 5 and 7, which means that the soils are slightly acidic to neutral and do not allow degradation in the moist soil or water. Guzsvány et al. [45] found that imidacloprid and thiamethoxam degrade faster at 23 °C in alkaline media, while they remain relatively stable at pH 7 and 4. Regarding residues of neonicotinoids in soil after the vegetation period, Table 3 shows that all residues were lower than LOQ in Lukač while in Tovarnik 0.17 mg/kg imidacloprid and 0.04 mg/kg thiamethoxam were detected. Such results can be explained by the dry conditions, low precipitation, and slightly higher air and soil temperatures prevailing in Tovarnik. The soils of Tovarnik also contain a large amount of soil organic matter as well as available phosphorus and potassium (Table 3), which prevents the leaching of residues and allows higher sorption in soils with high organic matter content, which is also in line with the results of [46]. Even though the results of the residues in soil are not statistically assessed, we may conclude that the faster reduction of residues in Lukač is most likely due to higher precipitation which is confirmed with the analyses of the residues in plants. The presence of a significant "treatment × location" (i.e., agroclimatic conditions) interaction for thiamethoxam in 2015 (when locations differ in temperature and precipitation) and the absence of a significant interaction for the same factors in 2016 (when locations differ only in temperature) implies that precipitation is an important factor in thiamethoxam leaching. The same logic could not be followed for the degradation of imidacloprid because there was a significant "treatment × location" (i.e., agroclimatic conditions) interaction for imidacloprid residues only in three out of seven samples in 2015 and in one out of six samples in 2016.

According to Bonmatin et al. [41], the half-life of imidacloprid for seed treatment in France was about 270 days, while [47] reported 83 to 124 days under field conditions and 174 days on bare soil. Under field conditions, thiamethoxam showed a moderate to fast degradation rate [48]. The calculated half-life in soil was between 7 and 335 days for thiamethoxam [49].

Uptake by the roots ranged from 1.6 to 20% for imidacloprid in aubergines and maize [50]. Krupke et al. [50] pointed out that the uptake of clothianidin by maize plants was relatively low and that plant-bound clothianidin concentrations followed an exponential decay pattern with initially high values, followed by a rapid decrease within the first ~20 days after planting. A maximum of 1.34% of the initial seed treatment rate (calculated as mg a.i./kg of seed) was successfully obtained from plant tissues (calculated as mg a.i./kg of plant tissue) and a maximum of 0.26% from root samples. Our study showed that 25 days to 27 days after planting in 2015, a maximum of 0.028% imidacloprid and 0.077% thiamethoxam was obtained from the raised plants (Figures 1 and 2). In 2016, the recovery rate from the raised plants 40 days after planting was 0.003% for imidacloprid and 50 days after planting up to 0.022% for thiamethoxam. These data confirm that the degradation scenario of imidacloprid and thiamethoxam in sugar beet crops is similar to the scenario established for clothianidin by [50].

Westwood et al. [51] found that the concentration of imidacloprid in the leaves of sugar beet grown from treated seed was 15.2 mg/kg 21 days after planting and degradation to 0.5 mg/kg 97 days after planting (25-leaf stage). Bažok et al. [52] found twice as high a concentration of 0.95 mg/kg imidacloprid in sugar beet leaves 42 days after planting using the HPLC method. Compared to HPLC, the LC -MS/MS method has a lower limit of determination (LOQ) and offers the possibility of a clear identification of the analyte [53]. Therefore, our results show more precise results confirming that there are no residues of neonicotinoids in the roots of sugar beet during harvest time. Nevertheless, the risk is not negligible in dry climates or after a dry period since results showed higher soil concentrations of imidacloprid than expected in Tovarnik. Results have shown [47] that field trials in Europe and the United States on the degradation of imidacloprid show that it does not accumulate in soil after repeated annual applications. Although sugar beet in Croatia is grown in crop rotation where neonicotinoids are already prohibited (maize, oilseed rape, wheat, etc.,), there should be a limited risk of bioaccumulation and transfer to other crops but the risk for succeeding crops needs to be further assessed.

Neonicotinoid seed treatment of sugar beet is still allowed in many other regions of the world (except the EU). Increase in the wide use of insecticides, in particular pyrethroid insecticides, against aphids and flea beetles (depending on the growing area) is expected in areas where neonicotinoids are banned. The status of neonicotinoids for sugar beet seed treatment will possibly be further investigated by various regulatory authorities around the world.

5. Conclusions

The residue levels of imidacloprid and thiamethoxam used for seed treatment of sugar beet plants were below the maximum permitted residue level at the time of harvest and were highly dependent on weather conditions, in particular rainfall. The results of this research show that the seed treatment of sugar beet leaves minimal trace in plants because of the complete degradation by the end of the growing season while higher residue concentration in the soil shows that there is risk in dry climates or after a dry period. The results of our study provide additional arguments for a possible risk assessment for sugar beet seed treatment in the succeeding crop and irrigation scenarios and provide further guidance for the assessment and/or reassessment of the use of neonicotinoids in sugar beet production. However, further investigation is needed to assess the possible neonicotinoids uptake by succeeding crops.

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Conflicts of Interest: The authors declare no conflict of interest.

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Neonicotinoid Residues in Earthworms and Ground Beetles under Intensive Sugar Beet Production: Preliminary Study in Croatia

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Abstract: Neonicotinoids are pesticides widely used for pest control in agriculture with undesirable effects on pollinators. However, other beneficial insects are exposed to insecticides that are not lethal to them but may accumulate and affect their vital characteristics. The objective of this study was to determine neonicotinoid residues in two types of beneficial soil organisms. The first group includes ground beetles (family: Carabidae, order: Coleoptera). They are important in the food web within existing ecosystems, especially in agricultural areas. The second group includes earthworms (family: Lumbricidae, order: Opisthopora) as humifiers, important members of the soil fauna. Fauna was collected at two sugar beet growing areas in Croatia under intensive sugar beet management. Ground beetles were collected from six plots of sugar beet fields treated with imidacloprid and thiamethoxam or left untreated with neonicotinoids. Earthworms were collected from the eight fields involved in four-year sugar beet crop rotation (sugar beet, maize, soybean, oilseed rape). Detection of neonicotinoid residues was performed by LC-MS/MS, SPE-QuEChERS method. The limit of quantification (LOQ) was 0.001 mg/kg. In ground beetles, the highest concentration of imidacloprid was detected at 0.027 mg/kg, while the residues of thiamethoxam and clothianidin were below LOQ. The highest concentration of imidacloprid in earthworms was 0.2141 mg/kg, while residues of thiamethoxam did not exceed 0.0008 mg/kg. This is the first study of this kind on Croatian territory and provides a valuable first insight into the ecotoxicological status of beneficial soil fauna. More comprehensive studies are needed to assess the extent of accumulation in and to take further steps regarding conservation programs for beneficial soil organisms.

Keywords: beneficial organisms; imidacloprid; liquid chromatography–mass spectrometry; neonicotinoid; pitfall; thiamethoxam.

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1. Introduction

Intensification and modernization of agricultural production has led to a decline in the number of individuals or species due to the negative impact of various factors [1]. Pest control exposes non-target organisms to insecticides that can affect their development, physiology, behavior, and communication [1]. Special concern is put on beneficial fauna. Beneficial fauna is a group of organisms that indirectly have a positive effect on crops by increasing soil fertility, regulating the water–air ratio, or feeding on pests and reducing their numbers. The beneficial soil fauna of agricultural lands includes insects, earthworms, nematodes, mites, and spiders. Insects that are part of the beneficial soil fauna and are important as indicators of habitat biological stability include ground beetles (family: Carabidae, order: Coleoptera) [2]. and earthworms (family: Lumbricidae, order: Opisthopora) [3] Intensive agriculture with high use of pesticides and fertilizers poses a threat to beneficial insects and leads to a loss of biodiversity [4].

Ground beetles are important predators of numerous pests, and they also feed on weed seeds and are a food source for animals at a higher trophic level [5,6]. The decline in ground beetle populations is explained by the higher use of agrochemicals, loss of grassland for foraging, and increasing average field size, the negative effect of which is even stronger than the effect of intensive cultivation [4–8].

Earthworms are important members of the fauna of agricultural soils, where they account for up to 80% of the total animal biomass [9]. They play a key role in the development and maintenance of physical, chemical, and biological soil properties [10]. In cultivated fields, earthworms are exposed to frequent and varied pesticide applications [7]. The seriousness of the problem of earthworms with pesticides is shown by the results of a study conducted in France. At least one pesticide was detected in 92% of the earthworms studied, both in treated crops and untreated habitats [11].

Neonicotinoids are highly toxic to most arthropods and have been widely used for pest control in agriculture and horticulture [12]. Although neonicotinoids are banned in Europe and the UK, they are still used for crop protection under special permits [13]. The top ten destinations for banned neonicotinoid exports from the EU, by weight of active ingredients, are Brazil, Russia, Ukraine, Argentina, Iran, South Africa, Singapore, Indonesia, Ghana, and Mali [14]. One of the most important reasons for the ban of neonicotinoids was the use of treated seeds and the use of pneumatic seeders, which create dust during sowing that gets onto the surrounding flowering plants and is carried by bees into the hive along with pollen [15,16]. For example, Krupke et al. [17] detected residues of thiamethoxam (68 to 13.240 mg/kg) and clothianidin (3.400–15.030 mg/kg) in dust from treated maize seeds. The undesirable effect on pollinators during foliar application, on treated areas and outside treated areas has adverse effects as well [16]. Exposure of beneficial or non-target organisms to insecticides need not be lethal to them, but can seriously affect their development, physiology, behavior, and communication [4].

The objective of this study was to determine pesticide residues in: (1) ground beetles (Carabidae) collected from sugar beet fields whose seeds had been treated with imidacloprid and thiamethoxam and from field without insecticide seed treatment, and (2) earthworms (Lumbricidae) collected from the fields involved in four-year sugar beet crop rotation to assess accumulation of neonicotinoids in the tested organisms.

Sugar beet was selected as a high-yielding crop that was frequently treated with neonicotinoids in the last decade and for which European Food Safety Authority (EFSA) assessed and permitted emergency neonicotinoid uses after general banning.

2. Materials and Methods

The investigation was conducted in the northern Croatia location Lukač (45.8739° N, 17.4191° E) and in the eastern Croatia location Tovarnik (45.1649° N, 19.1522° E). Average air and soil temperatures were higher in Tovarnik, while the amount of precipitation was higher in Lukač. The soils in Tovarnik have a higher content of soil organic matter, but on both locations, soils are classified as silty clay according to the soil particle size fractions [18]. On both locations' sugar beet fields, over 5 ha were chosen for setting up the experiment. Sowing of sugar beet included the untreated plot, a plot sown with seeds treated with imidacloprid at a dosage of 0.00091 a.i./seed, and a plot treated with a combination of 0.00036 thiamethoxam and 0.000036 a.i./seed tefluthrin, each sown on 1000 m^2.

All agrotechnical measures taken at both locations were standard for each investigated area, including the application of different plant protection products and fertilizers.

Samples of ground beetles were collected using 40 pitfall traps set in the form of a net (12 per plot + two indicative per location were initially sent to analysis to confirm if it is possible to detect residues in animal samples). Samples were collected three times during the growing season over a period of seven days in May (20.05), July (01.07), and September (22.09). In the meantime, the traps were closed with plastic covers. Ground beetle samples were deep frozen until analysis. Other organisms collected in the traps were not subjects of the study and were not considered for analysis.

Earthworm samples were collected at the same sugar beet fields and additionally, from the fields included in the sugar beet four-year crop rotation system (details on sugar beet crop rotation are in Table 1). Samples were collected three times on each field (autumn, spring, autumn) using the standard ISO method [19] that includes digging 60 × 60 cm holes filled with water and formalin. Per each field, four holes on randomly selected places were dug, and samples were handpicked. All collected samples per extraction hole presented a repetition in the experiment. All samples were deep frozen until analysis.

Neonicotinoid residue analysis was done by certified laboratory Euroinspekt Croatiakontrola Ltd. for Control of Goods and Engineering, Zagreb, Croatia, using a multiresidue method for the determination of pesticide residues by gas and liquid chromatography after extraction with acetonitrile and purification by solid-phase dispersive extraction (SPE)—Modular method QuEChERS (EN 15662:2018). The method is standardized for the analysis of foods of plant origin. However, since it covers a wide range of matrices in terms of chemical composition, including samples with a high protein and/or fat content, it is validated for samples of animal origin as well [20]. The limit of residue quantification, that is, the amount of active substance that could be detected by this method, was 0.001 mg/kg or ppm.

According to HRN EN 15662:2018 for multiresidue pesticide analysis, procedure includes homogenization of samples, which should not weigh less than 5 g each. Data on neonicotinoid residues were processed with ANOVA using ARM 9® GDM Software, Revision 2019.4; (B = 25105), SD, USA, [21] to determine the differences between sampling periods on both locations and crops involved in the research.

Table 1. Historical crop rotation at the locations included in the research.

Locality		Four Years Sugar Beet Crop Rotation System				
	Field	**I**	**II**	**III**	**IV**	**V**
Tovarnik	1.	maize *	**sugar beet**	soybean	wheat	**sugar beet**
	2.	wheat	maize	**sugar beet**	wheat	sunflower
	3.	**sugar beet**	wheat	sunflower	**sugar beet**	wheat
	4.	soybean	wheat	sunflower	barley	sugar beet
Lukač	1.	wheat	**sugar beet**	wheat	sunflower	maize
	2.	**sugar beet**	wheat	**sugar beet**	maize	maize
	3.	soybean	maize	wheat	**sugar beet**	bare soil
	4.	maize	oilseed rape	wheat	sunflower	**sugar beet**

* Fields marked with dark grey were under sampling during spring and autumn, I—sugar beet sown in testing year, fields marked with light gray were under sampling during autumn previous year, II—sugar beet sown one year ago. III—sugar beet grown before two years ago; IV—sugar beet sown three years ago; V—sugar beet sown four years ago.

3. Results

A total of number of collected ground beetles in sugar beet fields was 1.131 in Vukovar-Syrmia County and 1.250 Virovitica-Podravina County. On both locations, the species *Poecilus cupreus cupreus* Linnaeus, *Harpalus rufipes* De Greer, *Pterostihus melanarius melanarius* Illiger and *Pterostihus melas melas* Creutzer accounted for more than 80% of the individuals captured, while the remaining species were sporadic. A total of 14 homogenized ground beetle samples were analytically prepared for multiresidue analysis. Each sample contained an average of 150 beetles.

During total of 96 samplings, 419 earthworms were collected in Vukovar-Syrmia County and 650 in Virovitica-Podravina County. Distinguished species included *Allolobophora caliginosa* Savigny and *Lumbricus terrestris* Linnaeus. A total of 58 homogenized earthworm samples were analytically prepared for multiresidue analysis. Each sample contained an average of 30 earthworms.

The multiresidue method described above was used to determine the residues of 300 different active ingredients of plant protection products, but only the results of neonicotinoids are considered (Tables 2–4).

Residues of imidacloprid are present in all samples. The highest detected imidacloprid concentration was 0.027 mg/kg in Lukač during autumn sampling (Table 2). In most cases, the residues of thiamethoxam and clothianidin in the ground beetle samples were below LOQ. It can be observed that thiamethoxam is degraded faster than imidacloprid.

Table 2. Determined residues of neonicotinoids (in mg/kg) in ground beetle samples from different variants collected from sugar beet fields in Lukač and Tovarnik.

Lukač									
	imidacloprid residues			thiamethoxam residues			clothianidin residues		
Variant	S_1	S_2	S_3	S_1	S_2	S_3	S_1	S_2	S_3
V_1	0.004	0.002	0,011	0.002	<0.001	<0.001	0.001	<0.001	<0.001
V_2	0.004	0.004	0,027	0.002	<0.001	<0.001	0.001	<0.001	<0.001
V_3	0.004	0.006	0.008	0.002	<0.001	<0.001	0.001	<0.001	0.001
Tovarnik									
	imidacloprid residues			thiamethoxam residues			clothianidin residues		
	S_1	S_2	S_3	S_1	S_2	S_3	S_1	S_2	S_3
V_1	<0.001	0.002	0.002	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
V_2	0.001	0.008	0.003	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
V_3	<0.001	0.001	0.003	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

V_1—untreated sugar beet seeds; V_2—sugar beet seeds treated with imidacloprid; V_3—sugar beet seeds treated with a combination of thiamethoxam and tefluthrin, S_1—sampling in spring; S_2—sampling in summer, S_3—sampling in autumn.

Residues of imidacloprid in earthworms changed depending on the sampling period and their degradation dynamics depended on crop rotation. At location Lukač (Table 3), imidacloprid residues increased, especially towards the end of the growing season, even when no additional treatments were applied in the same vegetation. The highest concentration of imidacloprid measured in earthworm samples was 0.2141 mg/kg in Tovarnik during the final sampling in autumn (Table 4). Thiamethoxam and clothianidin residues are usually observed together because thiamethoxam is metabolized in the soil to clothianidin. This explains the fact that clothianidin was not used at seeding, but residues of clothianidin are still found in samples. At both locations (Tables 3 and 4) residues of thiamethoxam are not above 0.0008 mg/kg, and in most cases, results do not significantly differ between crops or sampling periods on both locations. Same as above, residues of clothianidin were somewhat elevated, but were still far below lethal doses.

Table 3. Determined residues of neonicotinoids (in mg/kg) in earthworm samples from fields with different crop rotations in Lukač.

Active Ingredient	**Sample Collection Period**					**LSD P = 0.05** [1]
	Crop Rotation	S_1	**Crop Rotation**	S_2	S_3	
Imidacloprid		0.0321		0.0184	0.0800	ns [2]
		0.0044 b		0.0049 b	0.0166 a	0.00495
		0.0493 a		0.0107 b	0.0663 a	0.01929
		0.0334 a		0.0067 b	0.035 a	0.01220
Thiamethoxam	sugar beet	0.0005	wheat	0.0002	0.0001	ns [2]
	oilseed rape	0.001 a	maize	0.0001 b	0.0001 b	0.00007
	wheat	0.0001	sugar beet	0.0003	0.0001	ns
	maize	0.0001	soybean	0.0001	0.0001	ns
Clothianidin		0.0084		0.0037	0.0001	ns[2]
		0.0077 a		0.003 b	0.003 b	0.00207
		0.0054		0.0218	0.0105	ns
		0.0133		0.0235	0.0070	ns

[1] Analysis of differences between fields in different rotations with respect to the time of sampling: values marked with the same lowercase letter belong to the same rank; [2] difference is not statistically significant (ns—no significant); S_1—sampling in autumn previous year; S_2—sampling in spring, S_3—sampling in autumn.

Table 4. Determined residues of neonicotinoids (in mg/kg) in earthworm samples from fields with different crop rotations in Tovanik.

Active Ingredient	Sample collection period					LSD P = 0.05 [1]
	Crop Rotation	S_1	Crop Rotation	S_2	S_3	
Imidacloprid	sugar beet	0.057 a	maize	0.0234 b	0.05 b	0.01609
	maize	0.0128 b	wheat	0.0958 a	0.1144 a	0.0289
	wheat	0.0058 c	sugar beet	0.0295 b	0.2141 a	0.00557
	wheat	-	soybean	0.0275	0.1191	ns
Thiamethoxam	sugar beet	0.0008	maize	0.0004	0	ns
	maize	0.0001	wheat	0.001	0.0001	ns
	wheat	0.0001	sugar beet	0.0001	0.0001	ns
	wheat	-	soybean	0.0001	0.0001	ns
Clothianidin	sugar beet	0.0073	maize	0.005	0.001	ns
	maize	0.0008	wheat	0.0048	0.0048	ns
	wheat	0.0053 b	sugar beet	0.018 a	0.0013 c	0.00285
	wheat	0.057 a	soybean	0.0182	0.0347	ns

[1] Analysis of differences between fields in different rotations with respect to the time of sampling: values marked with the same lowercase letter belong to the same rank; S_1—sampling in autumn previous year; S_2—sampling in spring, S_3—sampling in autumn; ns—no significant.

4. Discussion

Residues of imidacloprid were detected in all samples in our study, including those from the untreated plot. The reason for this is that ground beetles are very mobile insects, and individuals from one plot can easily be present in samples from the other plot or even neighboring fields. Within our study, the highest concentration of imidacloprid was 0,027 mg/kg in Lukač during the autumn sampling, while residues of thiamethoxam and clothianidin between <0.001–0.002 are negligible in all variants. In a study by Mullin et al. [22], almost 100% mortality of 18 ground beetle species and extreme sensitivity of ground beetle (*Poecilus cupreus* L.) larvae exposed to commercial corn seed treated with neonicotinoids at a dose of 700 g/kg were observed.

In the case of earthworms, toxicological studies show the risk of mortality of individuals of all known species when they ingest soil or organic material containing neonicotinoid residues at a concentration ≥ 1 mg/kg [3]. According to Gomez-Eyles et al. [23], imidacloprid can negatively affect the reproduction and growth of earthworms at 1.91 mg/kg. At a concentration of 3 mg/kg, 50% mortality of earthworms is expected [3]. Within our study, the highest detected residues of imidacloprid were far below the value of acute and chronic toxicity of the same pesticide (LC_{50} = 10.7 mg/kg). Increase of imidacloprid residues in earthworms at the end of sugar beet vegetation can be explained by their more active period toward the end of the vegetation season [10]. According to PPDB [24], imidacloprid is moderately toxic to earthworms with a low risk of bioaccumulating.

The use of neonicotinoids has become a major controversy because of their negative effects on pollinators. Studies by EFSA [25–27] have shown that neonicotinoids have negative effects on bees, other pollinators, and possibly other non-target organisms. EFSA was requested by the European Commission (EC) to provide technical assistance under Article 53(2) of Regulation (EC) No. 1107/2009 [25] to review the emergency authorizations in Croatia for pesticides containing the neonicotinoids (clothianidin, imidacloprid, or thiamethoxam) banned in May 2018 for use on sugar beets. EFSA was asked to evaluate whether the granting of this emergency authorization was necessary due to a hazard that could not be contained by other appropriate means. EFSA collected and evaluated the information related to the emergency authorization of neonicotinoids (thiamethoxam) in Croatia. The evaluation concluded that there are currently no sufficient alternatives for the tested sugar beet pests *Agriotes* sp., *Atomaria linearis* Stephens, *Bothynoderes punctiventris* Germar and *Chaetocnema* sp.

While clear results have been published on sugar beet pests, no relevant data were available on neonicotinoid influence on beneficial soil fauna on fields under intensive sugar beet production. According to EFSA, the treatment of sugar beet seeds with neonicotinoids poses a risk for the succeeding crop scenario where residue remains in the soil and can be absorbed [25–27]. High concentrations of neonicotinoids in soil are especially expected in cases of dry conditions, leaching incapacity, or irregular flushing into ground water [18]. Ground beetles feed on various economically damaging species [28] that have fed on the treated crop or through the treated surface on which they move [29–33] so they can easily be exposed to the elevated neonicotinoid residues. Earthworms, as organisms mostly living below the soil, have a specific way of feeding, leading them to ingest contaminated soil and organic particles [34] At higher neonicotinoid concentrations used to protect agricultural crops, the same neural pathways through which neonicotinoids affect invertebrates [35] may also affect those of earthworms [36].

5. Conclusions

In the two beneficial soil organisms studied, ground beetles and earthworms, the neonicotinoid residues were below concentrations reported as lethal. If the elevated concentrations of neonicotinoids remain in the soil after the growing season, residues in soil fauna can be expected. Considering the data presented in this preliminary study, approved seed treatments can be continued, but only under strict controls to minimize risks to the environment while providing effective and appropriate crop protection for key pests. The results of our study provide an important contribution and additional arguments for this and future assessment as well as conservation programs. More comprehensive studies are needed to assess the extent of accumulation in beneficial soil organisms.

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Plant protection products in agricultural fields – residues in earthworms and assessment of potentially toxic effects to the environment

Sredstva za zaštitu bilja na poljoprivrednim površinama – rezidue u gujavicama i procjena potencijalno toksičnih učinaka na okoliš

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ABSTRACT

The environmental risk assessment of plant protection products for soil organisms is mainly based on the results of laboratory and extended laboratory studies while the link from the laboratory to realistic field conditions over several seasons is not well established. The current environmental risk assessment is applied to a single active ingredient and does not consider that soil organisms are exposed to varying degrees to a mixture of active ingredients from different pesticides. In this study, earthworm samples were collected from eight fields in Croatia during two growing seasons and analyzed for 300 active ingredients. The concentrations of 26 analyzed active ingredients ranged between 0.000 and 0.247 mg/kg earthworm fresh weight with a mean of 0.005 mg/kg earthworm fresh weight. The percentage of samples with values below the limit of detection (LOD = ½ LOQ), values below the limit of quantification (LOQ = 0.001 mg/kg) and values above LOQ were 33, 44 and 23 %, respectively. Based on publicly available draft assessment reports from European Commission and European Food Safety Authority, degradation parameters (DT_{50}, DT_{90}) were used to calculate degradation curves and the current concentration in soil at the date of earthworm sampling. Subsequently, compound-specific bioconcentration factors in soil were determined by dividing the analyzed pesticide residues in earthworms by the calculated concentrations in soil. The results of the study showed that most active ingredients do not pose a risk to earthworms and have no secondary poisoning potential to birds and mammals that feed on them. The retrospective analysis method of analytically measured neonicotinoid residues in earthworm samples can be reliably used to calculate degradation and concentration curves in soil at the time of sampling.

