The sugar-beet weevil (Bothynoderes punctiventris Germar 1824., Col.: Curculionidae)

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Supervisor's Biography

Prof. Renata Bažok, PhD. was born in Bjelovar in 1965. She graduated in 1987 at the Faculty of Agriculture, University of Zagreb, Department of Plant Protection.
From 1988 to 1992 she was working in the "Radonja d.d.", Sisak, as head of biological laboratories in quality and market controlling and product's introduction based on *Bacillus thuringiensis*. From the same company she was sent to a ten-month specialization to the Department of Agricultural Zoology Faculty of Agriculture, University of Zagreb. From 1992 to 1993 she worked as advisor in agricultural pharmacies "Nikas d.o.o.", Rijeka.

At the Faculty of Agriculture in Zagreb she started working in 1993, as an assistant. Master thesis she defended in 1996, and the PhD degree she acquired in 2001 at the Faculty of Agriculture in Zagreb, mentored by prof. Jasminka Igrc Barčić PhD. As assistant professor she was elected in 2001 and as associate professor in 2007. In 2012 she became a full professor.

For undergraduate programs she teaches the modules of Phytomedicine, Protection of arable crops against pests and Zoocides, also she is a contributor to the modules of the biological factors of soil fertility and food safety. For graduate programs she teaches the module of Applied Entomology, Natural enemies and principles of biological control, Design and analysis of experiments in plant protection, Pests and diseases of medicinal and aromatic plants and Agricultural Consultancy in plant protection, also contributes to the modules Legislation in plant protection, Plant breeding for resistance to pests and abiotic stresses and protection of stored products from pests and diseases. She is teacher of the module Phytopharmacy with ecotoxicology and contributor to the modules Research of methods in agricultural entomology and Methods of fishery investigations of open water on postgraduate study and associate the module Fisheries ecotoxicology to postgraduate studies. Also, she is lecturer of the modules of Phytomedicine to study Mediterranean Agriculture, Split. Head of the graduate program Phytomedicine.
Under her mentorship six students defended their dissertations, and currently she is a mentor of four students. She was head of a master's thesis, and participated as a member of the committee in developing a doctorate and three master's degrees. So far she led 75 students in the development of diploma works, and was a member of the committee for the defense of a large number of graduate students.
She led four and was assistant to eight national research projects, and currently leads two international research projects. She was head of the four international scientific project assistant at three, and now leads an international research project. Leader in one, and was assistant to two TEMPUS projects.
She was at speciation in the United States three times, in 1994 with the Agricultural Research Service Center, 1997, at Purdue University as a scholarship winner Cochran and in 2012 At Purdue University as a recipient of a Fulbright scholarship.
She is the author or co-author of five books, 28 scientific papers cited in A1 databases, 56 papers referred to in A2 databases and 12 papers in conference proceedings with international review.
She is president of the Croatian Society of Plant Protection, a board member of the Croatian Entomology Society and a member of the Croatian Society of Agronomy. She is an active member of international organizations ESA, international IWGO working groups Working Group for *Ostrinia* and other Maize Pests and international scientific organizations and IOBC B.EN.A.

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To my parents, Zlatica and Krešo,
and sister Kristina

Summary

The sugar beet weevil (SBW) (*Bothynoderes punctiventris*, Germar 1824, Coleoptera: Curculionidae) is a very important pest of sugar beet. The life cycle and ecology of this pest has been studied in neighboring countries 20 and more years ago, while the data on SBW life cycle and ecology in the conditions of Croatia don't exist. Due to the specific morphological structure of SBW, their large feeding capacity and the small leaf area of plants at the time of insecticide application, insecticides often give very poor results and require repeated treatment, which is not in accordance with the principles of integrated pest management (IPM) nor with the rational use of pesticides in modern agriculture. Therefore, it is necessary to consider non-pesticide plant protection measures and other available methods that are compatible with all agricultural practices. Knowledge on the life table parameters of pests allows for the successful implementation of control in accordance with the principles of IPM. The area-wide mass trapping of SBW using aggregation pheromones in the previous year's sugar beet fields within a particular larger area might provide the possibility of reducing the pest population and reduces the need to apply insecticides what is in accordance with IPM. The entomopathogenic nematode (EPN) *Heterorhabditis bacteriophora* Poinar may have significant potential to reduce SBW population and shall be integrated with other measures into the strategy for SBW control.

During the four years (2012-2015) life table parameters and the population characteristics of SBW have been investigated. Area–wide (AW) mass trapping was implemented within a total area of 6 km^2 (in 2014 additional 8.8 km^2). The pheromone traps (15/ha) were installed on all previous years sugar beet fields in AW at the beginning of SBW emergence. The efficacy of three different doses of EPNs (3, 5 and 7 million of nematodes/10 m^2) on beet weevil larvae in two year field trials has been established.

The degree day accumulation (DDA) for SBW emergence can be calculated based on the soil temperature at 10 cm depth by the use the temperature of 5°C as the thermal threshold. The first emergence started when DDA reaches 20°C (first two decades of March). However, the emergence depends on the existing snow layer as well as on the availability of food. Weevils completed emergence when DDA reaches 428°C what usually happen in the first week of May. The largest proportions of specimens (which emerge from overwintering) were established in 14^{th} and 15^{th} week of the year (between 95 and 102 Julian day - JD). Males of SBW emerge first and dominate in the adult population up to 15^{th} week of the year when an equally sex ratio is present. Afterward adult population is dominated by females. SBW development in eastern Croatia is very similar with those in the neighboring countries (Serbia and Hungary). Overwintering adults are present in the fields up to the beginning of July. Newly developed adults emerge from the soil in July. Although the development stage of egg takes 10-15 days, due to expanded time of weevil emergence, in prevailing conditions, eggs were found on average in 102 days (between 112^{th} and 214^{th} JD), larvae development established up to 143 days (between 122^{th} and 265^{th} JD) and pupae development up to 102 days (between 143^{th} and 245^{th} JD). It is established that population growth positively correlate with air (r=0.9409**) and soil temperature (r= 0.9307**) during the vegetation period and negatively correlate with the amount of precipitation in vegetation period (r= -7971**) as well as with the amount of precipitation in May (r= -0.7794**). Population growth rate depends on the ratio between new and old sugar beet fields in marked area (r= 0.7813**). With increasing the share of newly sown sugar beet field, the population growth increases. Overwintering success depends on the air and soil temperature prevailed in the period of overwintering and doesn't depend on the amount of precipitation. In the conditions of very high population, baited traps were useful in terms of lowering SBW population. Mass trapping of SBW on the "old" sugar beet fields in marked area significantly reduced the number of insecticide

applications and the amount of used insecticides with keeping the damage and weevil infestation on the same or even lower level comparing to the fields outside AW. EPN, *H. bacteriophora* has a potential in suppressing the SBW. EPNs shows dose response in the conditions of moderate intensity attack. In such conditions, the highest dose resulted with the efficacy of 92.46 %. AW mass trapping shall be combined with other non-pesticide measures for control SBW. The EPN might serve as good tool to be implemented into AW programms.
The research results significantly contribute to the ability of sugar beet producers to introduce mandatory principles of integrated pest management in their production and enable environmentally acceptable control of SBW which almost became a limiting factor in the production of sugar beet.

Key words: aggregation pheromones, area wide, entomopathogenic nematodes, mass trapping, sugar beet, sugar beet weevil

Sažetak

Repina pipa (*Bothynoderes punctiventris* Germar 1824, Coleoptera: Curculionidae) predstavlja najznačajnijeg štetnika šećerne repe na području istočne Hrvatske. Životni ciklus i ekologija štetnika istražena je u susjednim zemljama prije više od 20 godina, a isti podatci u uvjetima koji prevladavaju na području Hrvatske ne postoje. Zbog specifične morfološke građe i velikog kapaciteta ishrane pipe te male lisne površine biljaka u vrijeme primjene insekticida, zadovoljavajući učinak insekticida često izostaje te su potrebni dodatni tretmani. Ponavljanje insekticidnih tretmana nije u skladu s načelima integrirane zaštite bilja (IZB) niti s racionalnom upotrebom pesticida u suvremenoj poljoprivredi. Stoga je neophodno istražiti i primijeniti ne-pesticide mjere u zaštiti bilja kao i sve druge raspoložive metode koje su kompatibilne dobroj poljoprivrednoj praksi. Neke od tih raspoloživih metoda su primjena entomopatogenih nematoda ali i masovni ulov agregacijskim feromonima. Iz povijesti i današnje prakse postoje brojni primjeri masovnog suzbijanja štetnika na velikim površinama engl. „Area-Wide Pest Management“ (AW). Masovno suzbijanje na velikim površinama predstavlja sustavnu organiziranu kontrolu ukupne populacije štetnika na širem području. Za razliku od pojedinačnih mjera suzbijanja koje provodimo s kratkoročnim ciljem trenutačnog smanjenja štete na određenoj površini, dugoročni je cilj ove metode je smanjiti napad štetnika u određenom području ispod one brojnosti koja može izazvati štete. Ova metoda u skladu je s načelima IZB jer ima za cilj populaciju štetnika svesti ispod praga odluke, a istovremeno se suzbijanje provodi nekom od ekološki prihvatljivih metoda. Suzbijanje masovnim ulovom koristi se na pojedinačnim poljima, ali i u programima suzbijanja na velikim površinama. Ono se zasniva se na korištenju atraktanta kojim se kukci privlače do klopke u koju se hvataju u velikom broju. Mamci s agregacijskim feromonima uspješno privlače pipe koje ostaju zadržane u mamcima. Repinu pipu prema brojnim autorima treba zadržati na mjestu prezimljenja i ne dozvoliti prijelaz na nova repišta.