Keywords: bioaccumulation, bioconcentration factors, earthworms, environmental risk assessment, pesticide residues, secondary poisoning, toxicity-exposure ratio

SAŽETAK

Procjena ekološkog rizika sredstava za zaštitu bilja za organizme u tlu uglavnom se temelji na rezultatima laboratorijskih i proširenih laboratorijskih studija dok veza između laboratorija i realnih poljskih uvjeta tijekom nekoliko sezona nije dobro utvrđena. Trenutna procjena rizika za okoliš primjenjuje se na pojedinačne aktivne tvari i ne uzima u obzir da su organizmi u tlu izloženi mješavini mješavini aktivnih tvari različitih pesticida. U istraživanju su prikupljeni uzorci gujavica s osam polja u Hrvatskoj tijekom dvije vegetacijske sezone. Analizirani su na 300 aktivnih tvari. Koncentracije 26 analiziranih aktivnih tvari kretale su se od 0,000 do 0,247 mg/kg svježe mase gujavica sa srednjom vrijednosti od

0,005 mg/kg svježe mase gujavica. Postotak uzoraka s vrijednostima ispod granice detekcije (LOD = ½ LOQ), vrijednosti ispod granice kvantifikacije (LOQ = 0,001 mg/kg) i vrijednosti iznad LOQ iznosio je 33, 44 and 23%. Na temelju javno dostupnih nacrta izvješća o procjeni Europske komisije i Europske agencije za sigurnost hrane, parametri degradacije (DT_{50}, DT_{90}) korišteni su za izračunavanje krivulja razgradnje i koncentracije u tlu u vrijeme uzorkovanja gujavica. Potom su određeni faktori biokoncentracije specifičnih za spoj u tlu dijeljenjem analiziranih ostataka pesticida u gujavicama s izračunatim koncentracijama u tlu. Rezultati istraživanja pokazali su da većina aktivnih tvari ne predstavlja rizik za gujavice i nema sekundarni potencijal trovanja za ptice i sisavce koji se njima hrane. Metoda retrospektivne analize analitički izmjerenih rezidua neonikotinoida u uzorcima gujavica može se pouzdano koristiti za izračunavanje krivulja razgradnje i koncentracije u tlu u vrijeme uzorkovanja.

Ključne riječi: bioakumulacija, faktori biokoncentracije, gujavice, procjena rizika za okoliš, ostaci pesticida, sekundarno trovanje, omjer toksičnosti i izloženosti

INTRODUCTION

The environmental risk assessment of plant protection products on invertebrate soil organisms is based on the European Commission (EC) Guidance Document on Terrestrial Ecotoxicology (EC, 2002) and EC Regulation No 1107 (EC, 2009) with additional recommendations given by the European Food Safety Authority (EFSA) Scientific opinion addressing the state of the science on risk assessment of plant protection products for in-soil organisms (Ockleford et al., 2017). In principle, acute and chronic effects of the active ingredient of a plant protection product or the plant protection product itself are tested by exposing a few soil species to treated artificial soil. If the toxicity-exposure ratio (TER) does not exceed the defined trigger value, the active ingredient in question is not considered to pose an unacceptable risk to soil organisms. Otherwise, the exposure scenario must be refined, or higher tier tests must be performed (e.g., terrestrial mesocosm or earthworm field studies) to study the potential impact of an active ingredient under more natural conditions. Only with the evidence of no effects at one level of the tiered testing approach, the active ingredient is allowed to be placed on market and used in the field following the recommended use pattern dependent on the crop species (Ockleford et al., 2017).

Whereas monitoring of a medicine after its approval (pharmacovigilance) is a requirement of the European Medicine Agency (Küster and Adler, 2014), post-registration monitoring of plant protection products (PPP's) is still not strictly required (Vijver et al., 2017). According to Hernandez-Jeret et al. (2021) if refined approaches have been used in the risk assessment of metal-containing PPP's, post-registration monitoring and controlled long-term studies should be conducted and assessed. For PPP's, residue data from monitoring studies in soil are rare in comparison to aquatic systems (Hommen et al., 2004, Rosenbom et al., 2016). In the case of heavy metals and a few persistent organic chemicals, historical data from permanent study fields are available (German Environmental Specimen Bank, 2018) and document the time series of concentrations in different matrices such as soil and earthworms. However, samples from different matrices are often not taken from the same site at the same time and cannot be compared directly, e.g., for using soil concentrations and earthworm concentrations to calculate bioaccumulation factors. Some monitoring projects measured soil biodiversity in relation to general land use pattern and not specifically dependent on soil concentrations of PPP's (Rutgers et al., 2009).

In this study, monitoring data on residues in earthworms are available from a two-year investigation in agricultural fields in Croatia, although most active ingredients were not analytically determined in the corresponding soils. Since the data on application time and amount of applied PPP's were delivered by the farmers, a retrospective analysis of analytically measured residues in earthworms and re-calculated soil concentrations was performed with the aim to answer the following questions:

a) can the concentrations of active ingredients in soil be reliably calculated based on information from farmers and soil dissipation studies from publicly available assessment reports;

b) are the "hybrid" bioaccumulation factors, calculated by using analytically measured residues in earthworms and recalculated soil concentrations, comparable to literature data;

c) are the "hybrid" bioaccumulation factors suitable for the assessment of the potential for secondary poisoning;

d) d) are the recalculated soil concentrations of active ingredients suitable for the assessment of their potential risk to the earthworms.

MATERIALS AND METHODS

Field site and cultivation

Four fields in each of two investigated regions (Tovarnik, Lukač) in Croatia were cultivated with alternative crops according to good agricultural practices in 2015 and 2016. The predominant crops were wheat, maize, or sugar beet. In the two seasons, 2 – 16 different pesticides were applied per field, namely 2 – 10 herbicides, 2 – 9 fungicides, and 0 – 6 insecticide active ingredients. Farmers provided information on the name of the pesticide used, application rate in the case of spray application or seed density in the case of sowing treated seed, as well as time of application.

Earthworm sampling and residue analysis

Earthworms were sampled three times during the two seasons (autumn 2015, spring 2016., and autumn 2016) following the sampling method of ISO 23611-1 (2006). The fresh weight of the earthworm samples was 5-17 g/sample. The earthworm samples were deep frozen until analysis. Analysis was done by liquid chromatography-tandem mass spectrometry (LC-MS/MS), with so called "QuEChERS" (Quick, Easy, Cheap, Effective, Rugged and Safe) pre-treatment sample purification method (Anastassiades et al., 2003). Limit of quantification (LOQ) was 0.001 mg/kg in case of earthworm fresh weight and limit of detection (LOD) = ½ LOQ. LC-MS/MS is one of the most widely used techniques for pesticide multiresidue analysis in food due to their high sensitivity and selectivity and their ability to screen many pesticides from different chemical classes in a very complex matrix in a single run. LC-MS/MS is suitable for both more polar pesticides and pesticide metabolites, which are often more polar and less volatile than the pesticide itself (Stachniuk and Fornal, 2016).

Recalculation of soil concentrations

Substance specific dissipation curves in soil were calculated by using soil concentrations at DT_0, DT_{50} and DT_{90} (DT = dissipation time when 0, 50 and 90% of the substance has dissipated from the soil). The soil concentration at DT_0 was derived from the application rate on a study field by converting the application rate (g a.i./ha) to soil concentration (mg a.i./kg dry soil), considering the soil density of 1.5 g/cm^3 and a soil depth of 30 cm. The values for DT_{50} and DT_{90} were taken from data of soil dissipation field studies, publicly available in EC review reports for active substances (1998 – 2016) and EFSA scientific reports on conclusion on the peer review of active substances (2005 – 2016). Based on the soil concentration at DT_0, the soil concentrations at DT_{50} (i.e., 50% of soil concentration at DT_0) and DT_{90} (i.e., 10% of soil concentration) were derived, and the three soil concentrations at three different times were used for construction of a logarithmic dissipation curve following the formula

$$y = a * e^{(-b * x)}$$

y = concentration in soil at day x; a = soil concentration at day 0; b = substance – specific slope; x = time after application.

Calculation of bioaccumulation/bioconcentration factors

Bioaccumulation is the general uptake and storage of substances, while uptake from the surrounding medium as part of bioaccumulation is defined as bioconcentration (Franke et al., 1994, Fent, 2013).

Bioconcentration is a measure of the amount of pesticide residues in an organism's tissues relative to the concentration in the organism's environment (Zartarian and Schultz, 2009). This includes the uptake of pesticides through respiration and contact, but not through food sources. Bioconcentration factors (BCF) are calculated by considering pesticide tissue concentrations relative to pesticide concentrations in the environment.

BCF Values > 1 indicate that the concentration in the organism is higher than that of the medium (e.g., soil or water) from which the pesticide was taken (USEPA, 2021). In this study, bioconcentration cannot be separated from bioaccumulation, so the two terms are used interchangeably. The ratio of concentration in earthworms and concentration in soil was defined as bioconcentration factor. For nine active ingredients, data from investigated fields allowed the calculation of bioconcentration factor, using analyzed residues in earthworms and recalculated soil concentrations.

Assessment of potential for secondary poisoning

Secondary poisoning is defined by the transfer of the active ingredient within the food chain from earthworms to earthworm-eating birds and mammals. The assessment of the potential for secondary poisoning followed EFSA (2009) procedure. In a five-step calculation scheme, the predicted environmental concentration in soil (PEC_{soil}) was determined. In EFSA (2009), the theoretical bioconcentration factor for earthworms ($BCF_{earthworm}$) is calculated using the substance-specific partition coefficient in octanol/water (as a measure of lipophilicity) and the substance-specific partition coefficient in soil organic carbon/water (as a measure of adsorption). In this study the bioconcentration factor can be derived from the ratio of measured residues in earthworms and the calculated soil concentration at the time of sampling. The residues in earthworms as predicted environmental concentrations (PECearthworm) were estimated by multiplying PEC_{soil} and $BCF_{earthworm}$. The estimated residues in earthworms were converted to daily consumption doses for birds (factor 1.05) and mammals (factor 1.28) and finally toxicity-exposure ratios ($TER_{seconday\ poisoning}$) were calculated by using No-Oberserved-Adverse-Effect-Levels (NOAEL) from chronic dietary studies with birds and mammals, taken from the above-mentioned EC review reports (1998 – 2016) and EFSA scientific reports (2005 – 2016). The calculated daily consumption doses for birds and mammals. $TER_{seconday\ poisoning}$ values < 5 indicate a potential risk for secondary poisoning and would require further refinement.

Assessment of potentially toxic effects to earthworms

Data on laboratory reproduction tests with the compost earthworm *Eisenia fetida* Savigny were available from above mentioned EC review reports (1998 – 2016) and EFSA scientific reports (2005 – 2016). The toxicity endpoint was the No-Observed-Effect-Concentration (NOEC) where the number of juvenile worms did not significantly differ from the control. A toxicity-exposure ratio (TER_{worm}) was calculated by using the NOEC from the worm reproduction test and the soil concentration at the time of application. TER_{worm} values < 5 indicate a potential risk to earthworms and would require further refinement.

RESULTS AND DISCUSSION

Residues found in earthworms

The sampling resulted in 58 individual earthworm samples over the two-year investigation. The fresh weight of the samples was 5 -17 g/sample. Screening for 300 active ingredients was performed for each sample, of which 26 active ingredients were detected (9%). From 1566 analytical measurements, 33.2 % were < LOD, 43.5 % between LOD and LOQ and 23.3 % ≥ LOQ (Table 1). Three active ingredients, boscalid, fipronil, and difenoconazole, were detected, although farmers reported that they had not been applied during the two study years and were residues from previous year's applications.

Seven active ingredients were detected in 100% of earthworm samples (i.e. the insecticides imidacloprid, clothianidin and thiamethoxam, the fungicides azoxystrobin and cyproconazole, the herbicides tembotrione and ethofumesate) (Figure 1).

Table 1. Residues of active ingredients in earthworm samples 2015 and 2016

Number of analysed earthworm samples, Location: municipality Lukač, Croatia (45.8739° N, 17.4191° E)	34
Number of analysed earthworm samples, Location: municipality Tovarnik, Croatia (45.1649° N, 19.1522° E)	24
Earthworm number per sample	2-62 (mean ± SD: 18.4 ± 11.4)
Earthworm fresh weight per sample	5.0 – 27.0 g (mean ± SD: 10.1 ± 4.6 g)
Limit of quantification (LOQ)	0.001 mg/kg earthworm fresh weight
Limit of detection (LOD = ½ LOQ)	0.0005 mg/kg earthworm fresh weight
No. active ingredients (a.i.s) analysed	300
No. active ingredients detected	26
No. analytical measurements	1566
• Percentage < LOD	33.2
• Percentage between LOD and LOQ	43.5
• Percentage ≥ LOQ	23.3
No. a.i.s detected in one earthworm sample	12 - 20
No. a.i.s quantified in one earthworm sample	3 - 12

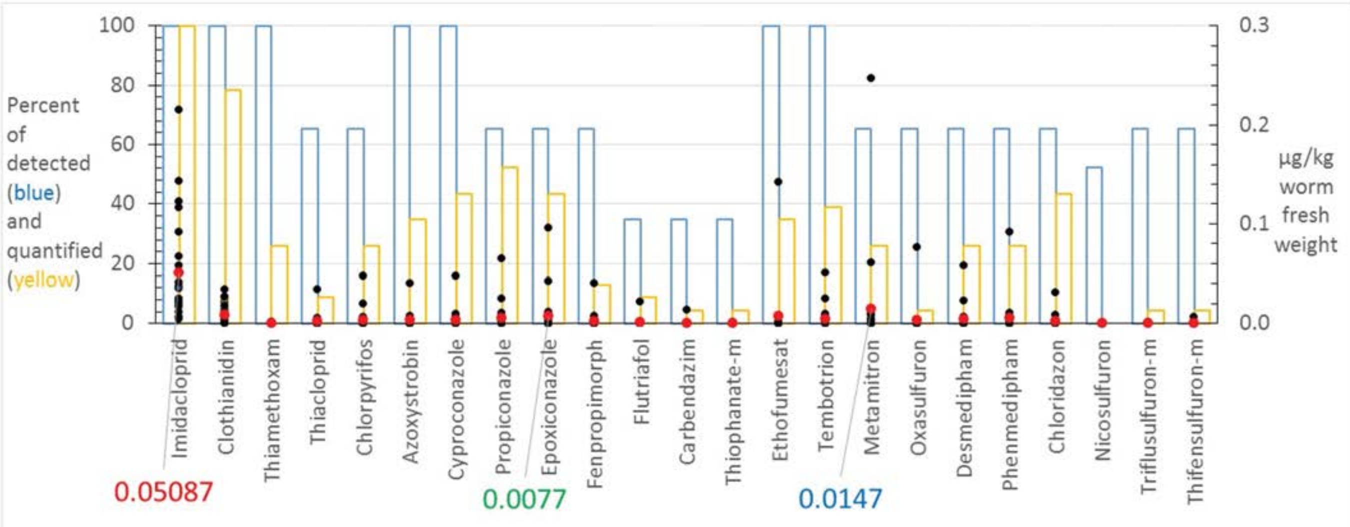

Figure 1. Frequency of detection and concentration of 23 active ingredients analyzed in earthworm samples from four plots of two fields (Lukač, Tovarnik) during three sampling dates (blue and yellow bars represent the percent of detection and quantification. Black dots denote individual concentrations per plot (only one subplot sampled on one sampling date) or mean concentrations per plot (2-4 subplots sampled on one sampling date. Red dots denote the overall mean of the analyzed concentrations)

Imidacloprid was the only active ingredient which was quantified in all earthworm samples. The highest mean concentrations of an insecticide, fungicide, and herbicide in one plot were: 0.05087 mg imidacloprid/kg earthworm fresh weight, 0.0147 mg metamitron/kg earthworm fresh weight and 0.0077 mg epoxiconazole/kg earthworm fresh weight, respectively.

Recalculation of soil concentrations

The dissipation behaviour of active ingredients in soil was calculated by using following data points: the soil concentration at the time of application as soil concentration at DT_0 and the soil concentrations at DT_{50} and DT_{90}, taken from EC reports (1998 – 2016) and EFSA reports (2005 – 2016). Examples are presented as Figure 2 for the herbicide ethofumesate, fungicide azoxystrobin and the insecticide imidacloprid. The coefficient of determination R^2 was > 0.95 for the majority of the active ingredients indicating that the used dissipation formula was reliable for estimating the soil concentration of an active ingredient at any time after application.

Calculation of bioaccumulation factors

Dividing the analysed residues of an active ingredient in earthworm samples by its corresponding calculated soil concentration at the time of earthworm sampling results in a ratio, the bioconcentration factor (BCF) (Figure 3). BCF values > 1.0 indicate an accumulation within the earthworms. For nine active ingredients, a variation of plot-spectific BCF values below and above the trigger value of 1.0 is observed. Therefore, the potential for bioconcentration cannot be considered as straight-forward but seems to depend on plots characteristics and the time between application and sampling. For imidaclopid, thiamethoxam, metamitron and phenmediphamthe the mean BCF value is > 1.0.

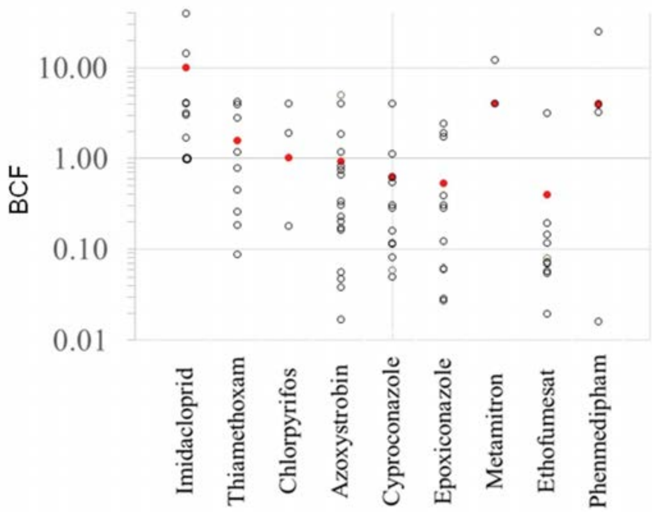

Figure 3. Bioconcentration factors (BCF) of nine active ingredients in earthworm samples derived from calculated soil concentrations at the time of sampling (white dots indicate BCF values from individual field plots; red dots indicate the resulting mean value)

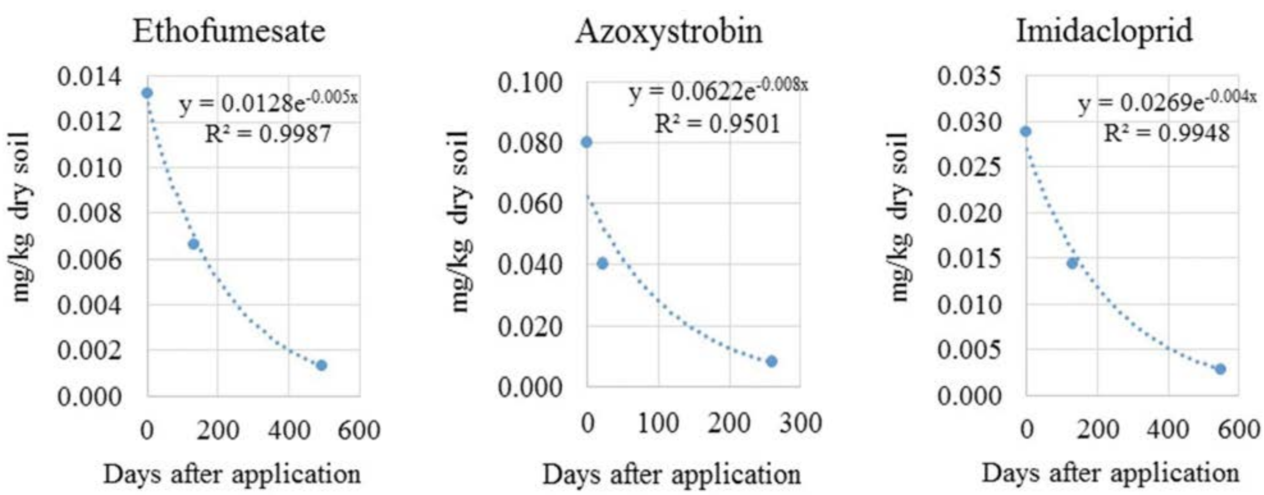

Figure 2. Three examples of soil dissipation curves for the active ingredients ethofumesate (left figure), azoxystrobin (middle figure) and imidacloprid (right figure)

The calculated BCF values of this study are comparable to values from the literature, as shown for imidacloprid (BCF = 15, Chevillot et al. 2017), thiamethoxam (BCF = 1-2, Douglas et al. 2015), azoxystrobin (BCF = low risk, EFSA 2009) and ethofumesate (BCF = 2.2, Xu et al. 2014).

Therefore, the information of farmers regarding the actual application rate and application time of a product is highly valuable for the calculation of the soil concentration at a specific time after the application and can be used for the calculation of bioconcentration factors.

Assessment of potential for secondary poisoning

Earthworms are considered as potential prey for mammals and birds. According to EFSA (2009), the predicted environmental concentration in earthworms (PEC_{worm}) is calculated based on a theoretical bioconcentration factor BCF(calc.) from substance-specific physicochemical data i.e., logarithm of the octanol-water partition (LogPow) and logarithm of the octanol-water partition (Koc) (Table 2.). For eight out of nine active ingredients, all $TER_{secondy\ poisoning}$ values were > 5 indicateing no potential for secondary poisoning to earthworm-feeding mammals and birds. In the case of chlorpyriphos, the high lipophilicity (LogPow = 7.0) triggers a high PEC_{worm} and accordingly a $TER_{secondy\ poisoning}$ value of < 5 meaning a high risk for secondary poisoning to mammals and birds.

As the previous section shows, the "hybrid" bioaccumulation factors derived from analytically measured earthworm concentrations and recalculated soil concentrations are reliable and can be used for further refinement. When replacing BCF (calc.) with the measured BCF, the $TER_{secondy\ poisoning}$ values for chlorpyriphos are > 5 and no longer pose a risk to birds and mammals. For the remaining eight active ingredients, $TER_{secondy\ poisoning}$ values generally decrease but still do not exceed the trigger of TER < 5.

Table 2. Potential of secondary poisoning using calculated BCF values

Active ingredient	Physchem data		Application		PEC_{soil} mg/kg dry soil at sampling	BCF (calc.)	PEC_{worm} = PEC_{soil} x BCF (calc.) mg/kg	Residue (mg/kg)		long-term NOAEL (mg/kg/day)		TER	
	Log Pow	Koc	g/ha	PEC_{soil} mg/kg dry soil				mamal	bird	mamal	bird	mamal	bird
imidacloprid	0.57	225	130	0.0289	0.0037	0.1966	0.0007	0.0009	0.0008	5.7	9.3	6123	12178
thiamethoxam	-0.13	56.2	36	0.0080	0.0001	0.7552	0.0001	0.0001	0.0001	2.6	29.4	26895	370740
chlorpyrifos	7.0	8151	850	0.1889	0.012	736.1	8.8	11.3	9.3	1.0	25.0	0.088	2.695
azoxystrobin	2.5	482	165	0.0367	0.0082	0.4808	0.004	0.0050	0.0041	20	1200	3963	289888
cyproconazole	3.09	711	64	0.0142	0.0118	1.0973	0.013	0.0166	0.0136	1.84	1.4	111	103
epoxiconazole	3.3	2647	112	0.0249	0.0049	0.4681	0.002	0.0029	0.0024	2.30	10.0	783	4152
metamitron	0.85	122	700	0.1556	0.0004	0.3791	0.0002	0.0002	0.0002	4.9	81.5	25246	511892
ethofumesat	2.7	147	60	0.0133	0.045	2.3314	0.1049	0.1343	0.1102	7.0	406	52	3686
phenmedipham	4.0	888	78	0.0173	0.0004	6.8041	0.0027	0.0035	0.0029	6.8	82	1952	28554

Log Pow: Lipophilicity (Log of partition between octanol and water);
Koc: Potential for adsorption (Distribution between organic carbon and water);
PEC_{soil}: Predicted Environmental Concentration in soil (30 cm soil depth, density 1.5 kg/L), BCF (calc.) Bioconcentration Factor (calculated: $BCF_{earth-worm}$ = (0.84 + 0.012 * Pow) / (foc x Koc));
PEC_{worm}: Predicted Environmental Concentration in worms (PEC_{worm} = PEC_{soil} x BCF(calc.));
Residue in mammals: PEC_{worm} x 1.28;
Residue in birds: PEC_{worm} x 1.05;
NOAEL: No-observed-adverse-effect level from chronic studies with mammals and birds;
TER: Toxicity-Exposure-Ratio from NOAEL/Residue (risk is TER < 5)

Table 3. Potential of secondary poisoning using measured BCF values

Active ingredient	Application		PEC_{soil} mg/kg dry soil at sampling	Residues in worms (mg/kg)	Measured BCF (max)	PEC_{worm} = PEC_{soil} x BCF	Residue (mg/kg)		long-term NOAEL (mg/kg/day)		TER	
	g/ha	PEC_{soil} mg/kg dry soil					mamal	bird	mamal	bird	mamal	bird
imidacloprid	130	0.0289	0.0037	0.1427	38.6	0.1427	0.1827	0.1498	5.7	9.3	31	62
thiamethoxam	36	0.0080	0.0001	0.0005	5.0	0.0005	0.0006	0.0005	2.6	29.4	4063	56000
chlorpyrifos	850	0.1889	0.012	0.0475	4.0	0.0475	0.0608	0.0499	1.0	25.0	16	501
azoxystrobin	165	0.0367	0.0082	0.0408	5.0	0.0408	0.0522	0.0428	20	1200	383	28011
cyproconazole	64	0.0142	0.0118	0.0475	4.0	0.0475	0.0608	0.0499	1.84	1.4	30	28
epoxiconazole	112	0.0249	0.0049	0.0177	3.6	0.0177	0.0227	0.0186	2.30	10.0	102	538
metamitron	700	0.1556	0.0004	0.0043	10.8	0.0043	0.0055	0.0045	4.9	81.5	890	18051
ethofumesat	60	0.0133	0.045	0.142	3.2	0.1420	0.1818	0.1491	7.0	406	39	2723
phenmedipham	78	0.0173	0.0004	0.0105	26.3	0.0105	0.0134	0.0110	6.8	82	506	7401

PEC_{soil}: Predicted Environmental Concentration in soil (30 cm soil depth, density 1.5 kg/L);
BCFmax: Bioconcentration factor (maximum calculated value from measured earthworm residues and calculated soil concentration at the time of sampling);
PEC_{worm}: Predicted Environmental Concentration in worms (PEC_{worm} = PEC_{soil} x BCFmax);
Residue in mammals: PEC_{worm} x 1.28;
Residue in birds: PEC_{worm} x 1.05;
NOAEL: No-observed-adverse-effect level from chronic studies with mammals and birds;
TER: Toxicity-Exposure-Ratio from NOAEL/Residue (risk if TER < 5)

Nevertheless, the comparison of Table 2 and Table 3 shows that environmentally relevant values can be derived from compound-specific characteristics but should be taken with caution and verified by measured values as far as possible.