Provedeno istraživanje polazi od hipoteza: (1) parametri životnog ciklusa repine pipe u uvjetima istočne Hrvatske nedovoljno su poznati te su pod utjecajem agro-ekoloških uvjeta, a njihovo poznavanje omogućava uspješnu provedbu zaštite bilja u skladu s načelima IZB. (2) masovni ulov repine pipe, korištenjem feromonskih trapova na prošlogodišnjim repištima (engl. „Area-Wide Pest Management“(AW) omogućava smanjenje populacije štetnika i smanjuje potrebu za primjenom insekticida, što je u skladu s IZB, (3) uporaba entomopatogenih nematoda (EPN) smanjuje populaciju repine pipe te se može integrirati s drugim mjerama u strategiji suzbijanja ovog štetnika.

Da bi se dokazale postavljene hipoteze postavljeni su ciljevi istraživanja: (1) utvrditi parametre životnog ciklusa repine pipe, (2) implementirati masovni ulov pipa putem feromonskih trapova na prošlogodišnjim repištima i utvrditi njegovu učinkovitost, (3) utvrditi učinkovitost EPN u smanjenju populacije repine pipe.

Istraživanje je provedeno tijekom četiri godine (2012.-2015.) na području istočne Slavonije (Tovarnik). Podatci o vremenskim uvjetima (srednja dnevna temperatura zraka i tla na dubini 10 cm i dnevna količina oborina) prikupljeni su s meteoroloških postaja Gradište i Vukovar. Dinamika izlaska odraslih s prezimljenja utvrđena je pomoću feromona agregacije postavljenih na mjestima prezimljenja. Suma efektivnih temperatura (SET) za izlazak repine pipe je izračunata temeljem termalnog praga od 5 °C na dubini od 10 cm. Temeljem utvrđene dinamike izlaska utvrđena je SET kod koje se javljaju prva imaga. Pregledima tla i vizualnim pregledima biljaka utvrđena je pojava pojedinih razvojnih stadija repine pipe kao i promijene seksualnog indeksa tijekom vegetacije. Temeljem utvrđene zaraze polja prije i nakon prezimljenja utvrđene su promjene u visini populacije te je utvrđen utjecaj klimatskih čimbenika na fluktuaciju populacije. Kreiran je fenogram razvoja repine pipe u uvjetima istočne Hrvatske. Masovni ulov pipe feromonima agregacije proveden je na površini od 6 km^2 u 2012., 2013. i 2015. godini a u 2014. godini je površina na kojoj je proveden masovni ulov

iznosila 14,8km^2. Korišteni su feromoni agregacije u količini od 15 klopki/ha, a postavljani su na sva polja u području masovnog ulova koja su u prethodnoj godini bila zasijana šećernom repom. Sva novo zasijana polja šećerne repe redovito su pregledavana jednom tjedno. Standardnim metodama pregleda biljaka utvrđena je zaraza/m^2 a pregledane biljke su obzirom na oštećenja klasificirane po skali 0-5. Utvrđena je zaraza po Towsend-Heubergeru. Na poljima u području masovnog ulova detaljno je vođena evidencija broju insekticidnih tretmana i o količini i vrsti primijenjenog insekticida. Radi usporedbe sa zarazom, štetama i primjenom insekticida na poljima izvan područja masovnog ulova na isti način je jednom tjedno utvrđivana visina zaraze i štete te je vođena evidencija o primjeni insekticida. Uspjeh masovnog suzbijanja utvrđen je (a) usporedbom ulova pipe u feromonskim mamcima i procijenjene visine populacije na području masovnog ulova; (b) usporedbom visine zaraze i visine šteta utvrđenih na poljima šećerne repe u području masovnog suzbijanja i izvan tog područja; (c) usporedbom broja tretiranja i utroška djelatne tvari insekticida za suzbijanje repine pipe na poljima u području području masovnog suzbijanja i izvan tog područja; Učinkovitost primjene entomopatogene nematode *Heterorhabditis bacteriophora* Poinar za suzbijanje repine pipe utvrđena je primjenom pripravka Nematop (e-Neema) u tri različite doze (3, 5 i 7 milijuna nematoda / 10 m^2) na polja šećerne repe u vrijeme pojave ličinki repine pipe u dvogodišnjim poljskim pokusima.

Vremenski uvjeti na području istočne Hrvatske značajno su varirali u godinama istraživanja (2012. - 2015.). Količina oborina u 2014. i 2015. bila je u skladu s 40-godišnjim prosjekom. Tijekom 2013. godine količina oborina bila je viša za 30 % od prosjeka. Za razliku od ostalih godina istraživanja, vegetacijsko razdoblje u 2012. godini je bilo obilježeno ekstremno visokim temperaturama, koje nisu povoljne za razvoj i razmnožavanje repine pipe.

Rezultati istraživanja utvrdili su detaljne informacije o životnom ciklusu repine pipe u uvjetima istočne Hrvatske, te glavne čimbenike koji utječu na rast populacije. Biologija repine pipe u uvjetima istočne Hrvatske vrlo je slična onoj u susjednim zemljama (Srbija i Mađarska). Prva pojava štetnika je zabilježena kada SET dosegne 20 °C (prve dvije dekade ožujka). Međutim, izlazak štetnika ovisi i o sloju snijega i o dostupnosti hrane. Repina pipa završava izlazak iz tla kada SET dosegne 428 °C, što je uobičajeno za prvi tjedan svibnja. Najveći udio populacije (koji je izlazio nakon prezimljenja) pojavio se tijekom 14. i 15. tjedna u godini (između 95. i 102. dana u godini). Mužjaci repine pipe prvi izlaze iz tla i dominiraju u populaciji odraslih do 15. tjedna u godini, kada se seksualni indeks izjednačava. Nakon toga u populaciji odraslih prevladavaju ženke. Temeljem podataka o pojavi pojedinih razvojnih stadija kreiran je fenogram razvoja repine pipe u uvjetima istočne Hrvatske. Potpuno razvijene jedinke repine pipe nakon prezimljenja izlaze na površinu i prisutne su na poljima šećerne repe do početka srpnja. Nova generacija repine pipe izlazila je iz tla u srpnju. Iako razvojni stadij jaja traje 10-15 dana, zbog produženog vremena izlaska pipe iz tla, jaja se mogu pronaći tijekom 102 dana (između 112. i 214. dana u godini). Utvrđeno je da se ličinke razvijaju u razdoblju do 143 dana (između 122. i 265. dana u godini), a kukuljice u razdoblju od 102 dana (između 143. i 245. dana u godini).

Uspjeh prezimljenja štetnika ovisi o temperaturi zraka i tla koje prevladavaju u zimskom periodu. Nije utvrđena korelacija između uspjeha prezimljenja štetnika i količine padalina.

Rast populacije štetnika je bio najmanji u 2014. godini, a najviši u 2012. Utvrđena je pozitivna korelacija rasta populacije s temperaturom zraka u vegetacijskom razdoblju (r = 0.9409 **) i tla (r = 0,9307 **) te negativna korelacija s količinom padalina u vegetacijskom razdoblju (r = -0.7971 **), kao i s količinom padalina u svibnju (r = -0,7794 **). Također, rast populacije repine pipe ovisi o zastupljenosti novo zasijanih polja i prošlogodišnjih polja šećerne repe (r = 0,7813 **). Stoga, sjetva novih površina šećerne repe na širem području treba biti pomno planirana kako bi se smanjila mogućnost rasta populacije repine pipe.