Assessment of potential toxic effects to earthworms

Recalculated soil concentrations, based on application information provided by farmers, are converted from application rates (g a.i./ha) to soil concentrations (mg a.i./ha). These expected soil concentrations directly after application are used for the assessment of the potential risk of plant protection products on earthworms in the field. The toxicity-exposure ratio (TER_{worm}) for earthworms was derived from the values of no-observed-effect-concentrations (NOEC) from earthworm laboratory reproduction studies and the expected soil concentrations directly after application (OECD, 1984; 2016).

For fungicides (Table 4), NOEC values from earthworm reproduction studies were available for all 12 fungicide active ingredients used and resulted in TER values of 1.5 - 241. The two fungicides epoxiconazole and thiophanate-methyl resulted in TER values of 1.5 and 4, respectively, and would need to be further evaluated for their potential risk to earthworms in the environment. Some fungicides are characterised by the same mode of action and may cause mixed toxicity to earthworms when applied in the same season. This needs to be further evaluated.

When replacing the expected soil concentration directly after application by the maximum calculated soil concentration at the time of earthworm sampling, the TER_{worm}-values increased as expected since the soil concentrations decreased continuously after application. This decrease was rather slow for epoxiconazole resulting into a still critical TER_{worm}-value.

Table 4. Fungicide active ingredients risk potential on earthworms in the field

Active ingredient	Number of fields	Number of applications	Application		Soil conc. at time of sampling	Toxicity to *Eisenia*			Mode of action
			g/ha	soil conc		repro NOEC	TER at DAT 0	TER at time of sampling	
				mg/kg dry soil					
azoxystrobin	8	8	165	0.083	0.0282	20	241	709	Respiration
carbendazim	1	1	250	0.125	0.0481	1	8.0	21	Mitosis and cell division
thiophanate-methyl	7	7	465	0.233	0.0001	0.85	3.6	8500	
chlorothalonil	2	2	500	0.25	0.0482	50	200	1037	Multi-site activity
copper oxychloride	2	3	750	0.375	0.0824	15	40	182	
cyproconazole	6	6	64	0.032	0.0118	0.75	23	64	Sterol biosynthesis
epoxiconazole	6	7	281	0.056	0.0274	0.084	1.5	3.1	
fenpropimorph	1	1	250	0.125	0.0544	4.7	38	86	
flutriafol	1	2	75	0.038	0.0164	6.1	161	372	
propiconazole	1	1	130	0.065	0.0285	0.833	13	29	
prothioconazole	2	2	100	0.05	0.0001	1.33	27	13300	
tebuconacole	2	2	100	0.05	0.0063	10	200	1587	

NOEC: Experimentally determined no-observed effect concentration from earthworm reproduction tests according to OECD 222;
TER: Toxicity-Exposure Ratio from soil concentration/repro-NOEC

Therefore, the environmental risk assessment on earthworms should consider that a slow degradation rate of an active ingrdient might impact earthworms over a longer time period.

CONCLUSIONS

Field dissipation curves (based on EU, EC and EFSA) reasonably predict the soil residue concentration of active ingredients at any time after application. Therefore, the analytically determined residues in earthworms from the two regions, Lukač and Tovarnik, can be reliably used for the calculation of bioconcentration factors. A comparison with literature data shows that these "hybrid" bioconcentration factors are reasonable and can be used for a basic assessment of the potential for bioaccumulation. Most active ingredients do not pose a risk to the earthworms and have no potential of secondary poisoning for earthworm-eating birds and mammals. The most important mitigation measure is to reduce the number of applications and/or the amount of application rates used.

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IMPACT OF ENVIRONMENTAL CONDITIONS AND AGRO-TECHNICAL FACTORS ON GROUND BEETLE POPULATIONS IN ARABLE CROPS

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Abstract. Ground beetles (Coleoptera: Carabidae) are the largest family of adephagan beetles. Their role in natural pest control is important due to their predatory polyphagous nutrition and bioindicative value since they are sensitive to environmental and anthropogenic changes. Therefore, the main objectives were to understand how common arable cropping systems in Croatia affect ground beetles abundance in respect to the environmental conditions. We hypothesized that environmental specifics (soil type and structure, climatic conditions) together with cultivation measures (tillage and insecticide application) would affect ground beetle activity and abundance. The research was conducted in two locations Lukač, Virovitica – Podravina County and Tovarnik, Vukovar – Sirmium County. Ground beetles were collected weekly, from May to September 2015, by epigeic pitfall traps and endogeic perforated probes from fields sown with typical arable crops in these areas. In total, 2,582 ground beetle individuals were collected using epigeic traps, and 323 ground beetles were collected using endogeic traps. Significantly lower ground beetle abundance has been recorded in Tovarnik than in Lukač. The crop and cropping history affect the abundance through modification of environmental conditions (soil characteristics, microclimate factors such as temperature and humidity), as well as trough disturbance factors such as tillage schedules and harvest/sowing schedules.
Keywords: *carabids, agro-technical measures, environment, plant cover, abundance*

Introduction

Ground beetles (Coleoptera: Carabidae) with over 40,000 species are the largest family of adephagan beetles (Lövei and Sunderland, 1996) inhabiting arable crops all over the world (Kromp, 1999). They are often used in cultivation experiments because they are one of the most abundant and diverse groups overwintering within cultivated fields (Holland and Reynolds, 2003). Ground beetles are bioindicators of agroecosystems quality (Cole et al., 2002; O´Rourke et al., 2008) and can be good ecological indicators of environmental change (Thiele, 1977; Maelfait, 1990). In term of environmental quality, arable land presents an anthropogenically influenced, unstable and devastated biotope with low contribution to farmland diversity (Baranová et al., 2013). Environmental change can cause a different kind of effects on the indicator species, including physiological changes or changes in species number and abundance (Raino and Niemelä, 2003). Increase or decrease of ground beetle abundance might be directly caused by the change in many abiotic and/or biotic factors (Blake et al., 1996). These factors include temperature and humidity (Lövei and Sunderland, 1996), soil characteristics, land heterogeneity and agricultural measures such as tillage, crop type, fertilization regimes, crop rotation and pest control (Stassart and Grégoire-Wibo, 1983; Kromp 1999; O´Rourke et al., 2008; Kos, 2010; Asteraki et al., 1992; 1995).

A further decisive factor for habitat selection considering soil specifics is soil particle size distribution (Thiele, 1977; Meissner, 1984). Vician et al. (2015) stated that content of organic matter and pH are the most significant factors that influence ground beetle diversity and abundance in agroecosystems. Some aspects of landscape heterogeneity (e.i. field size (Kromp, 1999), non-cropped habitat (Pollard, 1968; Sotherton, 1985) and land use diversity (Östman et al., 2001)), will also influence the ground beetles communities (Chapman, 2014).

Stassart and Grégoire-Wibo (1983) stated that depth of tillage is one of the major factors affecting ground beetle field fauna. Dobrovolsky (1970) and Baguette and Hence (1997) reported that deep cultivation had a detrimental effect on ground beetle abundance. Opposite to that, Cárcamo (1995) and Weibull et al. (2003) trapped significantly more ground beetle individuals under intensive tillage compared with reduces tillage.

Arable crops can affect ground beetles through modification of microclimatic factors, and trough disturbance factors (harvest and tillage schedules; Thiele, 1977; Witmer et al., 2003; O´Rourke et al., 2008). Although no ground beetles appear to be strictly related to certain crops, some studies reported a general difference between ground beetle abundance distributions in winter cereal versus root crops (beets) (Kromp, 1999). In root crops, the long period of bare soil in early spring present extreme soil-surface microclimate which has a negative influence on ground beetle abundance. In winter cereals, the less extreme microclimate is established in early spring and creates positive conditions for many ground beetle species (Kromp, 1999). Also, ground beetle abundance can be influenced by the crop-dependent timing of cultivation measures. Spring tillage and insecticide treatments can affect ground beetles at the beginning of their activity, but also, autumn tillage and insecticide treatments can disturb ground beetles overwintering (Hence et al., 1990).

However, the effects of soil tillage could not be clearly separated from the effects of different fertilization regimes (e.g. manure, mineral fertilizers) and may also vary with the crop, and among localities. Pietraszko and De Clercq (1982) and Hence and Grégoire-Wibo (1987) revealed organically manured fields to have higher ground beetle diversity and abundances. Similar results were reported by Bažok et al. (2007) and by Kos et al. (2011) in Croatian conditions. Kromp (1990) showed that the ground beetle abundance and diversity significantly decreased in the fields with the high amount of nitrogen applied as mineral fertilizer, manure and liquid manure.

The population of ground beetles in the agricultural landscape can be also influenced by the chemical pest control (Varvara et al., 2012). Basedow (1987) investigated ground beetle populations in winter wheat fields and found a significant decrease of ground beetles density as a consequence of intensive insecticide application against cereal aphids. Opposite results were established by Kos et al. (2010) who did not find significant differences in ground beetle abundance between treated and untreated fields. Negative effects of insecticides on ground beetles were recorded by Asteraki et al. (1992; 1995). Douglas et al. (2014) shown that insecticides (e.g. thiametoxam) can be poisonous to ground beetles due to their predatory nature. This means that chemical treatment of some agricultural pests can also affect ground beetles that consumed them (Jeschke et al., 2011; Szczepaniec et al., 2011).

In this study, the main objectives were to understand how common arable agroecosystems affect ground beetles populations in respect to the environmental conditions. We hypothesized that, within a region, environmental specifics (soil type and structure, climatic conditions) together with cultivation measures (tillage, insecticide application) would affect ground beetle activity and abundance. To test this hypotheses we compared ground beetle abundances and population dynamics in different arable agroecosystems in two different management regions.

Materials and methods

Sample sites

Ground beetles were collected during the arable crop growing season 2015 in two different counties of Croatia representing two distinct climatic and edaphic areas: Lukač (Virovitica – Podravina County) and Tovarnik (Vukovar-Sirmium County). In each county four fields with different cropping history were chosen for ground beetle trapping. The fields were chosen to represent common cultivation and crop rotation practices as well as the agro-technical measures in both areas. Since the soil type and soil characteristics differ between locations, the tillage is adapted to the given conditions. In Virovitica – Podravina County soils contain a great amount of fine sand and coarse silt which requires conservation tillage. This means that autumn ploughing on a depth of 20 – 25 cm is followed by the furrow closure for moisture conservation in spring. In Vukovar – Sirmium County soil contains a great amount of clay which requires deeper autumn mouldboard ploughing (30 – 35 cm). Chisel ploughing and tillage with the rotary harrow in spring and after harvest are usually followed by disk harrowing and again chisel ploughing. Characteristics of sample sites are introduced in *Table 1*.

Climatic and edaphic factors

Climate data used in this study (i.e., mean weekly air temperature, mean weekly soil temperature and the total amount of rainfall per week) were obtained from the Croatian Meteorological and Hydrological Service for the sampling period and analyzed per field site for period from May to September, 2015 (19th to 38th week of the year). From the fields at investigation area, soil samples were taken from the depth of a plow layer (30 cm) and regional physical and chemical soil properties have been analysed. Performed pedological procedures are explained in details in Kozina et al. (2015). Plant abundance is measured by plant cover, i.e., the relative projected area covered by a species (Kent and Coker, 1992). In our study, we used data from Kisić et al. (2005) about the percentage of plant cover, which are characteristic for part of Croatia where our study was conducted.

Table 1. *General information about fields where research has been conducted*

	Lukač (Virovitica-Podravina County)				Tovarnik (Vukovar-Sirmium County)			
Field label	0L	1L	2L	3L	0T	1T	2T	3T
Crop type	sugar beet	wheat	maize	oilseed rape	sugar beet	maize	wheat	wheat
Bare soil (mth)*	9	1	8	2	7	6	2	2
Fertilization	85 kg N 105 kg P 135 kg K	168 kg N 60 kg P 90 kg K	120 kg N 52 kg P 52 kg K	74 kg N 60 kg P 90 kg K	123 kg N 52 kg P 228 kg K	-	82 kg N	101 kg N
Insecticide treatment	Imidacloprid (seed treatment)	Lambda-cyhalothrin (foliar treatment)	-	Thiacloprid (foliar treatment)	Imidacloprid+ Thiamethoxam (seed treatment) Lambda-cyhalothrin x2 (foliar treatment) Cypermethrin + Chlorpyrifos ethyl (foliar treatment)	-	Lambda-cyhalothrin (foliar treatment)	Lambda-cyhalothrin (foliar treatment)
Pre-crop (2014)	wheat	sugar beet	wheat	wheat	soybean	sugar beet	sunflower	sunflower
Pre-crop (2013)	sunflower	maize	sugar beet	sunflower	wheat	wheat	sugar beet	barley
Pre-crop (2012)	maize	maize	fallow	sugar beet	sugar beet	sunflower	wheat	sugar beet

*number of months when fields were not covered after harvesting (in 2014) till soil preparing for crops which was grown in 2015 vegetation season

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Ground beetle trapping

Epigeic covered pitfall traps and endogeic perforated probes were used to collect adult ground beetles. On each of four fields on both locations four pitfall traps and four perforated probes (WB PROBE II ® Trap, Trece inc.) were placed. Polythene pots (Ø=12 cm, h=18 cm) were incorporated 18 cm into the soil and covered with PVC roofs (Ø=16 cm) approximately 2-4 cm above ground level. Four pitfall traps were placed into the center of the each field at 50 m apart and 100 m away from the field edges to minimize the edge effect on ground beetle catches. Each trap was half filled with salted water (20% solution) for captures conservation, with the addition of few drops of detergent to reduce the surface tension (no other chemicals were added). Trapping was performed from the 19th to the 38th week of the year. Perforated PVC probes were placed at 10 m distance from each pitfall traps and also inspected once a week. During weekly observation period, all ground beetles caught were collected from the traps and counted. For identifying ground beetles following keys were used: Auber, 1965; Bechyne, 1974; Harde and Severa, 1984).

Data analysis

Meteorological data (mean air temperature, mean soil temperature and the total amount of rainfall), the physical and chemical properties of the soil, and the average number of collected ground beetles were analyzed by a one-way analysis of variance (ANOVA; Gylling Data Management, Inc., USA, ARM 9® GDM software, Revision 9.2014.7.). A Tukey's posthoc test was used to establish climatic and edaphic differences among the fields and between the investigated areas. Where appropriate, data were $\sqrt{(x+1)}$ transformed. The ground beetle trapping results using both endogeic and epigeic traps for the selected intervals are presented as a mean number of individuals caught per field per week as a function of percent of ground cover with culture on every field. Ground beetle population dynamic results for the selected intervals are presented as the total number of ground beetles caught per traps (epigeic and endogeic) per field per week as a function of the average weekly air temperatures (°C), total weekly precipitation (mm) and average weekly temperature of soil (°C) at a depth of 10 cm. Values were determined from 19th to the 38th week of the year for epigeic caches, and from 22nd to the 38th week for endogeic caches.

Results

Climatic and edaphic factors

The both investigated locations were classified as belonging to the Cfwbx climatic type of the Köppen classification system (Penzar and Penzar, 2000), where temperate/mesothermal climates (Cf) with dry winters (w) dominate. In spite belonging to the same climatic type, locations in this research differ according to the climatic conditions. Significant differences in mean air and soil temperatures occurred as did the total amount of rainfall (*Table 2*). Edaphic conditions differ among locations also. Significant differences in the share of fine sand, fine silt, clay and pH values occurred. A detailed description of the regional physical and chemical soil properties are given in *Table 3*.

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Table 2. *Characteristics of the weather conditions prevailing in the two Croatia counties were ground beetles were sampled and corresponding ANOVA results*

County	Mean soil temperatures (˚C)	Mean air temperatures (˚C)	Total amount of rainfall (mm)
Virovitica-Podravina	23.40 b*	20.52 b	492.30 a
Vukovar-Sirmium	25.22 a	23.50 a	220.00 b
HSD P = 0.05	0.32	0.23	118.17

*means followed by the same letter are not significantly different according to the Tukey's HSD test (P<0.05)

Table 3. *Physical and chemical properties of the soil samples in two Croatia counties and corresponding ANOVA results*

Soil physico-chemistry	County		HSD P = 0.05
	Virovitica-Podravina	Vukovar- Sirmium	
Coarse sand	2.35	1.62	ns
Fine sand	11.83 a*	2.47 b	4.583
Coarse silt	38.42	35.87	ns
Fine silt	31.65 a	28.39 b	2,012
Clay	15.75 b	31.65 a	2,766
Soil pH in H2O	6.65 b	7.71 a	0,498
Soil pH in KCl	5,58 b	6.93 a	0.691
Humus	3.2	3.29	ns

*means followed by the same letter are not significantly different according to the Tukey's HSD test (P<0.05)

Ground beetle trapping

In total, 2,582 ground beetle individuals were collected using epigeic traps, and 323 ground beetles were collected using endogeic traps. Generally, significantly lower ground beetle abundance on all four fields in both types of traps has been recorded in Tovarnik than in Lukač (*Table 4 and 5*). In the whole sampling period, only one ground beetle has been collected with an endogeic trap on one out of the four fields in Tovarnik. Although slightly higher number of ground beetles was collected in Tovarnik on the field 3T, analyzing epigeic caches no significant difference among fields was recorded (*Table 4*).

Table 4. *The average number of ground beetles collected using epigeic traps in 20 weeks sampling period in two location of Croatia and the corresponding ANOVA results*

Fields	Location		HSD P = 0.05
	Lukač	Tovarnik	
0	62,37 b[1]A[2]	5,59 B	2,77t*
1	67,07 bA	11,21 B	1,99t
2	108,89 bA	10,38 B	2,03t
3	356,66 aA	12,59 B	0,595t
HSD P = 0.05	3,28 t*	ns	

*data were transformed by square root transformation of X+0.5; mean descriptions are reported in transformed data units and are not de-transformed; [1] small letters refer to differences among fields; [2]capital letters refer to differences among localities

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Table 5. *The average number of ground beetles collected using endogeic traps in 15 weeks sampling period in two location of Croatia and the corresponding ANOVA results*

Fields	Location		HSD P = 0.05
	Lukač	Tovarnik	
0	1,11 c[1]	0,00	ns
1	2,81 bc	0,19	ns
2	8,4 b	0,00	ns
3	59,04 aA[2]	0,00 B	0,385t*
HSD P = 0.05	0,567 t*	ns	

*data were transformed by square root transformation of X+0.5; mean descriptions are reported in transformed data units and are not de-transformed; 1 small letters refer to differences among fields; 2capital letters refer to differences among localities

At location Lukač significantly higher epigeic caches occurred on field 3L comparing with other three fields. There have been no significant differences between caches on 0L, 1L and 2L fields. Similar results occurred with caches by endogeic traps (*Table 5*). At field 3L significantly highest abundance occurred, while at other fields differences exist but not significant. At field 0L the lowest ground beetle abundance has been observed.

Generally, the highest ground beetle abundance was recorded at field 3 (sugar beet sown three years ago) on both locations using both trap methods. The lowest ground beetle abundance was recorded at field 0 (sugar beet field) on both locations (*Table 4 and 5*).

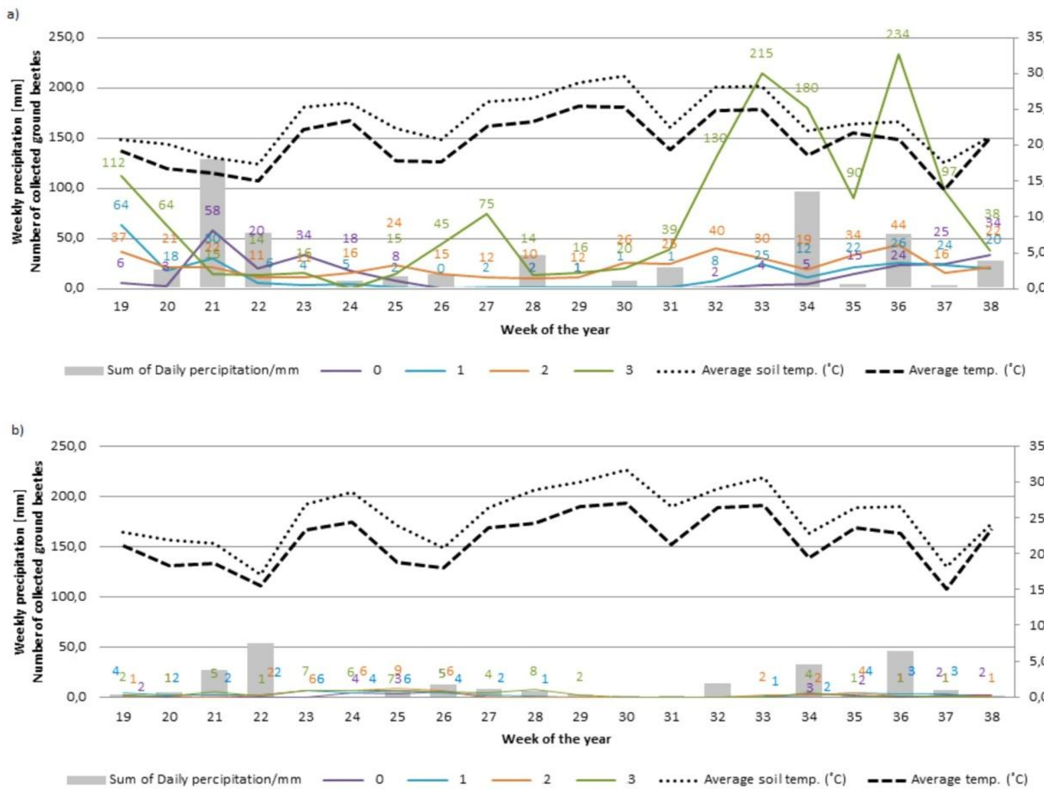

Figure 1a and b. *Number of collected ground beetles per week in Lukač and Tovarnik by epigeic traps in respect to the weekly precipitation and temperature*

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At location Lukač, the total number of ground beetles collected by epigeic traps per week was high at the beginning of collection period at week 19th and lasted till week 21st. The second maximum of the population started at the 32nd week and lasted till 36th week (*Fugure 1a*). The weekly endogeic caches at location Lukač were low but with an evident increase of ground beetle caches from 24th till 28th week, and again at 37th and 38th week (*Figure 2a*). At location Tovarnik caches in all period were low. Slight population increase in epigeic caches was observed from 23th till 28th week, and again from 34th till 37th week (*Figure 1b*). Only one ground beetle was collected with endogeic trap at location Tovarnik (*Figure 2b*).

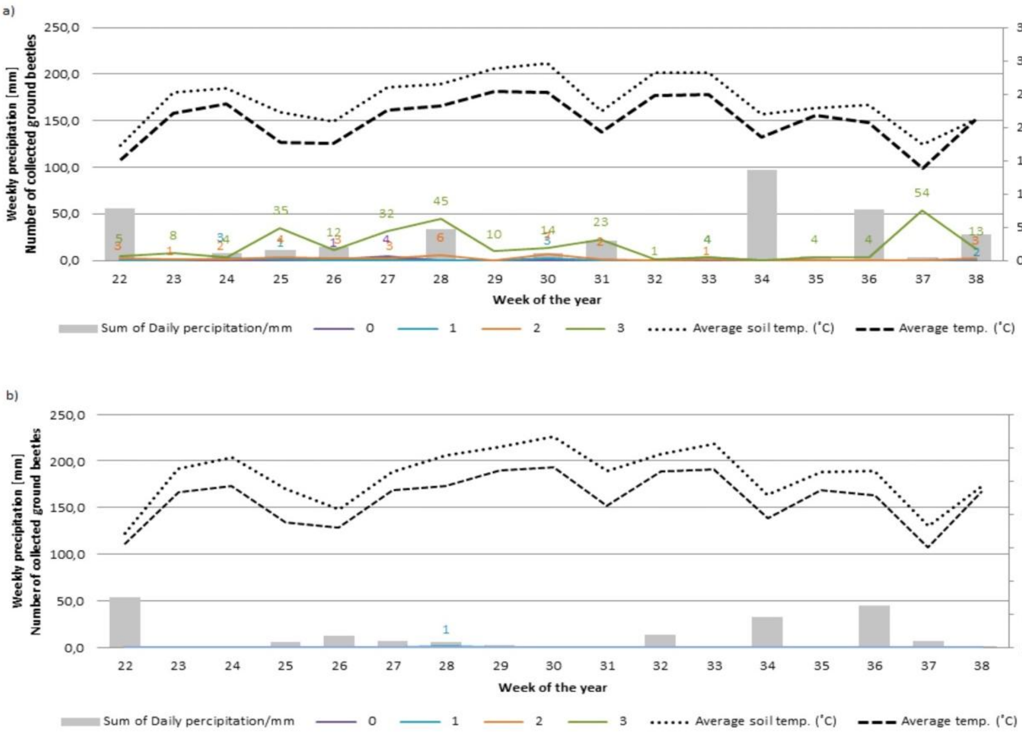

***Figure 2a and b.** Number of collected ground beetles per week in Lukač and Tovarnik by endogeic traps in respect to the weekly precipitation and temperature*

The relationship between the number of collected ground beetles per week and percentage of plant cover in investigated period on both locations was evaluated (*Figures 3 and 4*). Generally, the total ground beetle caches per week were higher in winter crops which were sown in autumn previous year, comparing with sugar beet and maize which were sown in spring after long bare soil period (*Figures 3 and 4*).