Populacija repine pipe na području masovnog ulova je bila visoka u sve četiri godine istraživanja. Utvrđena brojnost proljetne populacije štetnika na prošlogodišnjim repištima bila je između 28.000 i 78.000 pipa /ha, što je 10 – 20 puta veća brojnost od ekonomskog praga koji iznosi 1.000 do 3.000 pipa/ha. Masovnim ulovom agregacijskim feromonima uhvaćeno je od 0,7 do 11,59 % proljetne populacije. Iako je postignuto smanjenje populacije u pojedinim godinama bilo manje od 1 %, masovnim ulovom ostvareno je smanjenje brojnosti populacije repine pipe u 2014. i 2015. godini u odnosu na 2012. i 2013. godinu. Na području masovnog ulova broj tretmana i količina insekticida po jedinici površine značajno su smanjeni u odnosu na polja izvan područja masovnog ulova. Broj tretiranja insekticidima u području masovnog ulova bio je u skladu s načelima IZB dok je broj tretmana i količina primijenjenih insekticida izvan područja masovnog ulova znatno prelazila dozvoljene količine. Jedan tretman insekticidima primijenjen na rubovima polja i jedan po cijeloj površini unutar područja masovnog ulova dovoljan je za očuvanje šećerne repe te održavanje brojnosti štetnika ispod ekonomskog praga.
Entomopatogena nematoda *H. bacteriophora* ima potencijal u suzbijanju repine pipe. Ostvareni rezultati ukazuju na visoku učinkovitost sve tri doze EPN u uvjetima vrlo niskog intenziteta napada štetnika. U uvjetima umjerenog intenziteta napada EPN rezultira različitom učinkovitosti. Najniža doza nije bila učinkovita dok je najviša doza rezultirala sa 92,46 % učinkovitosti. Doza od 5 milijuna nematoda / 10 m^2 je preporučena od strane proizvođača u suzbijanju drugih pipa, no navedena doza ne daje zadovoljavajuću učinkovitost u uvjetima umjerene (ili čak i niske) brojnosti štetnika (42,86 %). Kao biološka mjera suzbijanja štetnika, entomopatogene nematode bi trebale biti usmjerene na suzbijanje odrasle populacije. Nužno je mjeru provesti u sklopu dobro razvijene strategije u kojoj trebaju biti obuhvaćene sve raspoložive mjere, uključujući masovni ulov na velikim površinama.
Nova saznanja o biologiji repine pipe omogućava razvoj novih strategija zaštite šećerne repe od repine pipe. Masovnim ulovom pomoću agregacijskih feromona na prošlogodišnjim repištima moguće je smanjiti visinu populacije repine pipe te smanjiti broj tretiranja insekticidima. Takav pristup omogučava poljoprivrednim proizvođačima zaštitu usjeva u skladu s načelima IZB s ne više od dva insekticidna tretmana unutar sezone. Upotreba entomopatogenih nematoda (*H. bacteriophora*) ima značajan potencijal u smanjenju brojnosti štetnika i može poslužiti kao dobar alat u provedbi AW programa.
Postignuti rezultati provedenog istraživanja značajno doprinose povećanju kapaciteta proizvođača šećerne repe za uvođenjem obaveznih načela integrirane zaštite bilja u proizvodnju i omogućavaju ekološki prihvatljivu zaštitu šećerne repe od repine pipe koja je postala limitirajući čimbenik u proizvodnji šećerne repe.

Ključne riječi: šećerna repa, repina pipa, feromoni agregacije, masovni ulov, entomopatogene nematode

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1. INTRODUCTION

Sugar beet cultivation in Croatia has increased from 21,000 to 27,000 ha in the past five years (Statistical Year Book of the Republic of Croatia, 2012). In the eastern part of Croatia, sugar beet has been sown since 1905. The first mass attack of sugar beet weevil (*Bothynoderes punctiventris* Germar 1824) was recorded in Osijek, Vukovar and Vinkovci in 1922 (Kovačević, 1929), and a high occurrence of pests was recorded from 1925 to 1931. Of the total sown area of sugar beets surrounding Vinkovci (Tovarnik) in 1964, 44% was damaged by the sugar-beet weevil. The population of the pest in the eastern part of Croatia was below the economic threshold until 2008 (Bažok, 2010).

The life cycle and ecology of the sugar-beet weevil have been studied by many authors in Romania and Bulgaria, whereas the life cycle and life table parameters of this pest in Croatia have not been explored. This pest produces one generation per year. At later stages of germination and the emergence of sugar beet and at the 2-4 leaf stage (Sivčev et al., 2006), beet weevils at an abundance of 0.1-0.3 weevils/m^2 (i.e., 1,000-3,000 weevils/ha) can cause economic damage and completely destroy crops, requiring re-sowing (Maceljski, 2002). Bažok et al. (2012) suggested that the occurrence of pests in eastern Croatia is the result of a warmer climate during the last 10 years, the absence of the secondary effects of insecticides used to control pests in sugar beets and the intensive cultivation of sugar beets.

Pest control is mainly based on the use of insecticides (Sekulić et al., 1997). However, chlorinated hydrocarbons (Čamprag, 1984), organic phosphorus insecticides (Radin, 1983) and pyrethroids in combination with organophosphorus (OP) insecticides (Bažok et al., 2012) have been used with varying degrees of success. Due to the specific morphological structure of weevils, their large feeding capacity and the small leaf area of plants at the time of insecticide application, insecticides often give very poor results and require repeat treatments (Bažok et al., 2012), which is not in accordance with the principles of integrated pest management (IPM) nor with the rational use of pesticides in modern agriculture. Therefore, it is necessary to consider non-pesticide plant protection measures and other available methods that are compatible with all agricultural practices.

Microbial insecticides based on entomopathogenic fungi have been shown to reduce the number of larvae and pupae by 85% (Bogdanov, 1961) and contribute to reduction of the population by 74% (Beratlief, 1979). The use of nematodes belonging to the genera *Steinernema* and *Heterorhabditis* (together with the symbiotic bacteria genus *Xenorhabdus* and genus *Photorhabdus*) for the suppression of weevils (Hassan, 2010; Susurluk and Ehlers, 2008) is currently under investigation. To date, the commercial use of products based on the aforementioned organisms has not been reported.

"Area-wide pest management" (AW) is the systematic organised control of all pest populations over a wide area (Hendrichs et al., 2007). Unlike individual control measures that result in short-term reductions and damage prevention in a particular field, AW has the long-term goal of decreasing the pests in a particular area below the threshold population level that can cause damage. This method is in accordance with the principles of IPM because it aims to reduce pest populations below the threshold while control is achieved via an environmentally acceptable method.

Mass trapping is used on individual fields but also in AW eradication programs. Mass trapping is based on the use of an attractant that draws insects to the traps in which they are caught in large numbers.

Scientists from Hungary (Tóth et al., 2002) developed mass trapping of the sugar-beet weevil using baits with aggregation pheromones. Weevils attracted by the pheromones walk up to and enter the traps, which are plastic boxes from which they cannot escape. Tomašev et al. (2007) propose that a density of 30 pheromone traps/ha shows good potential as a control method especially at population densities of 30 000 insect/ha or below, and may be capable of decreasing the population pressure of immigrating beetles to sites where sugar-beet is planted in the spring (Tomašev et al., 2007). Most authors (Čamprag, 1984; Sekulić et al., 1997, Maceljski, 2002) agree that sugar-beet weevils should be confined to the location where they overwinter and should not be allowed to enter new areas.

2. OBJECTIVE AND HYPOTHESIS OF RESEARCH

The proposed investigation is based on the following hypothesis:

There is a lack of data on the sugar beet weevil life cycle and life table parameters in the area of east Croatia. Life table parameters are under the impact of the agro-ecological conditions that prevail in that area. Knowledge of the life table parameters and pest life cycle allows for the successful implementation of control measures in accordance with the principles of IPM.

Area-wide (AW) mass trapping of beet weevils using aggregation pheromones in the previous year's sugar beet fields provides the possibility of reducing the pest population and reduces the need to apply insecticides. The entomopathogenic nematode (*Heterorhabditis bacteriophora* Poinar 1976) has significant potential to reduce the sugar beet weevil population and shall be integrated with other measures into the strategy for sugar beet weevil control.

The following tasks will be completed within the scope of this proposal:

1. To establish life table parameters of sugar beet weevil in east Croatia (including dynamic of the adult emergence, timing of the occurrence of different developmental stages of the pests, changes of sexual index during the vegetation, population fluctuation i.e. overwintering success and population growth during the vegetation and factors that influence population vegetation) and to compose a sugar-beet weevil phenology model in east Croatia;
2. To implement AW mass trapping by pheromone traps at all of the previous year's sugar beet fields within an area of at least 6 km^2 and to determine the success of area wide mass trapping (based on the: estimated population level and the number of captured weevils, the number of applications and the amount of the active ingredient of insecticides used to control sugar-beet weevils in fields outside and inside of the mass trapping area).
3. To establish the efficacy of EPN *H. bacteriophora* on sugar beet weevil larvae and estimate the possibility for its use for pest suppression.

3. LITERATURE REVIEW

3.1. SUGAR BEET (*Beta vulgaris* L. subsp. *vulgaris* var. altissima Döll.)

The Centre of origin of sugar beet is believed to be the area around the Mediterranean and Atlantic Sea, for around 2000 years. Historically, sugar beet have been used for both livestock and human consumption, likely bearing resemblance to the chard of today, cultivated in Assyrian, Greek and Roman gardens (Ford-Lloyd et al. 1991). Cultivated sugar beet is likely to have originated from wild maritime beet (*Beta vulgaris* L. subsp. *maritima* (L.) Arcang.) through breeding selection (Cooke and Scott, 1993). The sugar beet, as a biennial herbaceous dicotyledon, belongs to the order Caryophyllales, family Cheniopodiaceae and genus *Beta*, which is divided into 4 sections. With Corollinae, Patellares, Nanae section, the most familiar section is Vulgares with its species *B. vulgaris* L., *B. maritima* L. (sugar beets wild progenitor), *Beta macrocarpa* Guss., *Beta vulgaris* L. subspecies *patula (*Aiton*)* Ford-Lloyd and J. T. Williams, *Beta atriplicifolia* Rouy, and *Beta perennis* Freyn. Economically important species in this family include sugar beet, fodder beet/mangolds, red table beet, Swiss chard/leaf beet (all *B. vulgaris*), and spinach (*Spinacia oleracea* L.).