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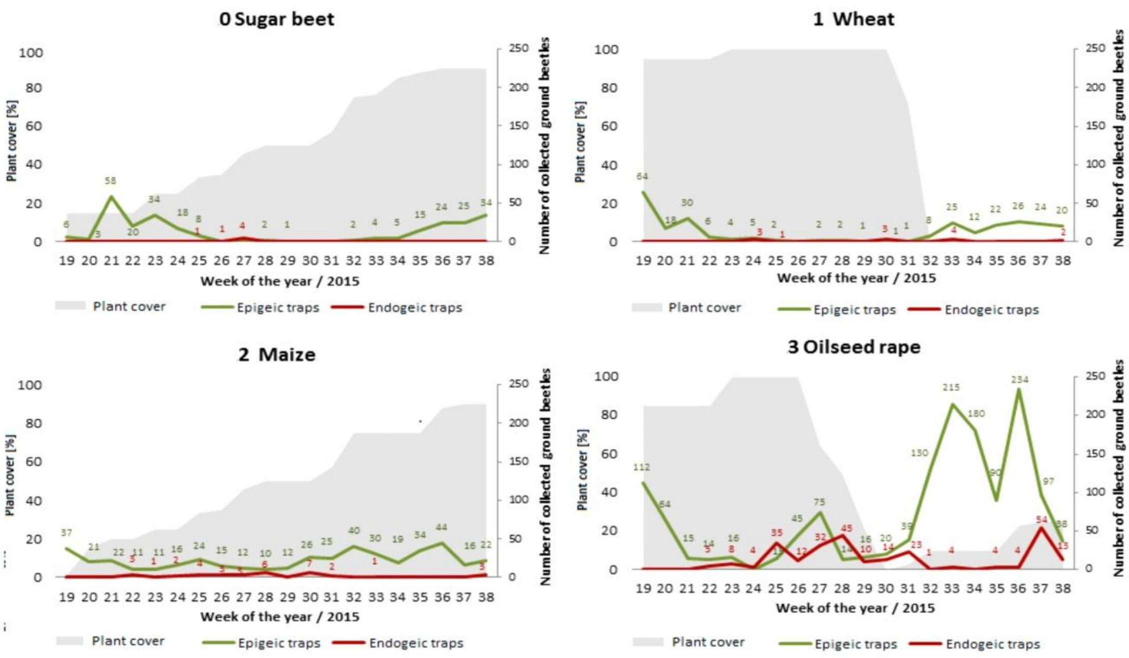

Figure 3. *Ground beetle dynamics in relation to plant cover in Lukač*

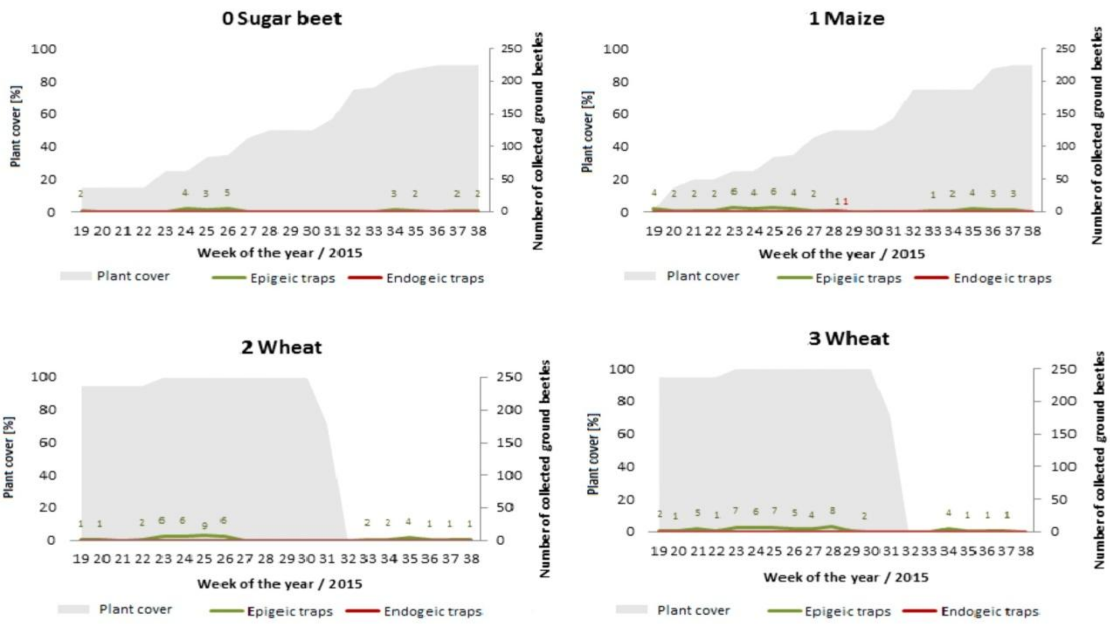

Figure 4. *Ground beetle dynamics in relation to plant cover in Tovarnik*

Discussion

This is the first detailed study in Croatia aimed at understanding how intensive arable crop production with their environmental and management specificities affects ground beetle communities. The abundance of the endogeic and epigeic ground beetles generally differed according to climatic and edaphic factors and specific environmental and cropping systems. Several key findings describe the impact of agro-ecological factors and agro-technical measures on ground beetle populations.

(i) The catches at locality Tovarnik were lower comparing to the catches at locality Lukač. The difference in the total catches between localities could be influenced by climatic conditions. Although the investigated localities are situated in the same climatic regions according to Koppen classification (Penzar and Penzar, 2000), the difference in climatic conditions during the investigation were established. At the location Tovarnik the growing season was characterized by exhibiting significantly higher mean air and soil temperatures than in Lukač (*Table 2*). At the location Lukač, the total amount of rainfall in the same period was significantly higher than in Tovarnik (*Table 2*). Presented results of ground beetle population dynamics shown that population increase follows air and soil temperature decrease. According to these information ground beetles seems to prefer humid areas and periods with lower air and soil temperatures. The ground beetle microclimatic preferences are far from uniform (Thiele, 1977) concretely, woodland species prefer dark and humid sites, whereas field species prefer warm and dry sites. Since we established higher ground beetle population at more humid and less warm locality we shall consider other factors which could influence the population level.

(ii) The difference in the total catches between localities could be influenced by edaphic conditions. Soils in Tovarnik have a significantly higher proportion of clay while soils in Lukač have a significantly higher proportion of fine sand and fine silt (*Table 3*). There are also differences in pH values between these locations. Tovarnik has neutral to slightly basic soils while Lukač has acidic to slightly acidic soils (*Table 3*). The content of humus on both locations was similar. Higher ground beetle abundance recorded at location Lukač is opposite to statements of previous researches (Barbercheck, 1992; Benitez et al., 2014; Hong et al. 2007; Turpin and Peters, 1971) who found that large proportion of sand could have a negative impact on larval survival. We can conclude that pH value and soil structure have the great influence on ground beetle abundance in our survey. According to edaphic factors prevailing at investigation areas ground beetles prefer slightly acidic soils with a great amount of fine silt and a small proportion of clay.

(iii) The intensity of ploughing was the main agro-technical difference between the studied locations so could be the cause of differences in ground beetle catches. The fields in Tovarnik were ploughed more often and on greater depth than the fields in Lukač. Ploughing is known that significantly influences psycho-chemical and biological soil properties and, along with other factors affects the abundance of various invertebrates (Vician et al., 2015). Generally, invertebrates tend to be enhanced under conservation tillage conditions because of reduced soil disturbance, increased surface residues and greater weed diversity. According to previous studies, higher ground beetle trapping rates were recorded on fields with reduced tillage or no tillage at all compared with conventionally tilled ones (House and All, 1981; Blumberg and Crossley, 1983; House and Stinner, 1983; House and Parmalee, 1985; Ferguson and McPherson, 1985; Stinner et al., 1988; Tonhasca, 1993). Presented results of this study confirm previous statements, meaning that conservation tillage, such as conducted on fields in Lukač, suit better to ground beetles survival than conventional tillage measures that are common in Tovarnik.

(iv) The soil factors are greatly influenced by weather conditions and ploughing but also are affected by crops growing at the specific area. Previous studies have shown that

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crops affect ground beetles through modification of microclimatic factors, and trough disturbance factors such as harvest and tillage schedules (Thiele, 1977; Holland, 2002). The strong effects of crops on ground beetles abundance seen in this research support the results of numerous other studies (Tonhasca, 1993; Zhang et al., 1998; Honek and Jarosik, 2000; Ward and Ward, 2001; Witmer et al., 2003; O´Rouke et al., 2008). Although no ground beetle species appears to be strictly bound to a certain crop, early agro-ecological studies in Europe reported a general difference between ground beetle abundance distributions in winter cereals versus root crops (Heydemann, 1955). The highest ground beetle abundance on fields 3T (wheat) and 3L (oilseed rape) can be affected by the characteristic of present crop but also can be the result of the specific crop rotation. O´Rourke et al. (2008) stated that thick stand crops, especially those which were sown in autumn, may provide important refuges for ground beetles in comparison with crops which were sown in spring. Wheat and oilseed rape sown at fields 3L and 3T in our survey confirm the importance of crop habitat for supporting ground beetle populations. These are overwintering crops which provided less extreme microhabitat in spring and created positive conditions for ground beetle survival.

(v) Beside crop specifics (thick stands and seasonal character), which obviously had great influence on abundance, a very long bare soil period can also be a significant factor that affect ground beetles. Our results confirm the statement of Kromp (1999) that very long period of bare soil in spring in sugar beet and maize crops (*Table 1*) present extreme soil surface microclimate which has a negative influence on ground beetle abundance. In root crops, the long period of bare soil in early spring creates a rather extreme soil-surface microclimate (high temperatures and insolation during the day), which changes with ongoing crop development towards being shadowy and humid. In winter cereals, the less extreme microclimate already established in early spring creates favorable conditions for ground beetles (Komp, 1999). Both locations in our study, where sugar beet was sown, have a period of bare soil for 7 months (0T) and 9 months (0L) which present exceptionally long period without plant cover. The low caches on these fields can be explained with extreme soil surface microclimate as a consequence of long bare soil period.

(vi) Our results shown correlation between plant cover and ground beetle catches since we recorded higher total ground beetle caches per week in winter crops which were sown in autumn previous year, comparing with sugar beet and maize which were sown in spring after long bare soil period.

(vii) Results of this study have also emphasized the influence of fertilization and insecticidal management practices on ground beetles. Generally, the fertilization was more intensive in Lukač, while insecticide treatments were more intensive in Tovarnik. Considering greater ground beetle abundance in Lukač than in Tovarnik our results confirm previous studies where has been concluded that insecticides have negative influence on the ground beetle populations (Asteraki et al., 1992; 1995). For the full conclusion of insecticide influence on ground beetles more detailed investigation should be conducted. Fertilization was considered to decrease ground beetle abundance exclusively when was applied with the high amount of nitrogen as mineral fertilizer (Kromp, 1999). The levels of nitrogen applied in all fields are compatible with permitted levels according to integrated plant production in Croatia (EU Directive 2009/128/EC) which provides minimal negative influence on all beneficial fauna. Organic manure has not been applied at investigated fields so possible positive effect

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(as in: Pietraszko and De Clercq, 1982; Hence and Grégoire-Wibo, 1987) on ground beetles cannot be confirmed.

(viii) At both locations, the crops have been grown in four-year crop rotation. The main focus in our survey was on sugar beet and its rearing in crop rotations on both locations due to the fact that, in Croatia, sugar beet is most intensive culture considering agro-technical measures and pest protection requirements. As it was presented in *Table 1*, fields where sugar beet was grown in 2015, underwent the most intensive tillage, fertilization and insecticide treatments (seed treated with neonicotinoids). As presented in *Tables 4 and 5*, our results shown the lowest ground beetle abundance on the sugar beet fields (0L and 0T) which is in accordance with results of Mullin et al. (2010) and Kos et al. (2013) who demonstrated the negative effect of neonicotinoids applied as a seed treatment on ground beetles. In Vukovar – Sirmium County besides seed treatment, three additional insecticide treatments have been applied on the sugar beet field 0T (*Table 1*) what may have additional negative influence on ground beetle abundance. Therefore, we estimated that sugar beet cultivation has the greatest negative influence on ground beetle populations but with the assumption that abundance can be restored in the years after sugar beet growing. Indeed in the years after sugar beet (four-year crop rotation: see detailed in *Table 1*) the ground beetle abundance increased.

Conclusions

Intensive agricultural production of arable crops generates different degrees of disturbances in the ground beetle life cycle trough specific environmental and management conditions. Clearly, there is more than just one factor which could significantly change the abundance of ground beetles. Sugar beet cultivation, which implies particularly intensive tillage and insecticide application, reduces ground beetle abundance, whose number recovered after the four-year crop rotation. It is shown that the arable agroecosystems influence the ground beetle community through the modification of environmental conditions (soil characteristics, microclimate factors such as temperature and humidity), as well as through disturbance factors such as tillage schedules and harvest/sowing schedules.

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THE GROUND BEETLE (COLEOPTERA: CARABIDAE) COMMUNITY IN AN INTENSIVELY MANAGED AGRICULTURAL LANDSCAPE

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Abstract. The effects of intensive agricultural management practices and environmental changes on biodiversity can be monitored by using the carabid beetles as biological indicators of agroecosystems quality. This study aimed to investigate the ground beetle species composition, abundance, dominance, diversity, zoogeographical types and distribution groups in an intensively managed agricultural field. Epigeic carabid fauna was collected weekly using pitfall traps on an arable crop field in Podravina, Croatia. Altogether, 1429 individuals belonging to 26 species and 15 genera were collected. The most abundant and eudominant were *Poecilus cupreus* (Linnaeus, 1758), followed by *Brachinus psophia* Audinet-Serville, 1821 and *Pterostichus melas melas* (Creutzer, 1799). Two species were dominant, two subdominant, four recedent and 15 subrecedent. The diversity of fauna was moderately high: Simpson diversity index 0.7875, Shannon-Wiener index 1.9654 and Pielou's evenness 0.6032. Zoogeographical analysis showed equal dominance of Euroasian and Palearctic species. Most (73%) of species belonged to E and 27% to A relict class. The majority of species were spring breeders (14 species), 8 species were autumn breeders and one species breeds in both seasons. In intensively managed agricultural landscape, ground beetle diversity was moderately high, because most of the species were eurytopic, i.e. capable of inhabiting strongly anthropogenically influenced landscapes.
Keywords: *Carabidae species composition, ecological factors, zoogeographical types, intensive crop production, agro-technical measures*

Introduction

Ground beetles are species rich and abundant in agricultural land all over the world (Lövei and Sunderland, 1996). As one of the most abundant and diverse groups overwintering within cultivated fields (Holland and Reynolds, 2003), they are often used in cultivation experiments. Carabids have also been successfully used for different kinds of indicator studies, serving as biological indicators of agroecosystems quality (Cole et al., 2002; O´Rourke et al., 2008). Most of these studies focus on beetles' response to agricultural management practices or changing environmental conditions (Rainio and Niemelä, 2003). According to Baranova et al. (2013), in terms of environmental quality, arable land represents an anthropogenically influenced, unstable and devastated biotope with low contribution to farmland diversity. Due to ground beetles' sensitive reaction to anthropogenic changes in habitat quality (Avgın and Luff, 2010), they have a bioindicative value for cultivation impacts, as well as for environmental change (Thiele, 1977; Maelfait, 1990).

Environmental change, through many abiotic and biotic factors, can cause different kinds of effects on the indicator species, including changes in species

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number and distribution (Blake et al., 1996; Rainio and Niemelä, 2003). Abiotic factors most often include temperature and soil moisture (Lövei and Sunderland, 1996; Holland, 2002). Other authors reported on many additional factors: landscape heterogeneity (Chapman, 2014), field size (Kromp, 1999), the presence of non-cropped habitat (Pollard, 1968; Sotherton, 1985) and land use diversity (Östman et al., 2001). Ground beetle abundance can be influenced by the crop-dependent timing of cultivation measures (Hence et al., 1990). According to Stassart and Grégoire-Wibo (1983), the depth of tillage is one of the major factors affecting the carabid fauna. Fertilization regimes (e.g. manure, mineral fertilizers) could also have a positive effect on ground beetle population (Pietraszko and De Clercq, 1982; Hence and Grégoire-Wibo, 1987) or a negative one (Kromp, 1990). Vician et al. (2015) considered the content of organic matter and pH as the most significant factors influencing ground beetle diversity in agroecosystems, while others stated soil particle size distribution can be a decisive factor in habitat selection (Thiele, 1977; Meissner, 1984).

Crop type can affect ground beetles through modification of microclimatic factors (i.e. temperature and humidity) and through disturbance factors (i.e. harvest and tillage schedules) (Thiele, 1977; Witmer et al., 2003; O´Rourke et al., 2008). The ground beetles population in the agricultural landscape can be also influenced by chemical pest control (Basedow, 1987; Asteraki et al., 1992; Jeschke et al., 2011; Szczepaniec et al., 2011; Varvara et al., 2012; Douglas et al., 2014).

Several studies in Croatia reported about epigeic ground beetles' assemblage, distribution and abundance in different vegetation types, including forests (Šerić Jelaska, 2005; Brigić et al., 2014a), wetlands (Brigić et al., 2014b), meadows (Durbešić, 1987; Durbešić et al., 2006) and parks (Durbešić, 1982; Marković, 2009). However, not many detailed studies about ground beetles on agricultural fields with intensive land cultivation have been done. Studies were performed on leguminous fields (Kovačević and Balarin, 1960; Balarin, 1974) and in wheat (Sekulić et al., 1973; Sekulić, 1977). The most comprehensive ground beetle faunal study on several different crop types was done in Podravina region more than 30 years ago (Štrbac, 1983), in which 31 species were identified. Since then, only few researchers investigated ground beetle assemblage in agricultural landscape, and these included research on sugar beet (Kos et al., 2013), maize (Kos et al., 2006; Bažok et al., 2007; Kos et al., 2011) and barley (Kos et al., 2010). The latest study on endogeic ground beetle communities in arable field in Podravina area revealed eight species (Drmić et al., 2016). Juran et al. (2013) investigated activity of the adult ground beetles in three differently managed fields in central Croatia and found that the endogaeic activity was highest in „organic" system, followed by the „conventional" and „integrated" system. Büchs et al. (2013) found 72 species on differently managed fields in a neighboring country. The authors, however, did not mention the species composition.

Different indices measure different aspects of the partition of abundance between species. Species evenness usually has been defined as the ratio of observed diversity to maximum diversity, the latter being said to occur when the species in a collection are equally abundant (Margalef, 1958; Patten, 1962; Pielou, 1966). Simpson's index, for example, is sensitive to the abundance only of the more plentiful species in a sample, and can therefore be regarded as a measure of "dominance concentration" (Whittaker, 1965). Used Shannon index is an

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information statistic index, which means it assumes all species are represented in a sample and that they are randomly sampled. This index estimates the affinity of different populations belonging to a community and, through the species composition, the similarity of the habitats (Popescu and Zamfirescu, 2004).

In modern intensively managed production in Croatia, there is still little knowledge on beneficial fauna importance (Bažok et al., 2015; Virić Gašparić et al., 2017). In order to preserve biodiversity in intensively managed arable land as much as possible, it is important to monitor the bioindicator species such as ground beetles, since they can indicate the anthropogenically influenced field quality. Detailed knowledge on their community in a specific agricultural landscape can give us a preview on agroecosystem stability. Therefore, this study aimed to investigate the ground beetle species composition, abundance, dominance and diversity, as well as zoogeographical types and distribution groups in an intensively managed agricultural field, with its specific agro-ecological factors and agro-technical measures.

Materials and methods

Location

Ground beetles were collected during the arable crop growing season in 2015 in Lukač (Virovitica–Podravina County, Croatia), on winter crop field with an intensive arable management and specific climatic and edaphic characteristics (field size 34.76 ha, coordinates: 45° 50′ 24″ N, 17° 24′ 0″ E). According to Köppen classification, this part of continental Croatia belongs to *Cfwbx* climatic type characterized with continental climate of cold winters and hot summers (Penzar and Penzar, 2000). The soils in the research area are gleyic luvisols (IUSS Working Group WRB 2015). These are hydromorphic soils, characterized by periodic or continuous wetting of part or whole of the profile, with stagnating precipitation or with additional surface or underground water that is not saline or alkaline. These soils contain a great amount of fine sand and coarse silt (sandy loams texture) (Bogunović et al., 1996) and often require conventional tillage.

The field was chosen to represent common cultivation practices as well as the agro-technical measures in this area. Considering the soil type and soil characteristics, the tillage was adapted to the given conditions and performed as follows: a) in autumn: ploughing on a depth of 20-25 cm was followed by the furrow closure for moisture conservation in spring; b) in spring: chisel ploughing and tillage with the rotary harrow; c) in summer: after harvest disk harrowing and again chisel ploughing. A description of the regional physical and chemical soil properties of investigated area as well as agrotechnical measures applied on the experimental field are given in *Table 1*. Performed pedological procedure consisted out of taking the soil sample from the depth of a plow layer (30 cm). Five sub-samples waging 300–400 g were taken and than pooled and homogenized for analysis. Analysis was performed by the pedology laboratory of the Department of Soil Science, Faculty of Agriculture, University of Zagreb and included sediment grain size and chemical properties analyses. Soil texture was determined by sieving following standard methods (ISO 11277 2004) (Kozina et al., 2015).

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Table 1. *Physical and chemical soil properties of arable field where research was conducted*

Location	Soil type	Soil pH	Humus (%)	Soil properties (mm)	Fertilization	Bare soil (mth)*	Insecticide treatment
Lukač (Virovitica -Podravina County)	gleyic luvisol	KCl 5.58 H2O 6.65	3.2	Coarse sand 2.35 Fine sand 11.83 Coarse silt 38.42 Fine silt 31.65 Clay 15.75	74 kg N 60 kg P 90 kg K	2	Thiacloprid

*number of months while field was not covered after harvesting till soil preparing for crops grown in folowing vegetation season

Climatic factors

Climate data (i.e., mean weekly air temperature, mean weekly soil temperature and the total amount of rainfall per week) were obtained from the Croatian Meteorological and Hydrological Service and presented for ground beetle collecting period from May to September 2015 (19^{th} to 38^{th} week of the year).

Ground beetle trapping

Epigeic covered pitfall traps were used to collect adult ground beetles. Polythene pots (Ø=12 cm, h=18 cm) were incorporated 18 cm into the soil and covered with PVC roofs (Ø=16 cm) approximately 4 cm above ground level. Each trap was half filled with salted water (20% solution) for captures conservation. Four pitfall traps were placed into the center of the field at 50 m apart and 100 m away from the field edges. Trapping was performed from the 19^{th} to the 38^{th} week of the year, from May to September 2015. Traps were inspected once a week and all ground beetles were collected and counted. The identification of the collected ground beetles to species level was based on the work of Auber (1965), Bechyne (1974), Harde and Severa (1984) and Freude et al. (2006).

Ground beetle composition analysis

The ground beetle trapping results using pitfall traps for the selected interval (from 19^{th} to 38^{th} week of the year) are presented as a mean number of individuals caught per field per week. Results of the ground beetle population dynamics are presented as the total number of ground beetles caught per week as a function of the average weekly air temperatures (°C), total weekly precipitation (mm) and average weekly temperature of soil (°C) at a depth of 10 cm.

The dominance values of carabids presented in percentage shares of a particular species in the community were calculated according to Tischler (1949) as follows: eudominant (10-100%), dominant (5-10%), subdominant (2-5%), recedent (1-2%) and subrecedent (<1%). To calculate the diversity of the carabid assemblages, Simpson (λ) and Shannon-Wiener indices (H') were used. Shannon-Wiener indices is an entropy, giving the uncertainty in the outcome of a sampling process key (Jost, 2006). Both

Shannon and Simpson diversities increase as richness increases, for a given pattern of evenness, and increase as evenness increases, for a given richness, but they do not always rank communities in the same order (Colwell, 2009). Evenness was estimated using Pielou's evenness. Analyses were carried out using the MATLAB program (The MathWorks Inc., 2015). Zoogeographical analysis adding new species records and contributing an understanding of the composition (Majka et al., 2007), was made according to Vigna Taglianti et al. (1999) and the database Fauna Europaea (Vigna Taglianti, 2013). The distribution/occurrence groups (relict classes E, A and R) were defined according to Hůrka et al. (1996).

Results and discussion

This study aimed at observation and description of a ground beetle fauna during one vegetation season in intensive arable crop production. During the sampling period, a total of 1429 individuals were collected using epigeic traps at Podravina region. Ground beetles collected belong to 26 species and 15 genera (*Table 2*) which in comparison with previous studies in arable agroecosystems can be classified as moderately high (Kos et al., 2006; Bažok et al., 2007; Kos et al., 2010, 2011; Drmić et al., 2016; Virić Gašparć et al., 2017). Despite the large number of species which may occur in agroecosystems, a relatively small number have been identified as being characteristic of arable areas and these are often the most abundant (Thiele, 1977; Holland and Luff, 2000).

The composition of recorded species in arable crops corresponds with results of similar investigations in Croatia (Kos et al., 2006; Bažok et al., 2007; Igrc Barčić et al., 2008; Kos et al., 2010; 2011; Drmić et al., 2016) and abroad (Bukejs and Balalaikins, 2008; Woodcock et al., 2010; Baranová et al., 2013). The most abundant species in the total catch was *Poecilus (Poecilus) cupreus cupreus* (Linneaus, 1758) (37.65%) followed by *Brachinus (Brachinus) psophia* Audinet-Serville, 1821 (21.06%) and *Pterostichus (Feronidius) melas melas* (Creutzer, 1799) (10.29%) (*Table 2*). The most abundant species accounted almost 70% of the total catch and belonged to the group of eudominant species. *Anchomenus (Anchomenus) dorsalis* (Pontoppidan, 1763) and *Harpalus (Pseudoophonus) rufipes* (DeGeer, 1774) were classified as dominant, *Amara (Amara) similata* (Gyllenhal, 1810) and *Pterostichus (Morphosoma) melanarius melanarius* (Illiger, 1798) as subdominant while others were recedent (4 species) or subrecedent (15 species). The species, which dominated the carabid assemblage in arable habitat (with the total collections), were *P. cupreus* (538), *B. psophia* (301), *P. melas* (147), *H. rufipes* (128) and *A. dorsalis* (97) (*Table 2*).

Species *P. cupreus* is considered as one of the most common species inhabitating winter crops (Alford, 2008), so these results strongly support this research. In Croatia, Štrbac (1983) also specified it among the three most dominant on arable land.

Drmić et al. (2016) investigated endogaeic ground beetle fauna in the same area in Croatia and detected *B. psophia* and *A. dorsalis* as the most abundant ones, therefore we may assume that these species are a typical arable ground beetle representatives in investigated region.

Species *P. melas* is also common in Croatia and was detected as dominant in agricultural land near the Nature park Lonjsko polje (Brigić et al., 2003).

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Table 2. *The composition, abundance, zoogeographical and geographical analysis of ground beetles collected in Lukač, 2015*

Species name	**N†**	**DV‡**	**Zoogeographical categories and faunal types§**	**Geographical distribution groups|**	**Reproduction period¶**
Calosoma (Campalita) auropunctatum auropunctatum Herbst, 1784	1	0.07	E-CAS	A	no data found
Brachinus (*Brachinus*) *crepitans* Linné, 1758	27	1.89	B-CAS	E	Sp
Brachinus (Brachinus) psophia Audinet-Serville 1821	301	21.06	E-CAS	E	no data found
Brachinus (Brachynidius) explodens Duftschmid 1812	3	0.21	E-CA-M	E	Sp
Clivina fossor fossor Linné, 1758	13	0.91	E-AS	E	Sp
Asaphidion curtum curtum Heyden 1870	3	0.21	OLA	E	Sp
Trechus (Trechus) quadristriatus Schrank, 1781	4	0.28	E-CA-M	E	A
Anisodactylus (Pseudanisodactylus) signatus Panzer 1796	1	0.07	E-AS	E	Sp
Harpalus (Harpalus) affinis Schrank, 1781	1	0.07	E-AS	E	Sp
Harpalus (Harpalus) dimidiatus P. Rossi, 1790	1	0.07	E-PAS	A	A
Harpalus (Harpalus) distinguendus distinguendus Duftschmid, 1812	2	0.14	PAL	E	Sp
Harpalus (Pseudoophonus) rufipes DeGeer, 1774	128	8.96	PAL	E	A
Stenolophus (Stenolophus) teutonus Schrank, 1781	16	1.12	E-MED	E	Sp
Agonum (Amara) viridicupreum viridicupreum Goeze, 1777	1	0.07	E-PA-M	E	Sp
Anchomenus (Anchomenus) dorsalis Pontoppidan, 1763	97	6.79	PAL	E	Sp
Abax (Abacopercus) carinatus carinatus Dejean, 1828	4	0.28	E-PAS	A	no data found
Abax (Abax) parallelepipedus parallelepipedus Piller & Mitterpacher, 1783	1	0.07	EUR	A	A
Poecilus (Poecilus) cupreus cupreus Linné, 1758	538	37.65	E-AS	E	Sp
Pterostichus (Feronidius) melas melas Creutzer, 1799	147	10.29	E-PAS	A	A
Pterostichus (Morphosoma) melanarius melanarius Illiger, 1798	54	3.78	E-SI	A	A
Pterostichus (Platysma) niger niger Schaller, 1783	1	0.07	E-AS	A	A
Calathus (Calathus) fuscipes fuscipes Goeze, 1777	19	1.33	PAL	E	A/Sp
Calathus (Neocalathus) ambiguus ambiguus Paykull, 1790	1	0.07	E-AS	E	A
Amara (Amara) aenea Degeer, 1774	3	0.21	OLA	E	Sp
Amara (Amara) ovata Fabricius, 1792	28	1.96	PAL	E	Sp
Amara (Amara) similata Gyllenhal, 1810	34	2.38	E-AS	E	Sp

† N-number of individuals; ‡ DV-dominance index; § I. Northern Holarctic and Euro-Siberian faunal type: OLA - Holarctic; PAL - Palearctic; E-SI - Eurosiberian; II. European faunal type: EUR - European; E-PAS - European-Neareastern; III. Euroasiatic faunal type: E-AS - Euroasiatic steppe complex; E-CAS - European and Central Asian; B-CAS - Balkan and Central Asian; IV. Mediterranean (s. lato) faunal type: E-CA-M - European-Centralasian-Mediterranean; E-PA-M - European-Neareastern-Mediterranean; E-MED - Eastmediterranean (Vigna Taglianti et al. 1999, the database Fauna Europaea (Vigna Taglianti, 2013)); | Relict classes: E-eurytopic species, A-adoptable species; ¶ A-autumn, Sp-spring.

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Kromp (1999) listed species *H. rufipes*, followed by *P. cupreus* and *P. melanarius* as the most abundant from agricultural fields of Eastern European countries, which is generally in accordance with our results. Similar investigations from Croatia (Bažok et al., 2007; Igrc Barčić et al., 2008; Kos et al., 2011) also stated species *H. rufipes* and *P. melanarius* in the group of the most abundant species in corn fields. Although they were not the most abundant species in our results, they were among species which generally dominated with the total scores.

This typically structured ground beetle community of arable land consists of a small number of dominant species represented with a large number of individuals and a large number of less commonly occurring species (subdominant, recedent and subrecedent) represented with a low number of specimens (Baranová et al., 2013).

The diversity of fauna was moderately high: Simpson (1-λ') diversity index was 0.7875, Shannon-Wiener index (H') was 1.9654 and Pielou’s evenness was 0.6032. Analysis of faunal types (zoogeographical analyses) showed the dominance of Euroasian (23.08%) and Palearctic (23.08%) species which corresponds with climatic and geographic characteristics of the investigated area (*Table 2*).

With reference to relict classes, 73% of determined ground beetles belonged to E relict class which consists of eurytopic species without special demands on habitat type and quality, and inhabiting strongly anthropogenically influenced landscapes (Hůrka et al., 1996). Species which belonged to A relict class were represented with 27% and this group included more adoptable species, which are found in more or less natural habitats (forests, meadows, pastures, standing and flowing water) (Hůrka et al., 1996). Neither one species was classified to relict class R, which was expected, because R class includes species with narrow ecological amplitude, which are rare and endangered, occurring naturally in undisturbed ecosystems which was not the case in our study (Hůrka et al., 1996). These results correspond to the results of Porhajašová et al. (2004) and Baranová et al. (2013) who reported that increasing human disturbances changes the composition to favor eurytopic species while reducing the number of specialized species with narrow ecological valences.

Abundance and diversity as well as the ratio of spring to autumn breeders varied between winter sown crops (cereals and oilseed rape) and spring sown root crops (potatoes, sugar beet, maize, carrots) (Kabacik-Wasylik, 1975 cit. Holland and Luff, 2000). Winter crops usually have higher abundance, diversity and more spring breeders with summer larvae (e.g. *P. cupreus*, *A. dorsalis*) which was confirmed with our results as well. These preferences are not, however, always apparent and even total numbers may vary (Holland and Luff, 2000). The majority of collected species were spring breeders (14 species), 8 species were autumn breeders and one species (*Calathus fuscipes fuscipes* (Goeze, 1777)) breeds in both seasons (*Table 2*). The domination of spring breeders could be a consequence of the cultivation measures. The depth of tillage is one of the major factors affecting field carabid communities, with superficial ploughing enabling a higher number of species and favoring spring breeders (Kromp, 1999 cit. Stassart and Grégoire-Wibo, 1983).

Species composition and the number of ground beetles in different agrocenosis differ and depend on edaphic factors (Bukejs and Balalaikins, 2008). Ground beetle species contribute significantly to the insect diversity in farmland because many species are adapted to agriculture and generally occur at high densities (Booij, 1994). According to Thiele (1977) and Kromp (1999) cultivated land is comprised of widely distributed, eurytopic ground beetle species, many of which have high tolerance to disturbances and

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chemical pollution. This means that cultivated land contains a typical ground beetle fauna, despite the regular implementation of cultivation measures (Kromp, 1999). For example, Thiele (1977) listed 26 species found at investigated arable habitats stretching from England over Central Europe.

In our survey the first population maximum was observed from week 19th to 21st which was also the beginning of sample collection period. The second population maximum was recorded from week 32nd to 36th (*Figure 1*). Presented results of ground beetle population dynamics show that population increase follows air and soil temperature decrease (*Figure 1*). In the whole investigation period the number of ground beetle decrease is followed by precipitation increase. Recording to Croatian Meteorological and Hydrological Service the Virovitica-Podravina County is described as mid worm area with intensive periods of rainfall especially in summer period.

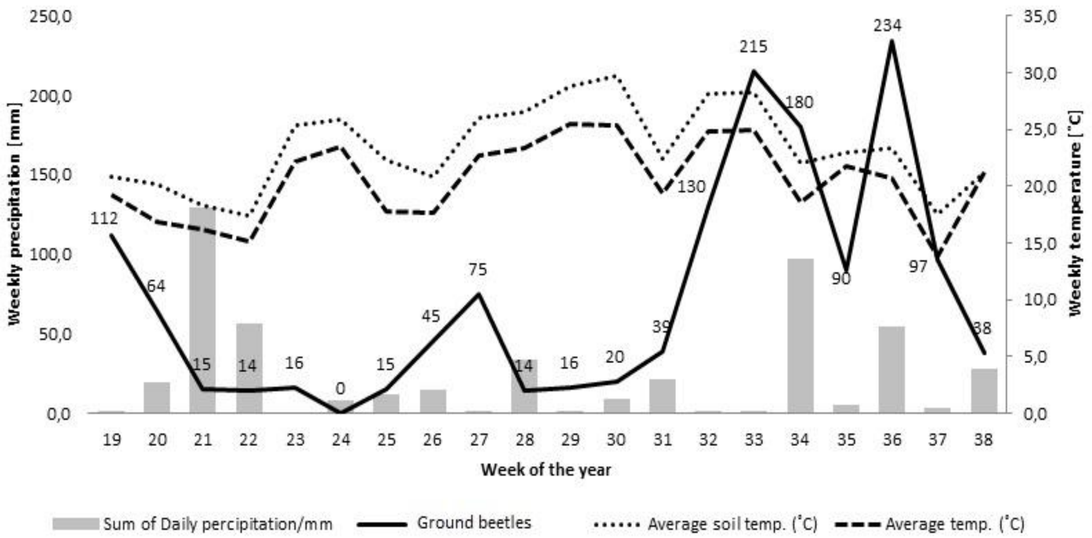

Figure 1. *Ground beetles weekly dynamics with prevailing climatic conditions*

According to edaphic factors prevailing at investigation area ground beetles inhabited slightly acidic soil with a great amount of fine silt and a small proportion of clay (*Table 1*). The intensity of ploughing was the main agro-technical specificity at studied locality. The field in Podravina has been ploughed often and on great depth during whole vegetation season. Ploughing is known to significantly influence physico-chemical and biological soil properties and affects the abundance of invertebrates (Vician et al., 2015). Generally, reduced soil disturbance, increased surface residues and greater weed diversity had positive impact on invertebrates (Kromp, 1999). According to previous studies, higher ground beetle trapping rates were recorded on fields with reduced tillage or no tillage at all compared with conventionally tilled ones (House and All, 1981; Blumberg and Crossley, 1983; House and Stinner, 1983; Ferguson and McPherson, 1985; House and Parmalee, 1985; Stinner et al., 1988; Tonhasca, 1993). Conventional tillage, such as conducted on the field in Podravina, could have an impact on established ground beetles abundance.

The soil factors are greatly influenced by weather conditions and ploughing but also could be affected by crops growing at the area. Previous studies have shown that microclimatic factors, such as temperature and humidity, and disturbance factors such as harvest and tillage schedules crops affect ground beetles communities (Thiele, 1977; Holland, 2002). Although no ground beetle species appears to be strictly bound to a certain crop, early agro-ecological studies in Europe reported a general difference between ground beetle abundance distributions in winter versus spring crops (Heydemann, 1955). O´Rourke et al. (2008) stated that thick stand winter crops provide important refuges for ground beetles in comparison with spring crops. The overwintering crop sown at the field in our survey may confirm the importance of crop habitat for supporting ground beetle populations by providing less extreme microhabitat in spring and creates positive conditions for ground beetle survival and the dominance of spring breeders.

Beside crop specifics, bare soil period can also be a significant factor that affects ground beetle communities. In winter crops, the less extreme microclimate already established in early spring creates favorable conditions for ground beetles (Kromp, 1999). Locality in our study had a very short period of bare soil (2 months period without plant cover; *Table 1*). No negative effect was observed in ground beetle populations regarding the extreme soil surface microclimate. The effect of intensively managed crop on ground beetles abundance which could be detected in this research support the results of numerous other studies (Tonhasca, 1993; Zhang et al., 1998; Honek and Jarosik, 2000; Ward and Ward, 2001; Witmer et al., 2003; O´Rourke et al., 2008).

As well, the fertilization in Podravina is generally intensive while insecticide treatments were common and in compliance with IPM. While previous studies had concluded that insecticides have negative influence on the ground beetle populations (Asteraki et al., 1992, 1995), more detailed investigation are needed for the full conclusion. Kromp (1999) shown that high amount of nitrogen used in fertilization process decrease ground beetle abundance. The levels of nitrogen applied in Podravina are under permitted levels (EU Directive 2009/128/EC, EUR-Lex, 2009) causing minimal negative influence on ground beetles. Only mineral fertilization has been used in Podravina so possible positive effect of organic manure recorded by Pietraszko and De Clercq (1982) and Hence and Grégoire-Wibo (1987) on ground beetle communities cannot be discussed.

Conclusions

The bioindicator species such as ground beetles have not received much attention by researchers in Croatia, although they can indicate the anthropogenically influenced field quality. In this study we gained detailed knowledge on their community in a specific agricultural landscape in northwest Croatia, Podravina region. In this investigation, a total of 1429 ground beetles were collected using epigeic traps, belonging to 26 species and 15 genera. Ground beetle diversity was moderately high, because most of the species were eurytopic, i.e. capable of inhabiting strongly anthropogenically influenced landscapes. In modern agriculture in European Union, conservation programs aimed to keep beneficial species and biodiversity are promoted as tool for ensuring sustainability. In order to measure the success of such programs, one has to have detailed knowledge on the initial situation. The results of this study significantly contributed to better understanding of initial situation about ground beetle communities in intensive agricultural landscape in northwest Croatia and will be a good entry point for future conservation programs.

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CARABIDS AS INDICATORS OF SUSTAINABILITY IN ARABLE CROPS

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Abstract. Intensive agriculture and crop production lead to a significant decline in biological control agents, their abundance and diversity. Ground beetles are important in reducing populations of pests and weeds. They are often used in environmental research as biological indicators of different habitats. The aim of this research was to analyse biocenotic and synecological indices of ground beetle populations collected from two remote sites differing in weather conditions, tillage, and types of arable crops. After detailed identification, 64 species were distinguished and classified according to the Catalogue of Palearctic Coleoptera. Biocenotic synecological analysis per crop in both Vukovar-Syrmia and Virovitica-Podravina counties showed that *H. rufipes*, *P. melas*, *P. melanarius melanarius* and *P. cupreus cuperus* were the most abundant species in the studied crops. Catches in Virovitica-Podravina County were significantly higher than catches in Vukovar-Syrmia County. Compared to the other crops, maize had significantly the highest. The highest catches were recorded in September, while catches were significantly lower in July. Catches were affected by location site, crop, and sampling date, as well as their combinations, proving that the abundance of ground beetles was significantly different at the two sites.
Keywords: *biological control agents, carabids, conservation, sustainable land use, tillage*

Introduction

Agricultural practices are thought to be responsible for the loss of species in many regions of Central Europe (e.g., Heydemann, 1986; Gall and Orians, 1992). Intensive farming, the use of broad-spectrum insecticides, and the cultivation of crops that lack weeds and field margins for food, shelter, and overwintering habitat are leading to significant declines in biological control agents, their abundance and diversity. Naturally occurring biological control agents are commonly referred to as biological conservation control. These include birds, bats, small mammals, but especially insects and other invertebrates which prey on or parasitize crop pests reducing damage. Most known are parasitic wasps, carabids, and ladybirds (EC, 2020).

As naturally occurring, predatory temperate organisms, carabids are often considered biological control agents in organic agriculture (Kromp and Meindl, 1997; Kromp, 1999). They are important in reducing populations of many pests and weed seeds, but they are also a food source for animals at a higher trophic level. Because of their large numbers, known taxonomy, and sensitivity to changes caused by external factors, they are often used in research (Lövei and Sunderland, 1996). Ground beetles that occur in arable landscapes are usually considered eurytopic. They are in direct contact with other

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soil dwellers as well as with higher agrochemical up-take, loss of greenbelts, and increasing size of croplands, which is often considered the main cause of declines in their populations (Fahrig et al., 2015).

Ground beetles are highly diverse, counting more than 3000 species in the Western Palearctic region (Rainio and Niemelä, 2003; Kotze et al., 2011). Compiled data on carabid density from 14 European countries between 1970 and 1994, indicated enormous temporal and spatial variation. In annual crops, for example, the total number of adult carabids averaged 32 per square meter and ranged from 1 to 96. Much higher densities were found at field margins, with an average of 233 and a range of 14.5 to 1113 beetles per square meter (Lövei and Sunderland, 1996). Partial assemblage of ground beetles in Croatian agricultural landscapes has recently been studied in annual crops (Bažok et al., 2007; Kos et al., 2010, 2011, 2013; Gotlin Čuljak et al., 2016; Drmić et al., 2016). The composition of the carabid fauna and the dynamics of their occurrence in arable crops in Croatia are not known, although it is often claimed that insecticides are the main factor for the decline in their numbers. Contact with insecticides may affect organisms that have fed on the treated plants, either directly or through treated surfaces on which they move (Albajes et al., 2003; Papachristos and Milonas, 2008; Moser and Obrycki, 2009; Prabhaker et al., 2011). Crop type determines shelter, microclimate, and food resource availability and is a key factor in carabid abundance and species richness (Brooks et al., 2003, 2008; Woodcock et al., 2014). Also, the timing of cultivation probably has the greatest impact on carabids, affecting population processes between fall and spring breeding (Holland and Luff, 2000; Marrec et al., 2015). According to Stassart et al. (1983) the depth of tillage is one of the major factors affecting ground beetle field fauna.

The objective of this study was to analyze biocenotic synecological indices of ground beetle populations collected from two remote regions that differ in weather conditions, tillage, and types of arable crops. The study will contribute to the general knowledge of ground beetles by providing a complete list of species found in four commonly grown crops in Croatia (and Europe).

Materials and Methods

Experimental site and agricultural practice

The survey was conducted in two remote regions of Croatia, Virovitica-Podravina County and Vukovar-Syrmia County. Regions belong to the same Cfwbx climatic type of the Köppen classification system (Penzar and Penzar, 2000), but differ according to agricultural practices regarding soil tillage (*Table 1*). Intensive agricultural practices are common in the fields of Vukovar-Syrmia County, including deep plowing and intensive use of agrochemicals and mineral fertilizers. There is a great number of large integrated farmlands used for commercial production. In Virovitica-Podravina County, arable farming is carried out according to good agricultural practices, which mostly include conservation tillage and lower use of agrochemicals. Smaller arable areas are cultivated on family farms. Woodland areas and water puddles/canals are common sight. Farmers provided information on farming practices. In each region, four fields of each crop (maize, wheat, sugar beets, and soybeans) were monitored during the 2016 growing season.

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Table 1. *Field cultivation on investigated locations*

	Vukovar-Syrmia County	Virovitica-Podravina County
crop	Tillage*	Tillage*
maize	CT	RT
wheat	CT	NT
sugar beet	CT	RT
soybean	CT	RT

*Tillage: conventional tillage (CT), reduced tillage (RT), no-tillage (NT)

Sampling method

Monitoring and collection of ground beetles was performed on each of the four fields included in the experiment. Forty traps were set in the form of a net per field. Total of 160 traps was used in each region. Traps were placed 20 x 20 m apart and 100 m from field edges to avoid marginal disturbance (adjacent field, roads, proximity to roads, etc.). The traps consisted of a PVC container (Ø = 12 cm, h = 18 cm) buried in the ground and half filled with salt water (50 g/l) a preservative with the addition of 20 ml/l unscented detergent to reduce surface tension. A PVC roof was placed over each hunting vessel at a height of 2 cm. Samples were collected four times during growing season over a period of seven days in May (20.05.), July (01.07.), August (19.08.), and September (22.09.). In the meantime, the traps were closed with plastic covers. Other organisms collected in the traps were not subject of the study and were not considered for analysis.

Trial assessment

Air and soil temperature and precipitation were monitored at both sites throughout the growing season by the Croatian Meteorological and Hydrological Service. Data on mean air and soil temperatures and total precipitation were evaluated for the nearest meteorological stations (Virovitica and Gradište), located no more than 20 km from the experimental sites (*Figure 1*). Adult carabid samples were identified to species level. The identification of the ground beetle was performed by a taxonomy expert (Teun van Gijzen, Zoological Museum Amsterdam and the Museum for Natural History “Naturalis” in Leiden) using standard keys (Freude et al., 2006).

Data analysis

To achieve the objectives of the study, we conducted a biocenotic synecological analysis that included the calculation of analytical ecological indices - species richness, dominance, and constancy index. Based on the calculated dominance, the represented species of the family Carabidae are classified according to Tischler and Haydeman cited in Balarin (1974). To determine the relationship between the dominance index and the constancy index, an ecological significance index (W) was calculated for each species (Varvara et al., 2012). The diversity and similarity of populations within the fields and among the fields are determined using the Shannon index (H) (Shannon, 1948) and the Sörensen coefficient (QS) (Sørensen, 1948) while the Shannon's equitability index (Shannon, 1948) measures the evenness of a community. Bray Curtis dissimilarity is used to quantify differences in species populations between two different sites. The formulas for each index can be found in *Table 2*.

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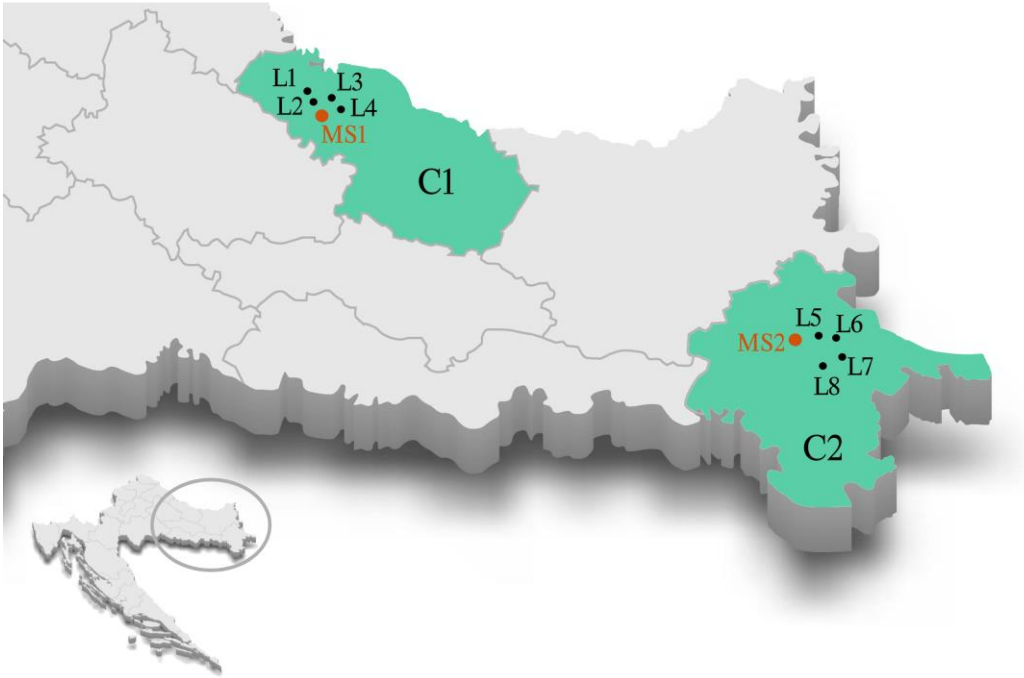

Figure 1. *C1: County Virovitica-Podravina (45.65, 17.79); MS1: Meteoroloigical station Virovitica, Taborište, (45.82, 17.41); L1 – sampling location 45.87, 17.49; L2 – sampling location 45.89, 17.39, L3 – sampling location 45.89, 17.42, L4 – sampling location 45.87, 17.45. C2: County Vukovar-Syrmia (45.13, 18.54), MS2: Meteoroloigical station Gradište, (45.15, 18.71); L5 – sampling location 45.19, 18.68; L6 – sampling location 45.22, 18.73; L7 – sampling location 45.16, 18.78; L8 – sampling location 45.24,* 18.74

The data on the average number of ground beetles per field collected using pitfall traps were analyzed by analysis of variance (ANOVA) with three factors. The first factor was site (i.e., location) which was considered as a fixed factor due to a characteristic weather conditions and similar tillage practices. The second factor was crop and the third factor was sampling date. Using ARM 9 software (Gylling Data Management Inc., 2019) a Tukey Post-Hoc test was used to determine which mean values of the variants were significantly different after a significant test result ($P < 0.05$). Where appropriate, data were log x+1 transformed.

Results

In general, Virovitica-Podravina County had lower mean air and soil temperatures while the amount of precipitation was higher. Climatic differences between sampling period of a) Virovitica-Podravina and b) Vukovar-Syrmia County during growing season 2016 are presented in *Figure 2*.