3.1.1. Economic importance of the sugar beet

Worldwide, 4.76 million hectares are sown with sugar beet every year. The largest parts of these areas are located in the Russian Federation, followed by the Ukraine, the USA, Germany, France, Turkey and Poland. The average root yield amounts to 49.73 t/ha, while the total sugar beet production worldwide is 236 million tonnes. The world's largest producer is in France with 32 million tonnes or 13.6% of the total world's production (Pospišil, 2013). In Croatia, sugar beet cultivation has increased from 21,000 to 27,000 ha in the past years (Statistical Year Book of the Republic of Croatia, 2012), from 20,245 ha in 2013, and to over 22,000 ha in 2014 (Statistical Year Book of the Republic of Croatia, 2013, 2014). In the eastern part of Croatia, sugar beet has been sown since 1905.

Sugar beet is grown for its thickened roots, which can comprise 14-20% sugar in the fresh state. This crop can be used as fodder or energy plants for ethanol and biogas production. Sugar (sucrose), as the main processing product, is a rich source of energy (170 kJ/100 g) and belongs to the category of easily-digestible food. A number of by-products are formed during the processing, such as leaves in the form of neck or beet pulp (also called cossettes), molasses and saturation sludge or raw juice. Out of a 50 t yield, following processing, an average of 6.25 tons of sugar, 30 tons of leaves with neck, 2.7 t of dried beet pulp, 2.1 tons of molasses and 2.5 t raw juices can be gained (Pospišil, 2013). The leaves and neck of sugar beet account for 35-65% of the total yield. Cossettes can be used in animal nutrition. Molasses is thick, viscous syrup created during the crystallisation of sugar, and contains 42-46% sugar. The residue which remains when juice is diffused and saturated (using quicklime, lime and CO_2) is called the saturation sludge, and if the amount of water declines below 30%, the saturation sludge is suitable for producing a calcium carbonate fertiliser (chalk).

The chemical composition of sugar beet roots depends on the varieties and hybrids, crop management, soil conditions, climate and other factors. A typical sugar beet root consists of 75.9% water, 2.6% non-sugars, 18.0% sugar and 5.5% pulp. In the sugar fraction, 83.1% is recovered as crystalline sucrose and 12.5% is recovered as molasses (Bichsel, 1987). The most common form of sugar found is sucrose; the sugar is not evenly distributed through the roots of beets, as the sugar content is higher in the middle part of the root than in other parts (head and root). Beside the root, beet contains sucrose and inverted sugar (a mixture of glucose and fructose). In healthy beet, inverted sugar is found in small amounts (about 0.1%). However, if the root is longer and stored in prisms, primarily within frozen and rotten roots, the inverted sugar content rises rapidly, and becomes a very harmful ingredient in the technological processing. The yield of sugar in the processing is greatly reduced by the share of minerals during growth, and their contents are affected by climate, soil, fertilisation and other factors. If the content of mineral substances increases, the utilisation of sugar decreases.

3.1.2. Biology and physiology of the sugar beet plant

The mature beet has an elongated pear-shaped body composed morphologically of three regions: the crown, the neck, and the root. The crown is the broadened, somewhat cone-shaped apex. It bears a tuft of large succulent leaves and leaf bases (Artschwager, 1926). The neck presents a smooth narrow zone which is the broadest part of the beet and which constitutes the ontogenetically thickened hypocotyl. The root region is cone shaped and terminates in a slender taproot. This region is also represented by the main bulk of the beet tissue. It is flattened on two sides, and is often more or less markedly grooved. There are two vertically persistent depressions arranged downwards, which make a shallow spiral, and include lateral rootlets that are indistinctly arranged in two double rows. The beet root area is covered by a thin layer of yellowish-white cork. Lateral roots are filamentous and originate from two ports: the xylem plates or the more peripheral growth rings. On average, one beet plant forms one taproot. Sometimes, branching of the main root occurs, which forms a number of thick stubby roots. From the two-arch xylem plate (or from the more peripheral rings of growth) the filiform lateral roots appear. On the crown, leaves are staggered in a close spiral. The cotyledons are arranged in opposite pairs. The leaf lamina is triangular and elongated, with a rounded tip and undulate margins. Unlike most cultivated plants, the beet shows a striking lack of uniformity in foliage characteristics. The most diverse types may be found growing side by side; plants with erect or flat foliage, short or long petioles, with triangular or oblong lamina, and with a straight or wavy margin and smooth or crinkly surfaces.

In the first growing season, the sugar beet plant is described as having glabrous leaves that are ovate to cordate in shape and dark green in colour; the leaves form a rosette from an underground stem. A white, fleshy taproot develops, which is prominently swollen at the junction of the stem (Duke, 1983). In the second growing season, a flowering stalk elongates from the root. This angular seed stalk forms an inflorescence and grows to approximately 1.2-1.8 metres in height. At the base of the stem, a large number of small petiolate leaves develop. Further up the stem, there are fewer petiolate leaves and sessile leaves are seen to develop. At the leaf axils, secondary shoots develop, forming a series of indeterminate racemes (Forster et al., 1997). These flowers are small, sessile and occur singly or in clusters. Sugar beets produce a flower that consists of a tricarpellate pistil surrounded by five stamens and a perianth of five narrow sepals. Petals are absent and each flower is subtended by a slender green bract (Smith, 1987). The ovary forms a fruit which is embedded in the base of the perianth of the flower. Each fruit contains a single seed whose shape varies from round to kidney-shaped. The ovaries are enclosed by the common receptacle of the flower cluster (Duke, 1983). When a flower occurs singly, a monogerm seed is formed. The multigerm beet seed is formed by the aggregation of two or more flowers (Cooke and Scott, 1993).

The vegetative and first phase is determined over a number of development stages (Figure 1). Germination begins with growing roots and takes place at the expense of reserve substances in the seed (endosperm). The process of germination continues intensively, as the division of the meristem tissue, and the formation of the hypocotyl. The root grows at depth and the hypocotyl with cotyledons is found at the soil surface. An optimum germination temperature is 25°C (minimum 4°C and maximum 30°C). The higher the temperature, the faster the germination process, and vice versa. During vegetative growth (30-40 days after emergence), root growth increases. The diameter of the root increases by the end of the growing season by 50 times, and increases in weight by as much as 400 times. The amount of sugar increases almost linearly from 0.5 g at the beginning of June to 150 g or more in late October. Thus, the processes of plant development and sugar accumulation run in parallel. The reproductive phase as a development stage has five different phonological periods: rosette phase (the appearance of the first leaf to the appearance of the first flower stalks); increased flower stalks (the appearance of the first flower stalks to the appearance of the first inflorescence); creation of the bourgeon (from the appearance of the first inflorescence to the opening of the first flower); flourishing (starts with the opening of the first flower and lasts until the end of flowering); and the formation of fruit (begins with the first fruit and lasts until the harvest). These root crops are planted in the spring and harvested in the autumn of the same year. The sugar beet plant develops a large succulent taproot in the first year and a seed stalk in the second year. For seed production, however, an overwintering period of cold temperatures from 4-7°C is required for the root to bolt in the next growing season and for the reproductive stage to be initiated (Smith, 1987).

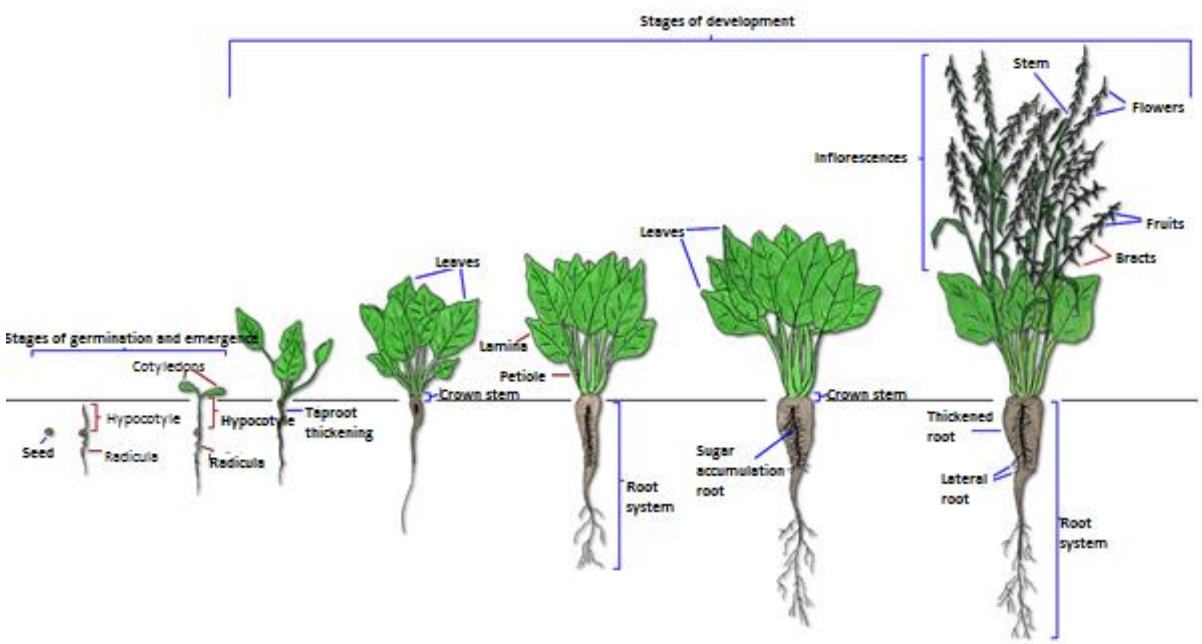


Figure 1. Sugar beet development (source: Remolacha, 2014)