During the 2016 growing season, a total of 11,763 ground beetle samples were collected from four different fields in each remote region of Croatia, Virovitica-Podravina County and Vukovar-Syrmia County. After detailed determination, 64 species were distinguished and arranged according to the Catalogue of Palearctic Coleoptera, Archostemata – Myxophaga – Adephaga, Revised and Updated Edition (Löbl and Löbl, 2017). Presence per each site and crop is presented in *Table 3*.

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Table 2. *Biocenotic synecological analysis indices with accompanying formulas and classifications used in research*

Index	Formula	Explanation	Classes
Abundance (A)	-	N – total number of individuals of all recorded species.	-
Dominance (D)	D = (nA / N) 100	nA – the number of individuals of species A N – total number of individuals of all recorded species.	D1 – subrecedent species (below 1.1%); D2 – recedent species (1.1-2%); D3 – subdominant species (2.1-5%); D4 – dominant species (5.1-10%); D5 – eudominant species (above 10.1%)
Constancy (C)	C = (nsA / Ns) 100	nsA – the number of samples that contained species A Ns – the total number of samples	C1 – accidental species (present in 1-25% of the samples); C2 – accessory species (present in 25.1-50%); C3 – constant (present in 50.1-75%); C4 – euconstant species (present in 75.1-100%).
Ecological significance (W)	W = (C x D) 100	C – the constancy of species A, D – dominance of species	W1 – for values < 0,1% (subrecedent species); W2 – for values between 0.1-1% (recedent species); W3 – for values between 1.1-5% (subdominant species); W4 – for values between 5.1-10% (dominant species); W5 – for values > 10% (eudominant species). The category W1 includes accidental species. The categories W2 and W3 include accessory species. The categories W4 and W5 include characteristic species.
Shannon's diversity index (H)	$\mathrm{H} = -\sum_{i=1}^{s}(p_i \ln p_i)$	p - proportion (n/N) of individuals of one particular species found (n) divided by the total number of individuals found (N), ln - natural logarithm, Σ - sum of the calculations, s - number of species	The bigger number is more diverse.
Shannon's equitability index (E_H)	$E_H = H/H_{max} = H/\ln S$	H - Shannon index, H_{max} - maximum diversity possible, S - total number of species in the community (richness)	Value between 0 and 1 with 1 being complete evenness.
Sörensen coefficient (Qs)	$DSC = \frac{2 \cdot \mathrm{c}}{\mathrm{S1} + \mathrm{S2}}$	c- the number of species common to both communities S1 - the number of species in community 1 S2- the number of species in community 2	Value between 0 and 1. The closer the value is to 1, the more the communities have in common. Complete community overlap is equal to 1; complete community dissimilarity is equal to 0.
Bray Curtis dissimilarity (BC_{ij})	$BCij = 1 - \frac{2\mathrm{ij}}{Si + Sj}$	i and j - two sites, S_i - total number of specimens counted on site i, S_j - total number of specimens counted on site j, C_{ij} - sum of only the lesser counts for each species found in both sites.	Number between 0 and 1. If 0, the two sites share all the same species; if 1, they don't share any species.

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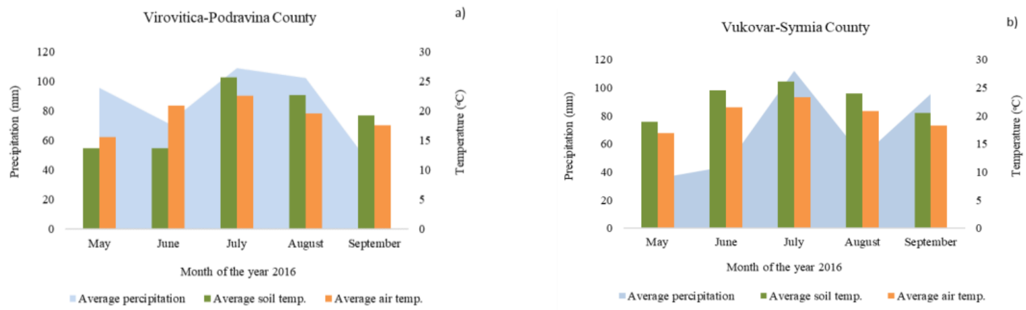

Figure 2. *Weather conditions during growing season 2016 monitored at nearest climate stations a) Meteorological station Virovitica in Virovitica-Podravina County and b) Gradište in Vukovar-Syrmia County*

In Vukovar-Syrmia County a total of 2,382 ground beetles were collected. After identification, 25 different species were distinguished. The largest number was collected in sugar beet fields (1,131), followed by wheat (656), maize (342) and soybean (253). The only eudominant (D5), characteristic (W5) species in maize was *H. rufipes* with 80.41%, followed by *P. melas* which was classified as dominant (D4) with 5.26%, accessory (W3) species. *H. distinguendus distinguendus* and *C. fuscipes fuscipes* were classified as subdominant species (D3) with no more than 3.51% but also accessory species (W3). Most of the remaining species were classified as subrecedent (D1), accidental (W1).

In soybean, *H. rufipes* was again the most common specie with 57.31%, followed by *A. dorsalis* with 16.21%, making those two species eudominant (D5) and characteristic species (W5 and W4). *C. fuscipes* and *H. distinguendus* were found to be dominant (D4) with 5.14 - 5.53%, accessory species (W3). *H. griseus*, *B. crepitans* and *P. melas* had no more than 3.95%, making them subdominant species (D1) but also accessory (W3). One species, *Z. tenebrioides* was recedent (D2) and remaining ten were classified as subrecedent (D1). Only *H. rufipes* and *H. distinguendus* were classified as constant species (C3). Most species (7) are accessory (W2), while remaining five are accidental (W1).

In sugar beet, *P. melas* was the only eudominant (D5) species with over 81.26%. *H. rufipes* was dominant (D4) with 9.46%. Two mentioned represent caracteristic species of sugar beet. *C. fuscipes* and *P. melanarius* were subdominant (D3) ranging from 2.3 to 3.54%, and accessory (W3) species. All four of the above species were found to be euconstant (C4). *A. dorsalis* was the only recedent (D2), constant (C3), accessory specie (W2), while remaining ten were subrecedent (D1) species and mostly accidental (C1, W1).

In wheat, *P. melas* (41.46), *H. rufipes* (26.22) and *P. melanarius melanarius* (10.21%) were eudominant species (D5), but according to the constancy index, *C. fuscipes* and *C. coriaceus coriaceus* were only euconstant species (C4). Among other species present in wheat four were classified as accidental (W1), seven as accessory (W2 and W3). All mentioned species belong to accidental (C1) or accessory (C2) category. Ecological significance confirmed the relationship between dominance and constancy and showed that *H. rufipes* and *P. melas* were the only two species classified as characteristic in all four fields studied (W4 and W5). A detailed biocenotic synecological analysis for each crop in Vukovar-Syrmia County is presented in *Table 4*.

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Table 3. *Complete list of identified ground beetle species in arable crop agricultural landscape*

Species /Location and crop	Virovitica-Podravina County				Vukovar-Sirmium County			
	Maize	**Soybean**	**Sugar beet**	**Wheat**	**Maize**	**Soybean**	**Sugar beet**	**Wheat**
Leistus (Leistus) ferrugineus Linnaeus, 1758	+							
Nebria (Nebria) brevicollis Fabricius, 1792	+	+		+			+	
Calosoma (Calosoma) inquisitor inquisitor Linnaeus, 1758	+							
Calosoma (Calosoma) maderae maderae Fabricius, 1775			+					
Carabus (Carabus) granulatus granulatus Linnaeus, 1758	+	+	+	+	+			
Carabus (Procrustes) coriaceus coriaceus Linnaeus, 1758	+	+		+	+	+	+	+
Carabus (Tachypus) cancellatus cancellatus Illiger, 1798	+	+		+				
Cylindera (Cylindera) germanica germanica Linnaeus, 1758		+		+				
Loricera (Loricera) pilicornis pilicornis Fabricius, 1775				+				
Clivina (Clivina) collaris Herbst, 1784	+							
Clivina (Clivina) fossor fossor Linnaeus, 1758	+	+		+				
Asaphidion flavipes Linnaeus, 1760	+			+				
Bembidion (Bembidion) quadrimaculatum quadrimaculatum Linnaeus, 1760	+	+		+				
Bembidion (Metallina) lampros Herbst, 1784				+				
Bembidion (Metallina) properans Stephens, 1828	+	+	+					
Bembidion (Peryphanes) dalmatinum dalmatinum Dejean, 1831	+							
Brachinus (Brachinus) crepitans Linnaeus, 1758						+	+	+
Brachinus (Brachinus) elegans Chaudoir, 1842	+	+	+	+				
Brachinus (Brachynidius) explodens Duftschmid, 1812								+
Callistus lunatus lunatus Fabricius, 1775		+						
Chlaenius (Chlaeniellus) nigricornis Fabricius, 1787	+							
Chlaenius (Chlaenites) tristis tristis Schaller, 1783				+				
Chlaenius (Chlaenites) spoliatus spoliatus P. Rossi, 1792	+		+					
Trechus (Trechus) quadristriatus Schrank, 1781	+	+		+		+		+
Drypta (Drypta) dentata P. Rossi, 1790				+				
Anisodactylus (Anisodactylus) binotatus Fabricius, 1787	+			+				
Anisodactylus (Pseudanisodactylus) signatus Panzer 1796	+	+	+	+				
Diachromus germanus Linnaeus, 1758		+		+				
Harpalus (Harpalus) affinis Schrank, 1781	+	+	+	+				
Harpalus (Harpalus) dimidiatus P. Rossi, 1790	+				+	+	+	
Harpalus (Harpalus) distinguendus distinguendus Duftschmid, 1812	+	+	+	+	+	+	+	+
Harpalus (Harpalus) tardus Panzer, 1796		+		+	+	+	+	

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Species /Location and crop	Virovitica-Podravina County				Vukovar-Sirmium County			
	Maize	**Soybean**	**Sugar beet**	**Wheat**	**Maize**	**Soybean**	**Sugar beet**	**Wheat**
Harpalus (Pseudoophonus) calceatus Duftschmid, 1812					+	+		+
Harpalus (Pseudoophonus) griseus Panzer, 1796			+		+	+	+	+
Harpalus (Pseudoophonus) rufipes De Greer, 1774	+	+	+	+	+	+	+	+
Harpalus (Pseudoophonus) signaticornis Duftschmid, 1812			+					
Parophonus (Parophonus) dejeani Csiki, 1932		+						
Stenolophus (Stenolophus) teutonus Schrank, 1781				+				
Demetrias (Demetrias) atricapillus Linnaeus, 1758				+				
Microlestes minutulus Goeze, 1777	+							
Oodes helopioides helopioides Fabricius, 1792				+				
Agonum (Amara) viridicupreum viridicupreum Goeze, 1777	+	+						
Anchomenus (Anchomenus) dorsalis Pontoppidan, 1763	+	+		+	+	+	+	+
Abax (Abacopercus) carinatus carinatus Duftschmid, 1812		+		+	+	+		
Poecilus (Poecilus) cupreus cupreus Linnaeus, 1758	+	+	+	+	+	+	+	+
Pterostichus (Argutor) vernalis Panzer, 1796				+				
Pterostichus (Cophosus) cylindricus Herbst, 1784								+
Pterostichus (Feronidius) melas melas Creutzer, 1799	+	+	+	+	+	+	+	+
Pterostichus (Morphosoma) melanarius melanarius Illiger, 1798	+	+	+	+		+	+	+
Pterostichus (Platysma) niger niger Schaller, 1783		+						
Stomis (Stomis) pumicatus pumicatus Panzer, 1796		+						
Calathus (Calathus) fuscipes fuscipes Goeze, 1777	+	+	+	+	+	+	+	+
Calathus (Neocalathus) ambiguus ambiguus Paykull, 1790	+							+
Calathus (Neocalathus) micropterus Duftschmid, 1812						+		
Dolichus halensis Schaller, 1783	+							
Laemostenus (Pristonychus) terricola terricola Herbst, 1784						+	+	+
Amara (Amara) aenea Degeer, 1774	+			+				
Amara (Amara) ovata Fabricius, 1792	+							
Amara (Amara) saphyrea Dejean, 1828	+			+				
Amara (Amara) similata Gyllenhal, 1810	+	+					+	
Amara (Zezea) chaudoiri incognita Fassati, 1946				+				
Amara (Zezea) kulti Fassati, 1947				+				
Amara (Zezea) plebeja Gyllenhal, 1810				+				
Zabrus (Zabrus) tenebrioides tenebrioides Goeze, 1777					+	+	+	+

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Table 4. *Biocenotic synecological analysis per crop in Vukovar-Syrmia County*

Crop	Species	*D (%)	**Class of D	*C (%)	**Class of C	*W (%)	**Class of W
Maize	*H. rufipes*	80.41	D5	100.00	C4	80.41	W5
	P. melas melas	5.26	D4	75.00	C3	3.95	W3
	H. distinguendus	3.51	D3	75.00	C3	2.63	W3
	C. fuscipes	3.22	D3	25.00	C1	0.80	W2
	A. carinatus carinatus	1.75	D2	75.00	C3	1.32	W3
	A. dorsalis	1.75	D2	50.00	C2	0.88	W2
	H. griseus	1.17	D2	50.00	C2	0.58	W2
	C. coriaceus coriaceus	0.58	D1	50.00	C2	0.29	W2
	H. tardus	0.58	D1	25.00	C1	0.15	W2
	A. saphyrea	0.29	D1	25.00	C1	0.07	W1
	C. granulatus granulatus	0.29	D1	25.00	C1	0.07	W1
	H. dimidiatus	0.29	D1	25.00	C1	0.07	W1
	P. cupreus cupreus	0.29	D1	25.00	C1	0.07	W1
	H. calceatus	0.29	D1	25.00	C1	0.07	W1
	Z. tenebrioides tenebrioides	0.29	D1	25.00	C1	0.07	W1
Soybean	*H. rufipes*	57.31	D5	75.00	C3	42.98	W5
	A. dorsalis	16.21	D5	50.00	C2	8.10	W4
	C. fuscipes	5.53	D4	50.00	C2	2.77	W3
	H. distinguendus	5.14	D4	75.00	C3	3.85	W3
	H. griseus	3.95	D3	25.00	C1	0.99	W2
	B. crepitans	2.37	D3	50.00	C2	1.19	W3
	P. melas melas	2.37	D3	50.00	C2	1.19	W3
	Z. tenebrioides tenebrioides	1.19	D2	50.00	C2	0.59	W2
	C. micropterus	0.79	D1	25.00	C1	0.20	W2
	C. coriaceus coriaceus	0.79	D1	50.00	C2	0.40	W2
	H. tardus	0.79	D1	25.00	C1	0.20	W2
	H. calceatus	0.79	D1	25.00	C1	0.20	W2
	T. quadristriatus	0.79	D1	25.00	C1	0.20	W2
	A. carinatus carinatus	0.40	D1	25.00	C1	0.10	W1
	H. dimidiatus	0.40	D1	25.00	C1	0.10	W1
	L. terricola terricola	0.40	D1	25.00	C1	0.10	W1
	P. cupreus cupreus	0.40	D1	25.00	C1	0.10	W1
	P. melanarius melanarius	0.40	D1	25.00	C1	0.10	W1
Sugar beet	*P. melas melas*	81.26	D5	100.00	C4	81.26	W5
	H. rufipes	9.46	D4	100.00	C4	9.46	W4
	C. fuscipes	3.54	D3	100.00	C4	3.54	W3

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Crop	Species	*D (%)	**Class of D	*C (%)	**Class of C	*W (%)	**Class of W
	P. melanarius melanarius	2.30	D3	100.00	C4	2.30	W3
	A. dorsalis	1.15	D2	75.00	C3	0.86	W2
	P. cupreus cupreus	0.71	D1	50.00	C2	0.35	W2
	C. coriaceus coriaceus	0.44	D1	75.00	C3	0.33	W2
	A. similata	0.18	D1	50.00	C2	0.09	W1
	B. crepitans	0.18	D1	25.00	C1	0.04	W1
	H. dimidiatus	0.18	D1	25.00	C1	0.04	W1
	H. distinguendus	0.18	D1	25.00	C1	0.04	W1
	L. terricola terricola	0.18	D1	50.00	C2	0.09	W1
	H. tardus	0.09	D1	25.00	C1	0.02	W1
	N. brevicollis	0.09	D1	25.00	C1	0.02	W1
	Z. tenebrioides tenebrioides	0.09	D1	25.00	C1	0.02	W1
Wheat	*P. melas melas*	41.46	D5	50.00	C2	20.73	W5
	H. rufipes	26.22	D5	75.00	C3	19.66	W5
	P. melanarius melanarius	10.21	D5	25.00	C1	2.55	W3
	A. dorsalis	7.01	D4	75.00	C3	5.26	W4
	C. fuscipes	2.90	D3	100.00	C4	2.90	W3
	H. distinguendus	2.90	D3	75.00	C3	2.17	W3
	C. coriaceus coriaceus	2.13	D3	100.00	C4	2.13	W3
	Z. tenebrioides tenebrioides	1.83	D2	50.00	C2	0.91	W2
	L. terricola terricola	1.37	D2	25.00	C1	0.34	W2
	P. cylindricus	1.22	D2	25.00	C1	0.30	W2
	C. ambiguus ambiguus	0.76	D1	50.00	C2	0.38	W2
	H. griseus	0.61	D1	50.00	C2	0.30	W2
	P. cupreus cupreus	0.46	D1	25.00	C1	0.11	W2
	T. quadristriatus	0.30	D1	50.00	C2	0.15	W2
	A. saphyrea	0.15	D1	25.00	C1	0.04	W1
	B. crepitans	0.15	D1	25.00	C1	0.04	W1
	B. explodens	0.15	D1	25.00	C1	0.04	W1
	H. calceatus	0.15	D1	25.00	C1	0.04	W1

*D - dominance; C - constancy; W - ecological significance. **For details on classes please see Table 2

In Virovitica-Podravina County, a total of 9,381 ground beetles were collected during the 20-week sampling period. After identification, 56 species were determined. The largest number was collected in maize (5,656), soybean (1,471), sugar beet (1,250) and wheat (1,004).

In maize *P. melanarius melanarius*, *H. rufipes*, and *P. cupreus cupreus* were eudominant species (D5), euconstant (C4), and characteristic species (W5) accounting

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over 50% of the represented species for the investigated area. *P. melas melas* was recedent (D2) but euconstant (C4), accessory (W3) specie. All other 30 species in maize were subrecedent (D1) and between accidental to accessory (W1 – W3).

In soybean eudominant species were *P. melas melas* (24,47%), *H. distinguendus* (23.79%) and *P. melanarius melanarius* (18.63%). Just as in maze, they were also euconstant (C4), characteristic species (W4). *H. rufipes*, *P. cupreus cupreus* and *B. elegans* were dominant species (D4) with a raging percentage of 5.71 to 7.68. All of them were euconstant (C4) and characteristic (W4), except for *B. elegans*, which is found to be accidental (C1), accessory (W4) species in soybean. *A. signatus*, *C. cancellatus cancellatus* and *A. dorsalis* are subdominant (D3), constant (C3), accessory species (W3). The other 19 species were subrecedent (D1) of which 13 are accidental species.

In sugar beet, the eudominant (D5), euconstant (C4) and characteristic (W5) species are *P. cupreus cupreus* (41.76%), *H. rufipes* (35.36%), and *P. melanarius melanarius* (10.40%). *P. melas melas* is a less common but classified as dominant (D4) (9.36%), yet euconstant (C4), characteristic species (W4) for sugar beet. The other 11 species present are subrecedent (D1) and mostly accidental (W1).

We found the highest number of eudominant (D5), characteristic (W5) species in wheat as follows *A. dorsalis* (24.70%), *P. cupreus cupreus* (19.62%), *H. rufipes* (18.63%) *P. melas melas* (17.93%) and *P. melanarius melanarius* (12.15%). All the above species are classified as euconstant (C4) except *A. dorsalis* which is constant (C3). The other 30 spices present are subrecedent ranging between accidental (20 - W1) and accessory (10 - W2). A detailed biocenotic synecological analysis for each crop in Virovitica-Podravina County is presented in *Table 5*.

The carabid species composition varies between the two different sampled locations (Bray Curtis Similarity Index: maize = 0.894, soybean = 0.7947, sugar beet = 0.7724) and share only little more than a third of the species (Sorensen Similarity Index: maize = 0.367, soybean = 0.478, sugar beet = 0.4). In wheat, Bray Curtis Similarity Index is 0.4289, while Sorensen Similarity Index is 0.3396 meaning that two sites share even less species than other mentioned crops.

Focusing on the locations separately, Shannon Diversity Index in Virovitica-Podravina County shows a higher overall diversity of carabid beetle species abundances as follows: soybean = 2.105, wheat= 1.9467, maize = 1.260 and sugar beet = 1.3572 than Vukovar-Syrmia County (Shannon Diversity Index in wheat = 1.7585, soybean = 1.5851, maize = 0.915 and sugar beet = 0.7817). When observing Shannon Evenness, both locations are mostly dominated by high abundances of single species. The trend is more pronounced in Vukovar-Syrmia County (wheat = 0.4228, soybean = 0.3811, maize = 0.22) with maximum diversity in sugar beet = 0.188. In Virovitica-Podravina County Shannon Evenness was between 0.5061 in soybean, 0.4681 in wheat, 0.3263 in sugar beet and 0.301 in maize). *Figure 3* shows the results of ANOVA for the average number of catches of ground beetles on the studied site (a), crops (b) and sampling dates (c).

The significantly highest captures were identified in maize comparing to other three crops ($HSD_{p=0.05} = 73.30$). The captures in Virovitica-Podravina county were significantly higher than the captures in Vukovar-Syrmia County ($HSD_{p=0.05} = 10.49$). The highest captures were recorded in September following with May and August. Comparing to September, significantly lower captures were recorded in July ($HSD_{p=0.05} = 62.64$).

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Table 5. *Biocenotic synecological analysis per crop in Virovitica-Podravina County*

Crop	Species	*D (%)	**Class of D	*C (%)	**Class of C	*W (%)	**Class of W
Maize	*P. melanarius melanarius*	51.18	D5	100.00	C4	51.18	W5
	H. rufipes	22.67	D5	100.00	C4	22.67	W5
	P. cupreus cupreus	21.76	D5	100.00	C4	21.76	W5
	P. melas melas	1.15	D2	100.00	C4	1.15	W3
	H. distinguendus	0.88	D1	50.00	C2	0.44	W1
	A. dorsalis	0.39	D1	50.00	C2	0.19	W1
	B. elegans	0.32	D1	75.00	C3	0.24	W1
	B. properans	0.21	D1	25.00	C1	0.05	W1
	T. quadristriatus	0.21	D1	25.00	C1	0.05	W1
	A. aenea	0.16	D1	25.00	C1	0.04	W1
	A. similata	0.14	D1	50.00	C2	0.07	W1
	C. fossor fossor	0.12	D1	50.00	C2	0.06	W1
	C. cancellatus cancellatus	0.11	D1	50.00	C2	0.05	W1
	H. affinis	0.09	D1	25.00	C1	0.02	W1
	C. ambiguus ambiguus	0.07	D1	25.00	C1	0.02	W1
	C. fuscipes	0.07	D1	50.00	C2	0.04	W1
	A. flavipes	0.05	D1	25.00	C1	0.01	W1
	H. dimidiatus	0.05	D1	50.00	C2	0.03	W1
	B. quadrimaculatum quadrimaculatum	0.04	D1	25.00	C1	0.01	W1
	C. spoliatus spoliatus	0.04	D1	50.00	C2	0.02	W1
	C. collaris	0.04	D1	25.00	C1	0.01	W1
	A. ovata	0.02	D1	25.00	C1	0.00	W1
	A. binotatus	0.02	D1	25.00	C1	0.00	W1
	A. signatus	0.02	D1	25.00	C1	0.00	W1
	B. dalmatinum dalmatinum	0.02	D1	25.00	C1	0.00	W1
	C. inquisitor inquisitor	0.02	D1	25.00	C1	0.00	W1
	C. coriaceus coriaceus	0.02	D1	25.00	C1	0.00	W1
	C. granulatus granulatus	0.02	D1	25.00	C1	0.00	W1
	C. nigricornis	0.02	D1	25.00	C1	0.00	W1
	D. halensis	0.02	D1	25.00	C1	0.00	W2
	L. ferrugineus	0.02	D1	25.00	C1	0.00	W1
	M. minutulus	0.02	D1	25.00	C1	0.00	W2
	N. brevicollis	0.02	D1	25.00	C1	0.00	W1
	A. viridicupreum viridicupreum	0.02	D1	50.00	C2	0.02	W1
Soybean	*P. melas melas*	24.47	D5	100.00	C4	24.47	W5
	H. distinguendus	23.79	D5	100.00	C4	23.79	W5
	P. melanarius melanarius	18.63	D5	100.00	C4	18.63	W5

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Crop	Species	*D (%)	**Class of D	*C (%)	**Class of C	*W (%)	**Class of W
	H. rufipes	7.68	D4	100.00	C4	7.68	W4
	P. cupreus cupreus	5.98	D4	100.00	C4	5.98	W4
	B. elegans	5.71	D4	25.00	C1	1.43	W3
	A. signatus	4.08	D3	75.00	C3	3.06	W3
	C. cancellatus cancellatus	2.65	D3	75.00	C3	1.99	W3
	A. dorsalis	2.18	D3	75.00	C3	1.63	W3
	N. brevicollis	1.02	D1	25.00	C1	0.25	W2
	H. affinis	0.75	D1	50.00	C2	0.37	W2
	C. granulatus granulatus	0.54	D1	75.00	C3	0.41	W2
	A. carinatus carinatus	0.41	D1	75.00	C3	0.31	W2
	C. fuscipes	0.41	D1	50.00	C2	0.20	W2
	C. coriaceus coriaceus	0.41	D1	25.00	C1	0.10	W2
	C. fossor fossor	0.27	D1	75.00	C3	0.20	W2
	B. quadrimaculatum quadrimaculatum	0.14	D1	25.00	C1	0.03	W1
	C. germanica germanica	0.14	D1	25.00	C1	0.03	W2
	S. pumicatus pumicatus	0.14	D1	50.00	C2	0.07	W3
	A. viridicupreum viridicupreum	0.07	D1	25.00	C1	0.02	W1
	A. similata	0.07	D1	25.00	C1	0.02	W1
	B. properans	0.07	D1	25.00	C1	0.02	W1
	C. lunatus lunatus	0.07	D1	25.00	C1	0.02	W1
	D. germanus	0.07	D1	25.00	C1	0.02	W3
	H. tardus	0.07	D1	25.00	C1	0.02	W1
	P. dejeani	0.07	D1	25.00	C1	0.02	W1
	P. niger niger	0.07	D1	25.00	C1	0.02	W1
	T. quadristriatus	0.07	D1	25.00	C1	0.02	W1
Sugar beet	*P. cupreus cupreus*	41.76	D5	100.00	C4	41.76	W5
	H. rufipes	35.36	D5	100.00	C4	35.36	W5
	P. melanarius melanarius	10.40	D5	100.00	C4	10.40	W5
	P. melas melas	9.36	D4	100.00	C4	9.36	W4
	C. fuscipes	0.88	D1	75.00	C3	0.66	W2
	H. distinguendus	0.72	D1	25.00	C1	0.18	W2
	H. griseus	0.64	D1	50.00	C2	0.32	W2
	A. signatus	0.32	D1	50.00	C2	0.16	W2
	B. properans	0.08	D1	25.00	C1	0.02	W1
	B. elegans	0.08	D1	25.00	C1	0.02	W1
	C. maderae maderae	0.08	D1	25.00	C1	0.02	W1
	C. granulatus granulatus	0.08	D1	25.00	C1	0.02	W1
	C. spoliatus spoliatus	0.08	D1	25.00	C1	0.02	W1

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Crop	Species	*D (%)	**Class of D	*C (%)	**Class of C	*W (%)	**Class of W
	H. affinis	0.08	D1	25.00	C1	0.02	W1
Wheat	*A. dorsalis*	24.70	D5	75.00	C3	18.53	W5
	P. cupreus cupreus	19.62	D5	100.00	C4	19.62	W5
	H. rufipes	18.63	D5	75.00	C3	13.97	W5
	P. melas melas	17.93	D5	100.00	C4	17.93	W5
	P. melanarius melanarius	12.15	D5	100.00	C4	12.15	W5
	L. pilicornis pilicornis	1.00	D1	50.00	C2	0.50	W2
	N. brevicollis	0.60	D1	25.00	C1	0.15	W2
	D. germanus	0.50	D1	25.00	C1	0.12	W2
	C. granulatus granulatus	0.40	D1	25.00	C1	0.10	W2
	P. vernalis	0.40	D1	50.00	C2	0.20	W2
	A. carinatus carinatus	0.30	D1	50.00	C2	0.15	W2
	A. plebeja	0.30	D1	50.00	C2	0.15	W2
	B. elegans	0.30	D1	25.00	C1	0.07	W2
	C. coriaceus coriaceus	0.30	D1	50.00	C2	0.15	W2
	H. affinis	0.30	D1	50.00	C2	0.15	W2
	A. flavipes	0.20	D1	25.00	C1	0.05	W1
	B. lampros	0.20	D1	25.00	C1	0.05	W1
	C. germanica germanica	0.20	D1	25.00	C1	0.05	W1
	D. atricapillus	0.20	D1	25.00	C1	0.05	W1
	D. dentata	0.20	D1	25.00	C1	0.05	W1
	H. tardus	0.20	D1	25.00	C1	0.05	W1
	A. chaudoiri	0.10	D1	25.00	C1	0.02	W1
	A. kulti	0.10	D1	25.00	C1	0.02	W1
	A. aenea	0.10	D1	25.00	C1	0.02	W1
	A. binotatus	0.10	D1	25.00	C1	0.02	W1
	A. signatus	0.10	D1	25.00	C1	0.02	W1
	B. quadrimaculatum quadrimaculatum	0.10	D1	25.00	C1	0.02	W1
	C. fuscipes	0.10	D1	25.00	C1	0.02	W1
	C. cancellatus cancellatus	0.10	D1	25.00	C1	0.02	W1
	C. tristis tristis	0.10	D1	25.00	C1	0.02	W1
	C. fossor fossor	0.10	D1	25.00	C1	0.02	W1
	H. distinguendus	0.10	D1	25.00	C1	0.02	W1
	O. helopioides helopioides	0.10	D1	25.00	C1	0.02	W1
	S. teutonus	0.10	D1	25.00	C1	0.02	W1
	T. quadristriatus	0.10	D1	25.00	C1	0.02	W1

D - dominance; C - constancy; W - ecological significance. **For details on classes please see Table 2

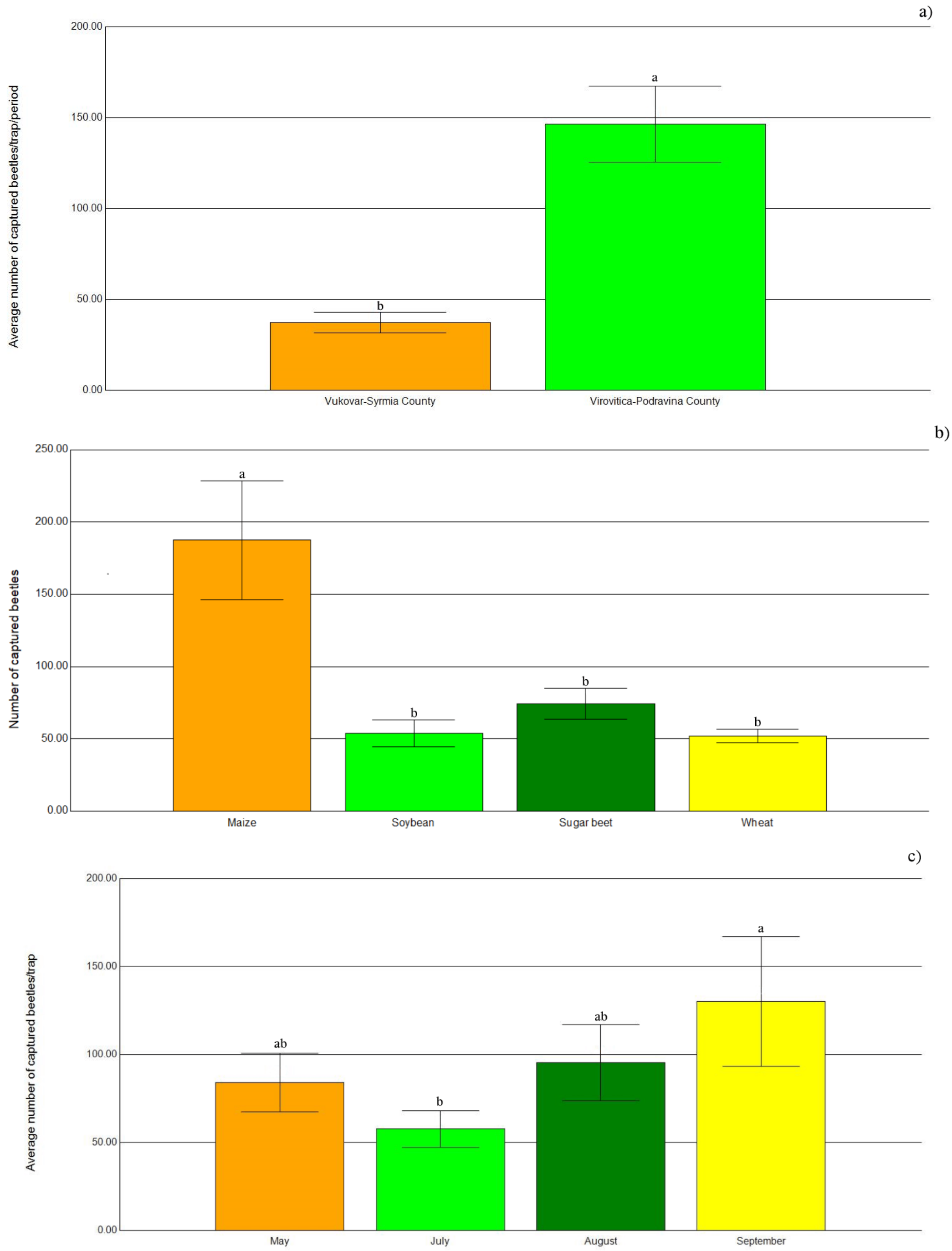

Figure 3. *Captures of ground beetles at different sites (a), in different crops (b) and on different sampling dates (c)*

The recording of ground beetles affected by site, crop and sampling date and their combinations, shown in *Table 6*, indicates that ground beetle abundance was significantly different at two sites and that crops and sampling date influenced ground beetle abundance under different environmental conditions.

Table 6. *Factorial analysis of the number of ground beetles collected in different crops. A Tukey post hoc test was used to determine which values of the ground beetles were significantly different after a significant test result (p < 0.05)*

Source of variation	df	F	Prob (F)	HSD $_{p=0.05}$
Total	127			
Rep	3	0.091	0.9648	
Locality (A)	1	569.774	0.0001	2.87
Crop (B)	3	26.850	0.0001	7.77
AxB	3	90.095	0.0001	5.72
Sampling date (C)	3	27.414	0.0001	7.85
AxC	3	61.544	0.0001	5.28
BxC	9	25.200	0.0001	10.03
AxBxC	9	13.978	0.0001	8.00
Error	93			

df–degrees of freedom; p–probability value; HSD–honestly significant difference

Discussion

Virovitica-Podravina County was characterized as region with less invasive agricultural practices. Most of investigated fields included reduced tillage or no-till practices as well as less use of agrochemicals. Compared to conventional practices, conservation tillage systems can reduce the number of tillages by 40% or more while improving soil aggregation, promoting biological activity, and increasing water-holding capacity and infiltration rates. Crop residues that remain in the soil throughout the year form a cover that reduces wind and water erosion, runoff, or particle and nutrient losses resulting in higher available soil moisture, better soil structure and higher organic matter content (UC Sustainable Agriculture Research and Education Program, 2017). Results of our study show significantly higher number of collected individuals as well as higher overall diversity of ground beetle species in Virovitica-Podravina County compared to Vukovar-Syrmia County. Such result is in line with previous studies where higher ground beetle trapping rates were recorded on fields with reduced tillage or no tillage at all compared with conventionally tilled ones (House and All, 1981; Blumberg and Crossley, 1983; House and Stinner, 1983; House and Parmalee, 1985; Ferguson and McPherson, 1985; Stinner et al., 1988; Tonhasca, 1993).

According to Geiger et al. (2010) and Postma-Blaauw et al. (2010) arable crops are characterized by the presence of depleted arthropod communities with low diversity, in which ground beetles have a highly heterogeneous spatial distribution (Holland et al., 1999). This is in accordance with our results obtained form Vukovar-Syrmia County where 6,999 ground beetles less were recorded during sampling period compared to Virovitica-Podravina County.

Climatic conditions in Vukovar-Syrmia County can be characterized as rather dry with higher average air and soil temperature, especially in May and June when most spring activity is expected. Ground beetles show an increase in population dynamics when air and soil temperatures decrease (Virić Gašparić et al., 2017). The results of this study show the same pattern, as the lowest catches in all fields in Vukovar-Syrmia County were recorded in May, when the lowest rainfall was recorded. Again, a decrease in the amount of ground beetles was observed during sampling in autumn, when average rainfall was lower. The largest number of collected ground beetles in Vukovar-Syrmia County was collected in sugar beet field, which is contrary to the research of

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Kromp (1999), who found that root crops have a negative impact on the abundance of ground beetles due to the long period of bare soil and extreme microclimate on the soil surface.

In Vukovar-Syrmia County *H. rufipes* was eudominant species with highest number of individuals in three out of four investigated crops (on sugar beet it was dominant). *H. rufipes* is species that usually occurs in cultivated lands, pastures, gardens, and polluted areas (Leibman, 1988; Brygadyrenko and Reshetniak, 2014; Cavaliere et al., 2019; Langraf et al., 2020). Other eudominant species were *P. melas melas*, *P. melanarius melanarius* and *A. dorsalis* which is in accordance with research done in Croatia (Bažok et al., 2007; Kos et al., 2010, 2011, 2013; Drmić et al., 2016; Lemic et al., 2017) as well as abroad. According to Lövei and Sunderland (1996) no more than 10 to 40 species are active in a habitat in the same season which is in line with findings form Vukovar-Syrmia County where each investigated arable crop had between 15 and 18 determined species.

Compared to Vukovar-Syrmia County, significantly higher abundance was found in Virovitica-Podravina County, which is characterized by conservation tillage. These results agree with those of Juran et al. (2014) who found that endogeic activity was highest in the organic system, followed by the conventional and integrated systems. In our results, the most abundant species were *P. melanarius melanarius, H. rufipes,* and *P. cupreus cupreus*. The same results in Eastern European countries were obtained by Kromp (1999) and in Croatia by Bažok et al. (2007), Igrc Barčić et al. (2008) and Kos et al. (2011). Higher abundance of ground beetles was found in fields with reduced or no tillage (House and All, 1981; Blumberg and Crossley, 1983; House and Stinner, 1983; House and Parmalee, 1985; Ferguson and McPherson, 1985; Stinner et al., 1988; Tonhasca, 1993). Our results confirm the findings of Lemic et al. (2017) stating that conventional tillage in Podravina location has an influence on the abundance of ground beetles.

Finally, because of this study, a detailed list of ground beetle species occurring in most of the common arable crops in Croatia was prepared. This list is a valuable result that complements previous research (Bažok et al., 2007; Kos et al., 2010, 2011, 2013; Drmić et al., 2016; Gotlin Čuljak et al., 2016; Lemic et al., 2017; Virić Gašparić et al., 2017) and to a better understanding of ground beetle communities in arable crops in Croatia. Such contribution can serve as a basis for conservation programs. The wealth of information on carabids provides an opportunity to use it to signal and predict changes in the environment because carabids can be easily and reliably collected. Standardized monitoring of environmental change using carabids may be possible (Niemelä et al., 2000).

Conclusions

Higher ground beetle abundance and diversity were found in fields with reduced tillage, lower temperatures, and more rainfall during vegetation. The results provide a better understanding of ground beetle communities in Croatian arable crops. Results can serve as a basis for conservation programs that should include reduced or no tillage as much as possible as well as reduced use of agrochemicals. This study also makes an important contribution to the overall knowledge of ground beetles with a comprehensive list of ground beetle species found in maize, sugar beet, wheat, and soybean crops in Croatia.

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Conflicts of Interests. The authors declare no conflict of interests.

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3.2. General discussion

3.2.1. Neonicotinoid efficacy on major pests and degradation dynamics in plants and soil

The **first objective** of the doctoral thesis was to determine the neonicotinoid efficacy on major pests and degradation dynamics in sugar beet plants grown from seed treated with imidacloprid and thiamethoxam under different weather conditions.

Field experiments were conducted to estimate efficacy of seed treatments with neonicotinoids (imidacloprid, and combination of thiamethoxam with tefluthrin) on the main sugar beet pests': wireworms (*Agriotes* spp., Coleoptera: Elateridae), flea beetles (*Chaetocnema tibialis*, Coleoptera: Chrysomelidae), sugar beet weevils (*Bothynoderes punctiventris*, Coleoptera: Curculionidae), caterpillars of noctuid moths (*Agrotis segetum*, *Agrotis ypsilon* and *Euxoa temera,* Lepidoptera: Noctuidae) and aphids (Hemiptera: Aphididae) in two-year experiments (2015 and 2016) on the territory of two dissimilar counties in Croatia, Virovitica-Podravina County at location Lukač (45◦52′26″ N 17◦25′09″ E) and Vukovar-Sirmium County at location Tovarnik (45◦09′54″ N 19◦09′08″ E) (*Publication No. 1*). When comparing the sites, the Tovarnik location had higher average annual air and soil temperatures and lower precipitation, although the amount of precipitation in 2016 did not differ significantly between the sites. Therefore, we can conclude that our study was conducted in two regions with different climatic conditions which is in accordance with results of Kozina et al. (2013, 2015) and Čačija (2015).

As expected, **(i)** neonicotinoid treatments-maintained crop stands and successfully suppressed wireworms although damage differed in terms of number of crop stands between sites and years, demonstrating that wireworms are serious pests at specific sites and in years with specific climatic conditions. The application of insecticides in 2015 resulted in an increase in plant stand of about 11% in Lukač and 69% in Tovarnik. The increase in plant population in 2016 ranged from 22% to 32% in Lukač and from 37% to 55% in Tovarnik.Therefore, insecticide treatments significantly maintained plant stand at both locations and in both years. In Croatia, the economic thresholds for wireworms in arable crops are 1–3 larvae/m^2 in dry areas and 3–5 larvae/m^2 in areas with more rainfall. The occurrence of wireworms in the studied fields as well as the data presented by Čamprag et al. (2006) show that in Croatia and in the neighboring countries the occurrence of wireworms could be significantly higher compared to north Europe, as presented by Hauer et al. (2017). According to Hauer et al. (2017) and Furlan and Kreutzweiser (2015) there is less than 10% occurrence of wireworms in sugar beet fields in north Europe and very low occurrence in the Netherlands, Belgium, Germany, Sweden, Denmark, and Italy. Furlan et al. (2017) reported that wireworm infestation was less than 15% in 70% of the fields observed over a period of 29 years. However, in more than 10% of the fields, the damage exceeded 40%. Poggi et al. (2018) reported damage above 15% in about half of the fields observed in northern France.

Further on **(ii)** neonicotinoid seed treatment significantly reduced flea beetle damage whose observed damage in both years and locations averaged 44% on untreated plots in BBCH 16 and 52% in BBCH 19 proving that flea beetles are a serious pest in Croatia, as in other neighboring countries (Kereši et al., 2006). The average percentage of damage at the Lukač site was significantly higher and amounted to 22.1 and 27.4% com-pared to the damage observed at the Tovarnik site, where it amounted to 17.4% and 18.6% in BBCH 16 and BBCH 19, respectively. At the same time, plant damage was significantly affected by insecticide treatments at all three stages of plant development (BBCH 12–19), proving that neonicotinoid seed treatments protect plants against flea beetle infestation. Kereši et al. (2004) also reported very severe damage by flea beetles in the experiment under extremely hot and dry weather conditions in Vojvodina, where seed dressing with thiamethoxam resulted in a fourfold increase in seedling weight. However, due to the other factors affecting yield, the increase in yield in the plots treated with thi- amethoxam was only 13%. Satisfactory protection of seedlings against beet flea beetle was achieved with thiamethoxam alone or in mixture with tefluthrin and a mixture of imidacloprid + tefluthrin (Kereši et al., 2006). These treatments yielded significantly lower percentages of damaged plants than the untreated, while significantly increasing yield. Nonchemical alternatives for beet flea beetle control in sugar beet are not available and the only alternative is foliar spraying with pyrethroids. Therefore, the need to control the pests by spraying with pyrethroids has increased after the ban of neonicotinoids in 2018. In Croatia, we have already observed resistance of the sugar beet flea beetles to pyrethroids (Bažok, unpublished data). Also, **(iii)** neonicotinoid seed coating provided adequate control at the most sensitive stages of sugar beet development against weevils under low population pressure (damage on untreated control plants between x and y (%). During BBCH 31–34 ne weevils were additionally observed. In 2016, the infestation was significantly higher, especially in the trial in Tovarnik. Damage on untreated plots was significantly higher than on treated ones. Under these conditions, seed treatment achieved satisfactory results in protecting sugar beet at the most sensitive stages of development. However, efficacy of neonicotinoides at higher weevil populations is insufficient and they are not the best solution for treatment. This pest occurs regularly in the eastern part of Croatia (Drmić et al., 2016). As Čamprag, (1984) stated, this species is the most important pest of sugar beet in Vojvodina. In the last 60 years, it has destroyed a total of more than 250,000 hectares of young sugar beet and caused reseeding of stands. Increased occurrence in Croatia, Ukraine and Vojvodina is associated with global climate change and increased temperatures and with the prohibition of effective insecticides (Fodorenko, 2005; Bažok et al., 2012; Vuković et al., 2014). As expected, **(iv)** neonicotinoid seed coating cannot reduce damage by noctuids at later growth stages of sugar beet since the first appearance of the caterpillars is usually in June, two to three months after sugar beet sowing. Due to the long period between sowing and their apperence in the fields, these pests are usually not controlled by seed dressing with neonicotinoids. Findings are in accordance with results of Hauer et al. (2017) who reported that neonicotinoid seed coating did not significantly reduce damage and that noctuid and moth caterpillars should be controlled by foliar application of insecticides. Finally, our research shoved that **(v)** at low population pressure of aphids, a solid conclusion on the effectiveness of neonicotinoid seed coating is not possible. Aphids damage the crop mainly by sucking, resulting in reduced assimilate availability for plant growth and leaf area production (Hauer et al., 2017). They can also transmit Virus Yellows (Schliephake et al., 2000) which can cause significant damage in some countries of southern and eastern Europe (Čamprag, 1973; Igrc Barčić et al., 2000). Based on the results of Igrc Barčić et al. (2000) and Altman (1991), we expected a high efficacy of seed coatings with neonicotinoids against aphids, but significant infestation of aphids was not detected in the experiment.

Within the **same objective** field and greenhouse trials were conducted to determine the degradation dynamics and residue levels of imidacloprid and thiamethoxam used as a seed treatment in sugar beet plants and soil collected from above mentioned regions to estimate environmental risk and possible transfer to succeeding crops (*Publication No. 2*).

In **field experiment,** at the end of sugar beet cultivation (180 days after planting), imidacloprid residues (0.17 mg/kg) and thiamethoxam residues (0.04 mg/kg) were found in the in **soils** of Tovarnik, while in Lukač all residues where below LOQ (Table 3). Tovarnik location is characterized with low amount of precipitation, higher air, and soil temperatures (Table 2) and such conditions are found to be more favorable for longer persistence of neonicotinoids (Bonmatin et al., 2005). Tovarnik soils are: (i) slightly acidic to neutral and do not allow degradation in the soil moist or water; (ii) saturated with high amount of organic matter and available phosphorus and potassium (Table 3), which prevents the leaching of residues and allows higher sorption in soils with high organic matter content (Bonmatin et al., 2005; Cox et al., 2008; Guzsvány et al., 2006). Our result is partially consistent with that of Bonmatin et al. (2005) who randomly sampled 74 soils after the cultivation of maize, wheat, and barley grown from treated seeds and proved that imidacloprid was found in all samples collected after cultivation. In both studied years the results on the degradation in sugar beet **plants** at the Tovarnik and Lukač show that the residues of imidacloprid fell below the maximum reside level (MRL) of 0.5 mg/kg 40-50 days after sowing while thiamethoxam degraded below MRL of 0.02 mg/kg 70-80 days after sowing. Westwood et al. (1998) found that the concentration of imidacloprid in the leaves of sugar beet grown from treated seed was 15.2 mg/kg 21 days after planting and degradation to 0.5 mg/kg 97 days after planting (25-leaf stage). Bažok et al. (2014a) found twice as high a concentration of 0.95 mg/kg imidacloprid in sugar beet leaves 42 days after planting using the HPLC method. Compared to HPLC, the LC -MS/MS method has a lower limit of quantification (LOQ) and offers the possibility of a clear identification of the analysis (Armbruster and Pry, 2008). Uptake by the

roots ranged from 1.6 to 20% for imidacloprid in auberges and maize (Krupke et al., 2017). Krupke et al. (2017) also pointed out that the uptake of clothianidin by maize plants was relatively low and that plant-bound clothianidin concentrations followed an exponential decay pattern with initially high values, followed by a rapid decrease within the first ~20 days after planting. A maximum of 1.34% of the initial seed treatment rate (calculated as mg a.i./kg of seed) was successfully obtained from plant tissues (calculated as mg a.i./kg of plant tissue) and a maximum of 0.26% from root samples. Our study showed that 25 to 27 days after planting in 2015, a maximum of 0.028% imidacloprid and 0.077% thiamethoxam was obtained from the raised plants. In 2016, the recovery rate from the raised plants 40 days after planting was 0.003% for imidacloprid and 50 days after planting up to 0.022% for thiamethoxam. Krohn and Hellpointer (2002) reported the half-life of imidacloprid under field conditions as 83 to 124 days, while Bonmatin et al. (2005) reported 270 days as the half-life of imidacloprid for seed treatment in France. These data confirm that the degradation scenario of imidacloprid and thiamethoxam in sugar beet crops is like the scenario established for clothianidin by Krupke et al. (2017).

In the **laboratory experiment**, higher neonicotinoid residues were found in both soil and plant samples (*Publication No. 2.,* Table 3). The sugar beet plants were sown at five times higher density than in the field so consequently **soil** from laboratory trials contained the average value of 5.34 mg/kg a.i. imidacloprid and 2.65 mg/kg a.i. thiamethoxam + clothianidin-treated variant (Table 4). The loss of neonicotinoids from agricultural soils is thought to occur through degradation or leaching in soil water (Gupta et al., 2008). EFSA's risk assessment (EFSA, 2018a, 2018b, 2018c) did not consider the results of Alford et al. (2017) on the low probability of residues of neonicotinoids remaining in soil for a longer period. Their findings, together with those of (Huseth and Groves, 2014) on the recycling of neonicotinoid insecticides from contaminated groundwater back to crops, point to the possible risk scenario of irrigation. Neonicotinoid residues determined in the sugar beet **plants** degraded more slowly compared to plants from field trials. Imidacloprid residues in plant parts fell below the MRL of 0,05 mg/kg about 60 days after sowing while thiamethoxam residues fell below the MRL of 0.02 mg/kg 70-80 days after sowing. Residues in the **roots** fell below the tolerance level about 80 days after sowing. In 2015, the residue of thiamethoxam roots was at the tolerance level (0.053 mg/kg), while the following year, 100 days after sowing, the concentration was below the MRL. At the time of sugar beet harvest (180 days after sowing), the residues of imidacloprid and thiamethoxam were below the MRL and were largely dependent on weather conditions, especially rainfall. Although sugar beet in Croatia is grown in crop rotation where neonicotinoids are already prohibited (maize, oilseed rape, wheat, etc.,), there should be a limited risk of bioaccumulation and transfer to other crops but the risk for succeeding crops needs to be further assessed. Similar results were obtained by Krohn and Hellpointer (2002) who showed no accumulation of imidacloprid in soil after repeated annual applications in Europe and the United States.