3.1.3. Environmental requirements for sugar beet production

Sugar beet is a widespread crop and grown in regions ranging from subtropical areas to the northern regions of Scandinavia. However, the most favourable area for the cultivation of sugar beet is a temperate zone. In general, we can say the weaker the growing practice, the greater the need for water in sugar beet will be. The vegetation period lasts for 160-200 days.

a) Climate

The total temperature required is 2200-3200°C. During growth, beet requires 500-600 mm (l/m^2) of rainfall. Sugar beet passes through a critical period of water deficit in June, July and August, when the greatest need for water appears (60-80 mm). According to the dynamics of growth and climatic requirements, sugar beet has a development cycle, which is divided into three equivalent periods of 60 days (Stanaćev, 1979). Each sub period is determined by sowing term, variety and environmental conditions of the growing areas. The first period lasts from emergence until the crop is closing rows (in our Croatian conditions, the first part of June), and the crop requires an average daily temperature of 10.7°C, with a total temperature of 650°C. The second period runs from closing lines to early August; during this period, the crop requirements are an average daily temperature of 18.8°C or a total temperature of 1150°C. The third period lasts from the beginning of August to the sugar beet harvest and is essential for the accumulation of sugars in the roots, with the need for a total temperature of 1000°C and an average daily temperature of 16.5°C. During sugar beet germination, the required soil temperature is 6-8°C, with a minimum of 4-5°C.

In our areas, sugar beets are usually sown in the period from 10th March until 10th April. Sowing is done using a 6-8°C heated layer (2-3 cm deep). Sugar beet belongs to plants with poor utilisation of sunlight, as only 2% is used; therefore, beets are sensitive to the lack of sunlight, and react by lowering their yield and quality (Stanaćev, 1979). In times of intensive formation, sugar beets prefer sunny and cloudy weather. During periods of diffuse light, carbohydrates formed in the leaves are quickly disposed of in the root. Sugar beet crop is a long day plant and beet needs 700 hours of solar insolation in the period of maturation. Root yield increases proportionately with the amount of rainfall and the number of hours of sunshine in May, June and July. For the continuous production of sugar beet, a total rainfall of 600 mm is sufficient. The ideal distribution of precipitation during the growing season (according to Wohltmann, 1904) is as follows: 240 mm during November and March, 40 mm in April, 50 mm in May, 80 mm in July, 65 mm in August, 35 mm in September and 40 mm in October. In our conditions, using a multi-year average, deficient rainfall occurs most often in July or August (Pospišil, 2013). From mid-July to mid-August, the root is in the process of weight gain, which is when sugar beet has the greatest need for water. If more rain falls at the time of maturation and sugar beet harvest, the sugar content in the root decreases. Low humidity with high temperatures and lower soil moisture can cause reduced turgor in the leaves, meaning that wilting occurs prematurely. Turgor increases overnight, however, so leaves appear normal in the morning (Stanaćev, 1979). Transpiration and sugar content in the roots reduce if humidity increases to above 75%. High air humidity also stimulates the development of leaf diseases. Due to the construction of thickened roots in the soil, sugar beets cannot tolerate any obstacles and seek generous fertilisation.

b) Soil

Beet requires deep, fertile and loose soils. For beet crops, in the upper layers of the soil it is necessary to have a stable, crumbly structure and neutral to slightly alkaline reaction (pH 6.8-7.2). For sugar beet production, chernozem, hydromorphic and alluvial loam soils are favourable. Less suitable soils for beet cropping are eutric brown soil, hydromorphic and vertic loessivised soil.

3.1.4. Soil management of sugar beet production

Sugar beet is a demanding industrial crop. It should be grown in rotation, on the same field, and should not be re-grown within five years. Narrow crop rotation leads to the accumulation of pathogens, nematodes and pests and the unilateral removal of nutrients. Each crop that leaves the field early is a pre-crop for beet. The best pre-crops for sugar beet are cereals (wheat, oats, and barley), potatoes and one-year legumes, while the worst pre-crops are corn, alfalfa, oilseed radish and canola. According to Pospišil (2013), the recommended crop rotation is as follows: sugar beet, wheat (soybeans or barley), corn, sunflower and wheat. Taking into account that sugar beet has the highest demand for the depth, time of performance and quality of primary tillage, pre-crop and soil properties determine the method of tillage. The basic tillage mode after cereal crops is as follows: shallow stubble treatment (immediately after harvest, farrowing at a depth of 12-15 cm), medium deep ploughing (a month after farrowing, with the introduction of mineral fertilisers or manure, to a depth of 20-25 cm) and deep autumn ploughing (in October, at a depth of 35-40 cm). Before sowing, beet requires a finely prepared shallow seeding layer; operations are needed to provide a 2-3 cm loose soil layer and a compacted soil layer 0.8-1.0 cm below this.

According to the empirical standard, the needs of sugar beet for phosphorus and potassium may be fulfilled with the amount of 80-130 kg/ha P_2O_5 and 150-250 kg/ha of K_2O (Pospišil, 2013) in most of the soils tested. Phosphorus and potassium fertilisers are fully incorporated in the basic tillage. It is thought that 1/3 of the nitrogen should be introduced in the fall as a fertiliser in sandy soils, and half of the nitrogen should be introduced in clayey soils. In fertilisation, the ratio of potassium and nitrogen fertilisers should be considered. A wider ratio of nitrogen versus potassium allows increased sugar content. The optimum ratio of N:P:K in sugar beet growth should be 1:0.8:1.6. Nitrogen fertilisation in the fall should not exceed 60 kg/ha, and 2/3 of the total nitrogen fertilisers should be incorporated in the form of ammonium or amide. The rest of the nitrogen (as calcium ammonium nitrate (KAN) or ammonium nitrate (AN)) should be incorporated in the spring (pre-sowing or in the form of a top dressing), no later than the 2-4 leaf development stage. Of the trace elements required for sugar beet, the most important are boron and manganese. If there is a deficit, trace elements should be applied with complex fertiliser-containing trace elements. The recommended foliar application of boron is in early June, in amount of 2-3 kg/ha, although the effect of this fertiliser will be visible only in dry years and in poor boron soils (Pospišil et al., 2005).

Sugar beet seed is processed (pelleted) as a one-germ seed. Seedtime is in spring, when the top soil layer at 5 cm reaches a temperature of 6-8°C. Early sowing deadlines have priority in the use of winter soil moisture for germination and a sufficient vegetation period. Shortcomings of earlier sowing are the risk of freezing plants and maintaining low germination. The optimal number of crops is 85,000-95,000 plants/ha, with a 45-50 cm row spacing and in row spacing 16-19 cm (Rešić, 2014). In the framework of less than 70,000 plants/ha decrease quality and yield of sugar beet. A reduction of 10,000 plants in the field results in a 2-5% yield loss and an approximately 0.2% lower sugar content in the roots (Pospišil, 2013). Sugar beet plants develop a large organic matter, with roots that constantly breathe; therefore, loose soil should be always provided for this crop. Inter-row cultivation is one of the measures of sugar beet crop care. During the growing season, this measure is carried out between 1 and 3 times. The depth of cultivation depends on the developmental stage of the root. First, inter-row cultivation is performed at a shallow depth, with the addition of a protective disc; therefore, young plants which do not have developed roots remain preserved. The last cultivation should be done before the beet close lines. Top dressing in sugar beet can start after the plants develop two pairs of leaves. Foliar fertilisation is common in stages after the beet close lines, with the aim of compensating for elements which are absent.

3.1.5. Pest control

Due to the low habitat and open circuit, sugar beet is exposed to problems with weeds for the entire vegetation period. The most common weeds in the crop are *Chenopodium album* L., *Polygonum persicaria* L., *Ambrosia artemisiifolia* L., *Abutilon theophrasti* Medik., *Xanthium strumarium* L., *Echinochloa crus-galli* (L.) P. Beauv, *Cirsium arvense* (L.) Scop., *Convolvulus arvensis* L., *Calystegia sepium* (L.) R. Br., *Sorghum halepense* (L.) Pers., *Agropyron repens* (L.) P. Beauv. and species of the genus *Setaria* and *Panicum* (Šćepanović and Galzina, 2010). Weeds in sugar beets are usually suppressed at the stage at which they do the most damage to the crop. The application of herbicides should therefore be carried out 2-4 times with lower doses (split applications).

In sugar beet crops, there are a large number of pathogenic organisms. During germination and emergence, beets are attacked in combination by pathogens known as blight and lodging young plants. According to Tomić (2010), the most significant pathogens are *Phoma betae* Frank, *Phytium* species, *Aphanomyces cochlioides* Drechsler, (1929), *Fusarium* species and *Rhizoctonia solani* Kühn. Every year, in our areas of sugar beet, crops suffer from leaf spot, which is a disease of sugar beet (*Cercospora beticola* Sacc.). Protection from this disease should be carried out on the basis of the plan protection forecast. Usually, this takes three treatments with fungicides; the first treatment should be carried out when 5% of the plants have about 10 spots (Cvjetković and Ivić, 2010). Chemical protection is a combination of systemic and contact fungicides. Sugar beet is also infected by Beet necrotic yellow vein virus (BNYVV). Under a strong attack of BNYVV, a yield loss of 50% can be incurred.

During the emergence of plants, sugar beet can be attacked by a large number of pests. The most important are from the family Elateridae, then *Atomaria linearis* Stephens 1830, *Chaetocnema tibialis* (Illiger 1807) and *Bothynoderes punctiventris* (Bažok, 2010). Sugar beet seeds are treated with insecticides during seed processing, so in the early stages of germination and emergence, the crop is protected from soil pests; however, this is only for a short time. If pest forecast highlights growing pest populations, a granular insecticide should be applied with depositors in strips during seed sowing. During vegetation, beets are susceptible to aphid, sugar beet moth and cabbage moth attack.

3.2. SUGAR BEET WEEVIL (*Bothynoderes punctiventris* Germar, 1824)

3.2.1. Systematic and morphological features

According to Maceljski (2002) sugar beet weevil is classified into:

Phylum: ARTHROPODA

Class: INSECTA

Order: COLEOPTERA

Suborder: POLYPHAGA

Superfamily: CURCULIONOIDEA

Family: CURCULIONIDAE

Genus: *Bothynoderes*

Species: *Bothynoderes*/*Cleonus*/*Lixus punctiventris* (Germar 1824)

The pest has a black basic body colour with many small grey peelings. Adult insects have a body length of 10-16 mm. Males are morphologically smaller, narrower and lighter than females and have final tentacles that are longer and thinner. The average male is 13.5 mm long and the average female is 14.5 mm. Males weigh 0.116-0.124 g, and females weigh 0.129-0.158 g (Pintér, 1953). Males have a longitudinal cavity on the border of two abdominal segments. The recognition of sex, according to Tielecke (1952), is achieved by monitoring characteristics of the dorsal end of the abdomen. This region can be observed when needle elytra are raised with insect preparation. Females can be recognised by a larger dorsal plate, which can be seen at the end of the abdomen. The last segment is not visible in females, while males have two visible dorsal plates. Adults of a smaller development size are formed in unfavourable conditions during the larval or pupal stages (Čamprag, 1954).

Adult weevils use chewing mouthparts to feed on the cotyledons and leaves of young sugar beet plants. On their elytra, longitudinal stripes are located which are made from point depressions, and there is also one strip in the middle of elytra that terminates with a white dot. Membranous wings are normally developed and adult insects can fly well. The ventral side of the body is lighter, with small scattered black dots. About 7250 individual insects weight one kilogram (Čamprag, 1984).

The egg is white to light yellow in colour (Figure 2e.), ovoid in form, and is 1.2-1.3 mm long and 1.0-1.1 mm wide (Čamprag, 1984). The larvae are typically cruciform, caterpillar-like and legless. The body is white with a tan or yellow head. The body of the larva is composed of 12 segments bent into an arc. Recently hatched larvae have a body that is covered with dense bristles, whereas fully developed larvae have no bristles. Larvae change 4 times and go through five development stages. The length of the back of old larvae is 27-30 mm. According to Petruha (1959), body dimensions of larvae through the various development stages are: first stage body length of 1.5 mm (and 0.5 mm wide sleeve head); second stage, 3.5 mm (1.0 mm); third stage, 5.0 mm (1.5 mm); fourth stage 7.5 mm (2.0 mm); and fifth stage, 12.5 mm (2.5 mm). Pupae are yellowish-white in colour, 10-15 mm long and 5-6 mm wide. The body of the pupae is elongated and egg-shaped with a pronounced head. This development stage consists of two pairs of wings and three pairs of legs.

3.2.2. Life cycle and ecology

The sugar beet weevil develops one generation per year (Figure 2).

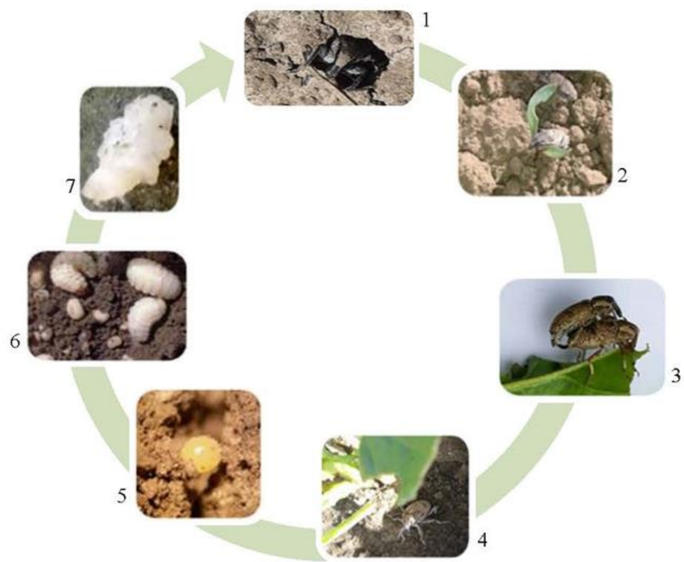


Figure 2. Sugar beet weevil development

(1. Out coming from overwintering (Photo: Drmić, 2014), 2. Weevil feeds on sugar beet cotyledons (Photo: Drmić, 2013), 3. Copulation (Photo: Šatvar, 2014), 4. Egg laying female (Photo: Drmić, 2013), 5. Egg (Photo: Šatvar, 2014), 6. Larvae (Photo: Drmić, 2013), 7. Pupae (Photo: Drmić, 2015).

The pest overwinters in the soil, almost exclusively as adults. Petruha (1959) stated that 80-90% of all individuals overwinter in the soil where sugar beet was sown in the same year. Also, 5-10% of weevils overwinter in the area where sugar beet was planted two years ago and 5-10% of the pest population overwinter in areas sown with another crop. Weevils can hibernate on fallow land, and on all places where plants of the Chenopodiaceae family are represented. Popov (1965) recorded a mass overwintering of pests in areas where sugar beet was produced two years previously, because of the depth of overwintering pests (50-70 cm). Auersch (1954) explained that the retention of the pest is a narcotic reaction to CO_2.

Adults appear in the spring, when the soil surface (10 cm) is warmed up to 8-10°C (Maceljski, 2002). The mass appearance of weevils occurs during sunny days, when the air temperature reaches 15-25°C, and the soil surface temperature (10 cm) warms up to 25-35°C (Petruha, 1959). The extent of the insect primarily depends on weather conditions, and in the first place on temperatures. Pests previously occur in areas which are left for the sowing of spring crops. The subsequent emergence of pests has been observed in fields where some winter crops (wheat, barley) were sown in the autumn. In several waves, weevils emerge. Individuals who have been overwintered in the upper layers of soil emerge first; then, individuals emerge from deeper layers. When it is rainy and cold at the time of pest emergence, individuals are found hiding under lumps of earth, cracks in the soil or down in the top soil layer. In the conditions found in Vojvodina (Sombor), most of the emerged weevils (32.65%) were collected on April 9th (Pyatnitzkiï, 1940). Further research found the same results; the largest collection was made in the first and second weeks of April.

The daily activity of pests starts at around 9-10 in the morning and lasts until the evening. If it is sunny and warm, the highest activity can be recorded between 11 am and 2 pm. By lowering the air temperature by 2°C, insects become depressed and stiff, and they do not move until the temperature rises to 5°C (Čamprag, 1984). Low activity begins at an air temperature of 5-10°C, and higher activity is seen when the air temperature reaches 15°C. The sugar beet weevil is found where the fewest micro relief obstacles are found, and then depending on the wind direction (if the wind speed is over 5 m/s). During one hour, weevils can walk over 10 m, or in one day up to 500 m per day (Čamprag, 1963). After emergence, insects are found concentrated in areas sown with sugar beet. The intensity of pest attack depends on the distance between the old and new sugar beet fields. Walking pests are found first in marginal parts of the field, and then gradually spread to the field interior. Overall, 90-95% of pests are concentrated on the sugar beet crop, with only 5-10% feeding on plants of the family Chenopodiaceae (Petruha, 1959).