3.2.2. Neonicotinoid residues in ground beetles and earthworms

The **second objective** was to determine the neonicotinoid residues in ground beetles and earthworms using the LC-MS/MS, SPE-QuEChERS method whose limit of quantification (LOQ) in case of animal samples was 0.001 mg/kg (*Publication No. 3*). Collecting of samples was done by standard methodologies in Lukač and Tovarnik. During the study, a total 14 ground beetle samples were collected from sugar beet fields grown form neonicotinoid-treated seeds and 58 earthworm samples collected from all fields included in the study during three sampling periods. The multiresidue method described above was used to determine the residues of 300 different active ingredients of PPPs, but only the results of neonicotinoids are considered in this survey. Clear results on sugar beet pests have been published, there were no relevant data on the impact of neonicotinoids on beneficial soil fauna in fields with intensive sugar beet cultivation. According to EFSA, treatment of sugar beet seeds with neonicotinoids poses a risk to the succeeding crop if residues remain in the soil and can be taken up (EFSA, 2018a, 2018b, 2018c). High concentrations of neonicotinoids in soil are most likely to occur during drought, incapacity of leaching, or irregular rinsing (Virić Gašparić et al., 2020). Thus, if significant concentrations of neonicotinoids remain in the soil after the growing season, residues in soil fauna can be expected.

Residues of imidacloprid in our study were present not only in the **ground beetles** collected on imidacloprid-treated variants but also in the other two variants. The reason for this is that the ground beetles are very mobile and individuals from one variant can be present in samples from the other. Within our study highest concentration of imidacloprid was 0.027 mg/kg on location Lukač during autumn sampling, while residues of thiamethoxam and clothianidin between <0.001 - 0.002 are negligible in all variants. In a study by Mullin et al. (2010), almost 100% mortality of 18 ground beetle species and extreme sensitivity of ground beetle (*Poecilus cupreus* L.) larvae exposed to commercial corn seed treated with neonicotinoids at a dose of 700 g/kg was observed. The amounts of residues in this research are extremely low, and we can consider that there are neither residues nor accumulation of neonicotinoids in ground beetles in Croatia.

In case of **earthworms**' toxicological studies show the risk of mortality of individual of all known species when they ingest soil or organic material containing neonicotinoid residues at a concentration ≥1 mg/kg (Pisa et al., 2014). According to Gomez-Eyles et al. (2009) imidacloprid can negatively affect the reproduction and growth of earthworm at 1.91 mg/kg. At a concentration of 3 mg/kg, 50% mortality of earthworms is expected (Pisa et al., 2014). Within our study the highest detected residues of imidacloprid were far below the value of acute and chronic toxicity of the same pesticide (LC_{50} = 10.7 mg/kg). Increase of imidacloprid residues in earthworms at the end of sugar beet vegetation can be explained by their more active period toward the end of the vegetation season (Piearce and Lee, 1987). According to Pesticide property data base (PPDB) of Agriculture & Environment Research Unit at the University of Hertfordshire. (AERU, 2022) imidacloprid is moderately toxic to earthworms with low risk of bioaccumulating.

Within **second objective** additional retrospective analysis of analytically measured residues in earthworms and re-calculated soil concentrations was performed to confirm was there a bioaccumulation potential (*Publication No. 4*). Bioaccumulation is the general uptake and storage of substances, while uptake from the surrounding medium as part of bioaccumulation is defined as bioconcentration (Franke et al., 1994; Fent, 2013). Bioconcentration is a measure of the amount of pesticide residues in an organism's tissues relative to the concentration in the organism's environment (Zartarian and Schultz, 2010). This includes the uptake of pesticides through respiration and contact, but not through food sources. Bioconcentration factors (BCF) are calculated by considering pesticide tissue concentrations relative to pesticide concentrations in the environment. BCF Values > 1 indicate that the concentration in the organism is higher than that of the medium (e.g., soil or water) from which the pesticide was taken (USEPA, 2021). Concentrations of 26 analyzed active ingredients from 58 earthworm samples ranged between 0.000 and 0.247 mg/kg earthworm fresh weight with a mean of 0.005 mg/kg earthworm fresh weight. The percentage of samples with values below the limit of detection (LOD = ½LOQ), values below the limit of quantification (LOQ = 0.001 mg/kg) and values above LOQ were 33.44 and 23% respectively.

Degradation parameters (DT_{50}, DT_{90}) were used to calculate degradation curves and the current concentration in soil at the date of earthworm sampling. Subsequently, compound-specific **bioconcentration factors** in soil were determined by dividing the analyzed pesticide residues in earthworms by the calculated concentrations in soil (Franke et al., 1994; Fent, 2013). For nine active ingredients, data from conducted research allowed the calculation of bioconcentration factors, using analysed residues in earthworms and recalculated soil concentrations. BCF values > 1.0 indicate an accumulation within the earthworms. For nine active ingredients, a variation of plot-spectific BCF values below and above the trigger value of 1.0. For imidaclopid, thiamethoxam and metamitron the mean BCF value is > 1.0. The calculated BCF values of this study are comparable to values shown in the literature for imidacloprid, whose BCF = 15 (Chevillot et al., 2017), thiamethoxam BCF = 1 - 2 (Douglas et al., 2015), azoxystrobin BCF = low risk, (EFSA, 2009), and ethofumesate BCF = 2.2 (Xu et al., 2014). Therefore, the information of farmers regarding the actual application rate and application time of a product is highly valuable for the calculation of the soil concentration at a specific time after the application and can be used for the calculation of bioconcentration factors.

Secondary poisoning is defined by the transfer of the active ingredient within the food chain from earthworms to earthworm-eating birds and mammals. The potential risk assessment of birds and mammals for secondary poisoning is done following EFSA protocol (2009). The predicted environmental concentration in earthworms (PECworm) is calculated based on a theoretical bioconcentration factor BCF (calc.) from substance-specific physicochemical data (i.e., LogPow and Koc). For eight of the nine active ingredients the Toxicity Exposure Ratio (TER) was calculated. TER is a risk indicator for the risk assessment of PPP's. It indicates the ratio of the harmful concentration to the estimated exposure concentration for an organism (acute, chronic) (Arapis et al., 2006). All calculated TERsecondy poisoning values were > 5 and indicate no potential for secondary poisoning to earthworm-feeding mammals and birds.

Assessment of potentially toxic effects to earthworms was re-calculated to soil concentrations (mg a.i./ha) based on information of application rates (g a.i. per ha) provided by the farmers. These expected soil concentrations directly after application are used for the assessment of the potential risk of PPPs on earthworms in the field. The toxicity-exposure ratio (TERworm) for earthworms was derived from the values of no-observed-effect-concentrations (NOEC) from earthworm laboratory reproduction studies and the expected soil concentrations directly after application. NOEC-values of earthworm reproduction studies were available from all 12 applied fungicide active ingredients and resulted in TERworm-values of 1.5 – 242. The calculation for epoxiconazole and thiophanate-methyl produced TERworm-values < 5 and would need further assessment of their potential risk to earthworms in the environment. Some fungicides are characterised by the same mode of action and may cause mixed toxicity to earthworms when applied in the same season. This also requires further consideration. When replacing the expected soil concentration directly after application by the maximum calculated soil concentration at the time of worm sampling, the TERworm-values increased as expected since the soil concentrations decreased continuously after application. This decrease was rather slow for epoxiconazole resulting into a still critical TERworm-value. Therefore, the environmental risk assessment on earthworms should consider that a slow degradation rate of an active ingredient might impact earthworms over a longer time.

3.2.3. Cenological analysis of ground beetles collected from fields included in the research

The **third objective** was determining the ground beetle's composition in sugar beet fields and in fields where beets were grown one, two or three years ago. By monitoring the ground beetle population at the studied sites, the factors influencing their activity and abundance were identified. During 2015, the influence of environmental specifics (soil type and structure, climatic conditions) along with cropping practices (tillage and insecticide use) on activity and abundance was evaluated. This was the first detailed study in Croatia aimed at understanding how intensive arable crop production with their environmental and management specificities affects ground beetle communities. (*Publication No. 5*). **First set of samples** was collected during 2015 in Virovitica-Podravina County, and Vukovar-Syrmia County. Catches of ground beetles were significantly lower in Tovarnik than in Lukač. The difference in total catch between localities was influenced by: **(i) different climatic conditions**, although the studied localities are located in the same climatic regions according to the Koppen classification (Penzar and Benzar, 2000); **(ii) edaphic conditions** more favorable at the Lukač site, where acidic soils with a high percentage of fine silt and a low percentage of clay are typical; **(iii) type of tillage**, with conservation tillage, as practised in the Lukač fields, resulting in higher ground beetle capture rates compared to conventionally tilled fields (House and All, 1981; Blumberg and Crossley, 1983; House and Stinner, 1983; Tonhasca, 1993; Ferguson and McPherson, 1985; House and Parmelee, 1985; Stinner et al., 1988); **(iv) type of crop stand** that, according to O' Rourke et al. (2008) may provide important refuges for ground beetles, especially thick stand crops sown in autumn, in comparison with crops which were sown in spring. In our study, the highest ground beetle abundances were found on wheat in Tovarnik and oilseed rape in Lukač. These were overwintering crops that provided less extreme microhabitat in spring and created positive survival conditions, confirming the importance of crop habitat in supporting ground beetle populations; **(v) period of bare soil,** which in root crops creates an extreme microclimate at the soil surface with high temperatures and insolation during the day, in contrast to winter cereals, where an already established crop stand in early spring creates favorable conditions for ground beetles (Kromp, 1999). The lowest caches on the sugar beet fields in our studies can be explained by the extreme microclimate on the soil surface as a result of the long period of bare soil, which was 7 months in the case of Tovarnik and 9 months in Lukač; **(vi) plant cover density,** which influenced higher total number of ground beetle caches per week in winter crops sown in the autumn of the previous year compared with sugar beets and maize which sown in the spring after a long period of bare soil; and **(vi) fertilization and insecticide applications.** The amounts of nitrogen applied on all fields are compatible with the allowed amounts according to the integrated crop production in Croatia (European Comission, 2009), which has a minimal negative impact on all beneficial insects, which is consistent with the higher abundance of ground beetles in Lukač, since in our studies fertilization was more intensive at this site. On the other hand, insecticide treatments, which generally have a negative impact on ground beetle populations (Asteraki et al., 1992, 1995), were more intensive in Tovarnik, and accordingly, ground beetle numbers were significantly lower at the Tovarnik site compared to Lukač. Finally, **(viii) crop rotation** affects ground beetle abundance. In Lukač, the significantly highest catches were recorded in wheat in both epigeic and endogeic traps, and on that field sugar beets were grown three years ago. There was no significant difference between the other crops. The lowest catches were recorded in sugar beet at both locations and with both types of traps confirming that sugar beet planting has the greatest negative impact on beetle populations due to intensive processing and frequent insecticide use. Ground beetle numbers were found to increase in years following sugar beet planting, i.e., in a four-year crop rotation, confirming the assumption that abundance can be restored in the years after sugar beet growing (four-year crop rotation: see detailed in Table 1).

In the framework of the **same objective** the **initial cenological analysis** was carried out for ground beetle species collected in 2015 in wheat from Virovitica-Podravina County *(Publication No. 6)*. The collected ground beetles belong to 26 species and 15 genera which can be classified as moderately high compared to previous studies in agricultural agroecosystems (Bažok et al., 2007; Kos et al., 2006, 2010, 2011; Drmić et al., 2016; Virić Gašparić et al., 2017).

By **dominance class** the most abudant and **eudominant species** (almost 70% of the total catch) were *Poecilus cupreus* (Linnaeus, 1758), *Brachinus psophia* Audinet-Serville, 1821 and *Pterostichus melas melas* (Creutzer, 1799) (Table 2). Species *P. cupreus* is considered as one of the most common species inhabitating winter crops (Alford et al., 2007), so these results strongly support this research. In Croatia Štrbac (1983) also specified it among the three most dominant on arable land. *Anchomenus (Anchomenus) dorsalis* (Pontoppidan, 1763) and *Harpalus (Pseudoophonus) rufipes* (DeGeer, 1774) were classified as dominant. Drmić et al. (2016) investigated endogaeic ground beetle fauna in the same area in Croatia and detected *B. psophia* and *A. dorsalis* as the most abundant ones, therefore it is confirmed that these species are typical arable ground beetle representatives in investigated region. Species *P. melas* is also common in Croatia and was detected as dominant in agricultural land near the Nature Park Lonjsko polje (Brigić, 2012). Other species were classified as subdominant (2), recedent (4) and mostly subrecedent (15). This structure is typical of a ground beetle community on farmland, consisting of a small number of dominant species represented by many individuals, and many fewer common species (subdominant, recedent, and subrecedent) represented by a small number of specimens (Baranová et al., 2013).

The **diversity** of investigated species was moderately high: the Simpson diversity index was 0.7875, the Shannon-Wiener index was 1.9654, and the Pielou evenness was 0.6032. Zoogeographical analysis showed an equal dominance Euroasian (23.08%) and Palearctic (23.08%) species which corresponds with climatic and geographic characteristics of the investigated area. By **relict class**, most species (73%) were eurytopic (E), i.e., capable of inhabiting landscapes under strong anthropogenic influence. Adaptive (A) species included 27% and this group included more species found in natural habitats (forests, meadows, pastures, standing and flowing waters) (Hůrka et al., 1996). Not a single species was assigned to relict class R (rare and endangered species). These results are consistent with those of Porhajašová et al. (2004) and Baranová et al. (2013) who reported that increasing human disturbance is changing the composition in favor of eurytopic species, while decreasing the number of specialized species with narrow ecological value.

Ratio of spring to autumn breeders of species collected in wheat was in favor of spring breeders (14 species), 8 species were autumn breeders and one species (*Calathus fuscipes fuscipes* (Goeze, 1777)) breeds in both seasons (Table 2). Such finding is in line with those of Holland and Luff (2000) who found that winter crops usually have higher abundance, diversity and more spring breeders with summer larvae (e.g., *P. cupreus, A. dorsalis*). The domination of spring breeders could be a consequence of the cultivation measures. The depth of tillage is one of the major factors affecting field carabid communities, with superficial ploughing enabling a higher number of species and favoring spring breeders (Stassart et al., 1983; Kromp, 1999). The results of this study significantly contributed to better understanding of initial situation about ground beetle communities in intensive agricultural landscape in Croatia.

As a **final contribution to Objective 3**, the **second set of ground beetle samples** was collected in Virovitica-Podravina and Vukovar-Syrmia counties in 2016. At each site, four fields were included in the study where sugar beet was sown in four-year crop rotation system. A biocenological-synecological analysis was performed, which included the calculation of analytical ecological indices - species richness, dominance, and constancy index. During the survey, 64 species of ground beetles belonging to 33 genera were collected and identified. The species are classified according to the Catalog of Palaearctic Coleoptera Archostemata - Myxophaga - Adephaga, revised and supplemented edition (Löbl and Löbl, 2017) *(Publication No. 7).*

Catches in Virovitica-Podravina County, characterized by conservation tillage, were significantly higher than catches in Vukovar-Syrmia County (HSD p=0.05 =10.49). This result is consistent with previous studies that found higher trapping rates for ground beetles in fields with reduced or no tillage compared to conventionally tilled fields (Blumberg and Crossley, 1983; Ferguson and McPherson, 1985; House and All, 1981; House and Parmelee, 1985; House and Stinner, 1983; Stinner et al., 1988; Tonhasca, 1993).

In Vukovar-Syrmia County, a total of 2,382 individuals of ground beetles (25 species) were collected during the 20-week sampling period, of which the highest number was found in sugar beet fields (1,131). Such result can be explained by the fact that **no sugar beet has been grown in rotation in this field for at least five years** (data available as of 2012). *P. melas* was classified as the eudominant species with 81.26%. *H. rufipes* was classified as the dominant species (9.46%), while *C. fuscipes* and *A. dosrsali*s were eudominant with proportions ranging from 2.3 to 3.54%. In wheat crops, 656 individuals were determined - sugar beet grown two years ago; in maize 342 – sugar beet grown in previous year; and the fewest in soybean (253) - sugar beet grown four years ago. In Virovitica-Podravine County, a total of 9,381 individuals (56 species) were collected. The significantly highest number was collected in maize field (5,656) where sugar beets were grown in 2012, **confirming again the recovery of the ground beetle fauna in a four-year crop rotation**. Such results are in line with those of Virić Gašparić at al. (2017) and Lemić et al. (2017). *P. cupreus* (41.76%), *H. rufipes* (35.36%) and *P. melanarius* (10.40%) were classified as eudominant, euconstant and characteristic species. *P. melas* was recedent but euconstant, accessory species. All other 30 species in maize were subrecedent and between accidental to accessory. In the remaining crops, the number of ground beetles was much lower: in soybean (1471) – sugar beet grown three years ago; sugar beet (1250) - sugar beet grown two years ago and then wheat (1004) - sugar beet grown in previous year.

Cenological analysis by crop in both sites showed that *H. rufipes, P. melas, P. melanarius* and *P. cupreus* are the most represented species in all studied crops. The eudominance of certain genera with many members of a given species is characteristic at both sites studied. The species composition varies between the two sites, with the Bray Curtis similarity index showing that the sites have no more than one-third of the species in common. The Shannon index of diversity in Virovitica-Podravina County shows greater overall diversity of species richness than in Vukovar- Syrmia County. When looking at Shannon evenness, both sites are mostly dominated by large numbers of individual species, with a more pronounced trend in Vukovar- Syrmia County. The highest number was recorded in September, while it was significantly lower in July due to high temperatures. The number of ground beetles was influenced by the location, culture, and date of sampling period.

Finally, as a result of this study, a detailed list of ground beetle species occurring in most of the common arable crops in Croatia was prepared. This list is a valuable result that complements previous studies (Bažok et al., 2007; Kos et al., 2010, 2011, 2014; Drmić et al., 2016; Gotlin Čuljak et al., 2016; Lemic et al., 2017; Virić Gašparić et al., 2017) and contributes to a better understanding of ground beetle communities in arable crops in Croatia. The wealth of information on carabids provides an opportunity to use it to signal and predict changes in the environment because carabids can be easily and reliably collected. Standardized monitoring of environmental change using carabids may be possible (Niemelä et al., 2000).

4. Conclusions and perspectives

Based on the research conducted, the following **conclusions** can be drawn:

1. Research results on the efficacy of seed treated with neonicotinoids (imidacloprid and thiamethoxam) against major sugar beet pests showed satisfactory protection against wireworm, flea beetle, and sugar beet weevil present at lower pressures regardless of weather conditions. Caterpillars and aphids were present in lower numbers, so it is not possible to conclude with certainty the degree of efficacy of the insecticides tested. Efficacy can be expected about seven weeks after sowing, which is consistent with the degradation dynamics.

2. Degradation dynamics showed that residue levels of imidacloprid and thiamethoxam in sugar beet plants were below maximum residue level at the time of harvest. The seed treatment as a plant protection measure leaves minimal trace in plants because of the complete degradation by the end of the growing season. The degradation dynamics are highly dependent on weather conditions, especially rainfall, so elevated residue concentrations in the soil indicate possible uptake of neonicotinoids by succeeding crops, especially in dry climates or after a dry period. However, further studies are needed to evaluate the potential uptake by succeeding crops.

3. Research on neonicotinoid residues in beneficial organisms, ground beetles and earthworms, showed that the highest concentration of imidacloprid and thiamethoxam residues detected was below lethal levels throughout the sampling period, so it can be assumed that there is no accumulation of neonicotinoids in these organisms.

4. The bioconcentration factors calculated in this study were comparable to published bioconcentration factors. The reconstructed soil concentrations were suitable for evaluating the risk of potentially toxic effects from individual active ingredients and from mixtures of active ingredients with the same mode of action. Most active ingredients do not pose a risk to earthworms and have no secondary poisoning potential to birds and mammals that feed on them. The bioaccumulation and secondary poisoning risk assessment method can be reliably used to calculate degradation and soil concentration curves at the time of sampling.

5. The composition and abundance of ground beetles in sugar beet fields is strongly influenced by numerous factors during the growing season. Insecticide use adversely

affects ground beetle populations, while reduced tillage, lower temperatures, and more rainfall result in higher ground beetle abundance and diversity. Cultivation of sugar beet in a four-year crop rotation ensures recovery of the ground beetle population.

6. Finally, a comprehensive list of 64 determined ground beetle species from maize, sugar beet, wheat, and soybean crops in Croatia represents a valuable finding that complements previous studies. The overall result contributes significantly to a better understanding of the baseline situation about ground beetle communities in intensive agricultural landscapes and serves as a good starting point for conservation programs that have become the standard in the European Union.

Based on the conclusions, the following **perspectives** emerge:

Given the data presented in the above studies, seed treatment (especially of sugar beet) with neonicotinoids should be reconsidered as a safe and effective crop protection measure. The use of neonicotinoids should be allowed but with strict usage controls and frequent ecotoxicological evaluation, to minimize risks to the environment and beneficial organisms. Monitoring of neonicotinoid residues (as well as other pesticides used) should become a necessary environmental protection measure under the new European policy. Data on pesticide residues in plants and soils, as well as in organisms that live in the soil (beneficial fauna), should become part of the strategy and procedure on the basis of which decisions are made to ban or authorize the use of existing (and new) agrochemicals in agriculture.

5. References

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Autobiography

Helena Virić Gašparić, M.Sc., was born in Zagreb on February 23, 1988. She majored as a chemical technician from Vladimir Prelog High School of Natural Sciences in Zagreb. In 2006, she enrolled in Plant protection undergraduate studies and then in graduate studies of Phytomedicine at the University of Zagreb Faculty of Agriculture. During her studies, she actively participated in scientific work under the supervision of Prof. Renata Bažok, Ph.D., resulting in the Dean's Award and publication of a scientific paper in 2011. In 2012, she received a semester scholarship to study at the University of Natural Resources and Applied Life Sciences in Vienna and a one-year scholarship from the Faculty of Agriculture Foundation for her academic success. She graduated with the highest honors in 2012. (*Summa cum laude*).

In 2013, she was employed at the Faculty of Agriculture as a Professional Associate in the International Relations Office, where she acquired knowledge in the areas of international cooperation, exchange programs and project administration. In 2014, she continued as Administrative Coordinator for the TEMPUS project, where she gained knowledge in management of project activities. In 2015, she was employed as an Expert Associate for the ESF project, where she handled administration and scientific research on the topic of her PhD thesis. In 2017, she was employed as an Assistant at the Department of Agricultural Zoology. She is involved in teaching 14 courses in Croatian and English, organizing professional internships and fieldwork for students, conducting experiments, and immediate supervision of undergraduate and graduate theses (8). She acquired her teaching competences by participating in four pedagogical training courses with a total duration of five months. She is registered in the Researchers Register of the Ministry of Science and Education under the registration number 360783.

Her scientific interests include the application of integrated and biological pest management, entomology, beneficial fauna, pesticide residues, ecotoxicology, and innovative methods in plant protection. In a total period over seven months, she received scientific training at: (i) Julius Kühn Institute (Berlin) in the analysis of pesticide residues using the LC-MS /MS method, SPE-QuEChERS (2016), (ii) University of Zagreb Faculty of Agriculture in the identification of species of the family Carabidae (ground beetles); (iii) Institute of Plant Protection (Sarajevo) in the work with VIPS models for pest infestation assessment and new technologies in plant disease diagnosis (2016/2017); (iv) Szent Istvan University (Poland) in the field of scientific publishing (2017); Department of Phytomedicine and Environmental Protection (Novi Sad) in the work with liquid chromatography-HPLC in the process of determining the presence of pesticides in samples (2022). She was additionally trained in entrepreneurial skills through three programs (SPOCK, BAIF, SF).

Collaboration with foreign and domestic scientists resulted in the publication of an author's book, a handbook, a book chapter, 22 scientific papers indexed in group a1, 14 papers indexed in group a2, and three papers from group a3. As an author or co-author, she participated in 28 international and eight national scientific and professional conferences with a total of 64 presentations, eight of which were invited lectures. She also published seven professional articles and nine popular science articles. She actively participated in the implementation of six scientific research projects (IPA, ESF, MP, HRZZ, ESF, BTM), four professional projects, one of which she coordinated (VIP, SuS, VEZfarm., KWS.), two teaching projects (TEMPUS, ERASMUS+) and one "Citizen Science" project (Krešo Krijesnica).

She is a member of the Croatian Plant Protection Society, Croatian Entomological Society, International Organization for Biological Pest Control (IOBC), Royal Entomology Society and Zasadi stablo ne budi panj association. She is one of the founders and leaders of the Entomology Group, an extracurricular activity in which she additionally mentors up to 10 students per year and has made significant contributions (Rector's Award 2015, work with gifted children in the "Panda" project and participation in the European Researchers' Night). She is a member of the Editorial Board of the scientific journal Entomologia Croatica. She is a member of the Faculty Council. She regularly and actively participates in all promotional activities of the Faculty.

She is the recipient of numerous awards and recognitions (Silver Medal for Innovation, AgroArca ('22); DIGIAward plaque ('21); Young Researcher Scientific Excellence Award ('21); UNESCO and L'Oreal Award "For Women in Science" and Recognition for Outstanding Scientific Work ('21), Faculty of Agriculture Foundation Annual Award ('17), Best Presentation Award at IOBC conference “Pesticides and Beneficial Organisms" ('16).