When sugar beet individuals emerge, they do not fly immediately. Flying usually starts after 9-17 days of walking on the soil surface, providing the maximum insect activity. The flight of the weevil is conditioned by a complex series of meteorological conditions, especially temperature and insolation. According to Auersh (1954), the minimum temperature for flight is

19.5°C. Most of the flight takes place at 20-25°C. In this pest, two types of flight are distinguished: migratory flight results in populations being spread out through an overwintering field, with both sexes are affected, while the second flight type affects sexually mature females, which are partly fertilised (Čamprag, 1984). Females in their flight spread population search for food and look for a suitable place for egg laying. This type of flight lasts for 2-3 days, but can sometimes reach several weeks. Intensive flight occurs at times of sexual maturation, mating and egg laying. During sunny and fair weather, insects fly between 11 am and 2 pm. Flight takes place at altitudes higher than 3-5 m, or even over 10 m if conditions are favourable. Daily overflights of pests can be up to 10 km (with the help of wind), and the flight period lasts 2-3 days, but sometimes up to 40-50 days (Petruha, 1971; Čamprag, 1984). During years characterised as humid and cold, mass flight is not reported.

In the spring, during migration, sex ratio changes. Initially, the ratio is dominated by males, then compensates, and by the end of the migration (and after), the relationship increases in favour of females. Research by Bogdanov (1965) in Bulgaria found a relationship between sexes, where 87% of the individuals captured in the last week of March were male (13% female), in the first week of April, 65% were male (35% female), in the second week of April 58% were male (42% female), in the third week of April, 41% were male (59% female), in the first week of May, 27% were male (73% female) and in the second week of May, 22% were male and 78% were female. Two to three weeks after pest emergence from overwintering, weevils initiate multiple copulations. Insects mate once, sometimes twice per day; Čamprag (1984) recorded the copulation of weevils three weeks after leaving the overwintering field. Females lay eggs shortly after mating (average of 4 weeks after appearance on the soil surface). Oviposition takes place in the first week of May in years of favourable weather conditions, when beet weevils start feeding early.

For egg-laying females, using their proboscis, they perforate a hole near the sugar beet; in rainy years, this is done between the lines in sugar beet, fodder beet, or on fields which are contaminated with Chenopodiaceae plants. Holes have a depth of 2-3 mm (in dry year conditions, this can be up to 10 mm). After drilling, females turn their abdomen and lay 1-2 (maximum of 10) eggs in the holes (Figure 3, 4 and 5). Eggs are reinforced with droplets of liquid secretions from the female and the hole is backfilled well with the front legs. The average female deposits 94-120 eggs in one year (Čamprag, 1984), although that number varies from 20-30 to high fertility levels of 200-300 eggs. A maximum of 740-950 deposited eggs was recorded by Petruha (1971). The intensity of egg laying is directly correlated with temperature, number and diet of individuals, type and amount of insecticides applied in weevil control, relative air humidity, and of the environmental conditions conducive to growth and development. The minimum temperature must be above 15°C, and egg laying is more intensive when it is warmer

Figure 3, 4, 5. Egg laying female (Photo: Drmić, 2013)

During the absence of pests for up to 20 days, the number of deposited eggs is reduced. The diet which is a combination of sugar beets and plants from the Chenopodiaceae family results in the largest number of deposited eggs. In contrast, nutrition with sugar beet seed reduces the number of deposited eggs, and an exclusive diet of sugar beet cotyledons contributes to the opposite (Žitkevič, 1959). Taranuhae (1956) reported similar data. Weed-infested fields (a combination of sugar beet plants and plants from the Chenopodiaceae family) contribute to increased egg laying. Relative air humidity during oviposition should range from 55-65%. According to the research of Gromova (1965), reduced and sub-lethal doses of organochlorine and polychloroprene insecticides contribute to greater egg depositing capacity for females, sometimes by up to 43%. According to Čamprag (1984), eggs show xerophytic characteristics, and low air humidity increases the development time and contribute to decay. Egg development takes 10-15 days at 16-26°C.

Although the number of hatched larvae only corresponds to the number of deposited eggs, their number soon declines. Young, just hatched larvae are very sensitive to high levels of soil moisture. The pest larvae are highly mobile in soil; they move in the direction of sugar beet, because their secretions are used as attractants. Most of the larvae found in the early stages of development were located at a depth of 10-20 cm. The further development of larvae continues in deeper soil layers. The number of larvae is also influenced by the soil structure. Compact soils present places where larvae cannot be found; in areas characterised as humic (lighter layers of soil), a significant number of larvae can be found. The larvae mainly feed on the roots of sugar beet and also on plants from the Chenopodiaceae family (Čamprag, 1984). According to Petruha (1959), the development of larvae lasts for 45-91 days, while in the research of Bogdanov (1965) on the territory of Bulgaria this period was only 29-62 days.

Upon the completion of larval development, larvae move away from the roots and create a vertical chamber with smooth interior walls (Čamprag, 1984), in which it metamorphoses into a pupae. According to Petruha (1959), the development of pests from the egg to the adult form takes 67-148 days.

The research of Tielecke (1952) in eastern Germany showed the entire duration of sugar beet weevil development to last for 2.5 months. In Bulgaria and Romania, the development of pests is shown over a shorter period, of 75 days and 70-82 days, while the period in Hungary extended for 3 months (Čamprag, 1984). In the area of Vojvodina, which is similar to Eastern Slavonia, the development of pests lasts from 2.5 to 3.5 months (Čamprag, 1984). The transformation in the adult form of pests during the average year begins in the second half or at the end of August (Čamprag, 1984), while deadline is shifted in wetter years to the beginning of September. The depth of overwintering pests depends on the area where they are located. For wetter years and compacted soils, overwintering was observed as a mass phenomenon of hibernation at only 10 cm deep (Tielecke, 1952). In the Soviet Union, according to Zverezomb-Zubovskij (1956), the main mass of pupae was found at a depth of 20-30 cm. In Turkey, Steiner (1936) found the greatest number of pests at 20-40 cm. Bogdanov (1965) found pests at a depth of 15-30 cm in the territory of Bulgaria. The depth of overwintering pests (according to Čamprag, 1984) is justified by abiotic factors prevailing in a particular area for that period. After transformation to the adult form, they remain in the soil until the following spring, and part of the population can remain in diapause for two years (Čamprag, 1984).

3.2.3. Pest distribution and harmfulness

The original habitat of sugar beet weevil is considered the Solonchaks area around the Caspian Sea (Čamprag, 1984). The pest is oligophagous, and with the development of industry, from nutrition found in plants of the family Chenopodiaceae, weevils easily adapted to a diet of sugar beet plants. Insects have a wide distribution area in Europe and Asia: 0-105º longitude and from north to south about 54-30º north latitude (Figure 6). The largest part of the population was recorded in the southeast part of Europe.

The number of pests depends on the abiotic factors of climate and soil. The most favourable soil for the sugar beet weevil is chernozem. Areas suitable for mass reproduction are within the limits of the annual isotherms of 6-8ºC, January temperatures are lower, from 4-6ºC, May temperatures are 14-15ºC, June temperatures are 19-21ºC, and the medium temperature in vegetation is 15-16ºC; the mean annual precipitation is 300-400 mm (Petruha, 1959).

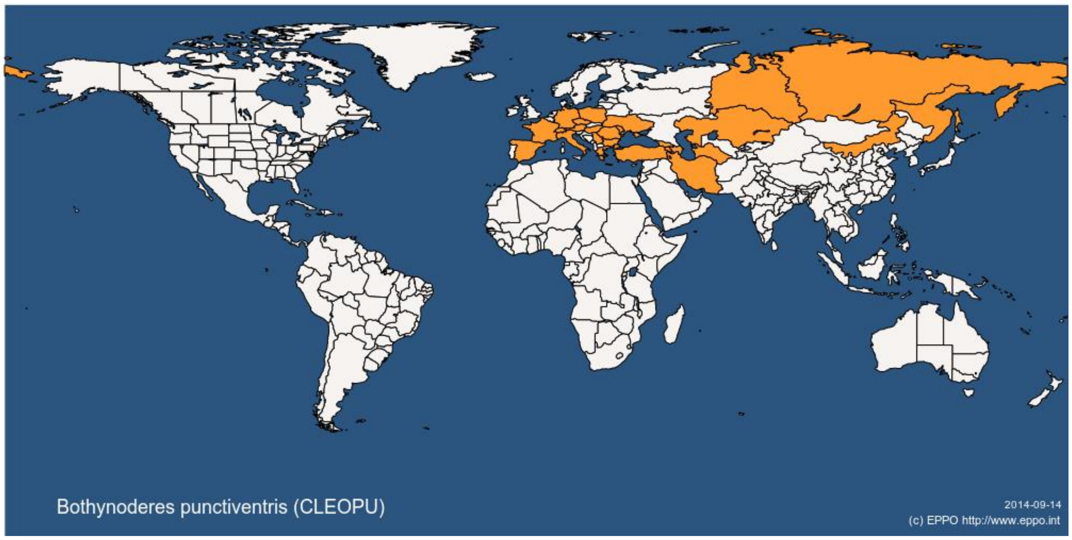


Figure 6. Map of *B. punctiventris* Distribution (source: EPPO Global Database, 2015)

The pest is abundant in high populations across the dry countries of Turkey, Bulgaria, Hungary, Romania, Ukraine, Serbia and Croatia. In these countries, sugar beet weevil is considered one of the most dangerous pests of sugar beet. In eastern Slavonia (Tovarnik), the pest is known to occur from the start of the cultivation of sugar beet. Beet weevil was a periodic pest until 2008, from then it has regularly appeared and causes significant economic damage to crops (Bažok et al., 2012). In Germany, Poland and Austria, the pest is rarely reported, causing no significant damage.

The greatest damage is caused by adults. Adults eat the stem, cotyledons and fully developed leaves. According to Maceljski (2002), in one day, a single individual can damage and destroy 50% of the plants that have emerged per m^2.

The damage varies depending on air temperature. At 10°C weevils feed on 4 mm^2 of leaf, while at an air temperature of 32°C, pests eat more than 143 mm^2 of leaf per day (Stanković and Maceljski, 1973).

3.2.4. Monitoring, forecast and control

Predicting the pest primarily provides the basis for the organisation and execution of all preventive and curative measures for the protection and control of pests. The timing, location, intensity, further development as well as the extent of damage is predicted. To forecast the pest population, it is necessary to collect data on the meteorological, biological and ecological characteristics of the pest, host phenology, phenology of weeds and soil type (Čamprag 1954). Forecasts can be positive or negative. When a forecast is positive, it gives information on the absence of pests and their damage. In forecast modelling, the point of view begins with principles that a small number of pests cannot proliferate rapidly to present a risk. Mass propagation takes 5-6 generations, and often 10-20 (Manninger 1968 cit. Čamprag, 1984).

Multi-year forecasts provide data related to the level around which fluctuations of distribution can be expected and also provide abundance data of pests. According to Polyakov, and Tanskii (1975), multi-year forecasts are a prerequisite for the development of plant protection strategies.

Long-term forecasting of adult weevils determines the appearance of the expected expansion of pest population size and probable losses due to attacks. Long-term forecasts can be made in the summer on the analysed weather conditions from the spring. A final long-term prognosis is given in the fall based on density data of overwintering adult weevils, which are determined by soil examination on fields in which sugar beet is sown (Čamprag, 1984). Old sugar beet fields are examined by digging pits, sized 50 × 50 × 50 cm, in late summer or early autumn. The excavated soil is examined for the presence of weevils. In the late winter and early spring, the soil survey is repeated. In addition to a long-term (basic) forecast, a supplementary forecast is conducted, usually early in the season. Then, data are determined by the number of pests investigated before and after the summer.

The short-term forecast and signalisation is about the density of overwintered pests which were determined by soil examination. Also, this type of forecast is based on distance data between last year's sugar beet fields and new sown, dynamic release, migration of pests in newly sown beet fields, evidence of plants damage and their development stage, the number of weevils in the sugar beet fields and weather conditions in the period from April to May (Čamprag, 1963). Short-term forecasts are more accurate, being predictive up to twenty days. They provide signals of where and when the safeguards should be placed for optimal results. The time sampling area for making short-term forecasts is the moment at which the weevils are rising to the soil surface (Čamprag, 1984). This time is the best to determine the migration dynamics of pests by soil examination. The larger the soil sample, the more accurate the data are. Forecasts give a picture of the prevalence of pests, from which we can predict the movement of pests, hence facilitating access to protective measures. It is a very important relationship between areas of old and new sugar beet fields and their distance from each other. The advantage comes in years when new fields of sugar beet are more represented than the old sugar beet fields, because it means that the pest concentrate on areas closer to the old sugar beet field. Additional inspections of soil and crop residues can be performed during growing seasons, with surveys on overhead and underground plant parts.

a) Threshold decisions in sugar beet weevil suppression

In pest control, except its population, there are weather conditions essential too. In a cold spring with a lot of moisture after crop emergence, it is very unlikely to see large-scale damage. In old sugar beet fields, a critical number for the most damaging sugar beet weevil is one adult weevil/m^2. In the same young crops, depending on the stage of plant development (Čamprag and Kereši 2003), the threshold decision is presented as 0.1-0.3 weevils/m^2 (representing 1,000-3,000 weevils per hectare). Pending establishment of the first two true leaves of sugar beet, the threshold is 0.1 weevils/m^2. The period of emergence and development of the first leaves is the most critical, and the greatest damage is reported during this period. In the later development stages of beet, from four leaves onwards, weevil does not seem to inflict economically significant damage, so the threshold increases to 0.3 weevils/m^2. Of course, the threshold is subject to revision depending on the distance of old and new sugar beet fields, weather conditions during the emergence of sugar beet, the development stage of the plants and access control.

b) Agro-technical suppression measures

Control of pests is carried out through a number of agro-technical, biological, mechanical and chemical measures (Čamprag, 1984). Implementation of these measures should be initiated on those fields on which sugar beet was cultivated last year and continued on fields in which beets are currently being planted. In last year's sugar beet fields, the suppression of adults is a priority, as this will prevent the movement of pests into new sugar beet fields, where weevils do the greatest damage during the emergence of sugar beet. As curative measures, insecticides are used on young sugar beet crops in order to prevent adult pests from feeding and thus propagating and ovipositing.

The first step in the control of pests is the effective implementation of agro-ecological principles. Some agro-technical measures can reverse pest attacks. The practice involves the widespread sowing of winter crops in the year after sowing of sugar beet. The advantage of the culturing plants at a high density is the fact that the adult forms of pests have difficulties walking and migrate through such crops. Furthermore, when planning the structure of sowing, the distance of fields of sugar beet from the previous and current year should be taken into account. According to observations of Petruha (1982), in sowing of sugar beet fields near last year's sugar beet, pests inhabit new fields that are within 100 m of last year's beet field. Fields of sugar beet which are far from last year's fields, by more than 1000 m, are not significantly damaged. Since the pest overwinters in the field on which the sugar beet was grown and since the pest passes into new fields of sugar beet by walking, new fields should be sown as far from last year's sugar beet as possible.

Sowing should be performed as early as possible. Ščegolev (1938) indicates reduced damage during early sowing. If cultivated plants reach the stage of true leaves before the pest attack, pests will cause less damage, and the point of plant growth will remain undamaged. Systematically performed early sowing affects the simultaneous appearance of seedlings in all areas. This contributes to the possibility of converting crops as hunting plantations and reducing pest attacks (Zverezomb-Zubovskij, 1956). Late sowing was seen as a cause of intense attacks and severe damage by pests in Slavonia from 1931 (Kovačević, 1929). The quantity of seeds sown may also contribute to crop protection. In areas of high abundance, sowing to the edges can be performed more densely, with a space of 10 cm between the rows. The pest in diet is thinning circuit, so dense sowing ensures better plant density per unit (Stanaćev, 1979). When a new field is sown close to last year's field, denser planting along the entire length is recommended. Areas in which a larger number of plants emerge are very attractive to pests and provide a generous and easy diet. Excess plants in the final part will be eliminated during the inter-row cultivation. According to Zraževskij (1951), when settling an area with lower density of plants, the beet weevil chooses topical loose soil and well lit, heated habitats. In experiments with different sugar beet row spacings (22 and 44 cm), results showing 5-6 times more pests were achieved in rarely observed areas. When cultivated plants are grown to the stage where leaves cover the soil surface, pests leave the field, regardless of whether egg laying has been completed.

When entering the soil, liquid nitrogen fertiliser adversely affects larval development in pests. The toxic effects of adsorbed ammonia (bound to the colloidal system of the mineral and organic components of the soil) are reflected in the increased gas exchange and reduced level of fats, which impair the physiological condition of the larvae (Grigorieva et al., 1971). Fertilisation of liquid ammonia led to reductions in larval population pests by 8-22 times. The present data relate to clayey soil, such as chernozem soil, while the light, sandy soils did not achieve satisfactory results. Part of pest populations (that diapauses over two years) after leaving the soil, feeds on plants that are mainly representatives of plant family Chenopodiaceae and Amaranthaceae (Čamprag, 1984). Destruction of weed flora, especially of Chenopodiaceae, reduces the number of pests on cultivated plants and tends to decrease female fertility.

Inter row tillage and cropping around the plants in the period after oviposition can also reduce the number of pests. During the development of larvae and pupae in the topical area of soil, inter-row cultivation can be carried out. In this way, the larvae are removed to the surface and some collapse due to overheating (direct action of the sun's rays), while others are destroyed by natural enemies. Up to 86% of the population can be destroyed by row cultivation (Petruha, 1971). At the time of oviposition, loosening of the soil contributes to drying eggs, distorting the capillary soil moisture. The research of Zaraževskij (1962) indicates less damage to the crop in terms of chopped and smooth surface soil (measure rolling is an example).

All cultural practices that contribute to the faster and better development of sugar beet plants (measures that contribute to water collection and storage in the soil, proper nitrogen fertilisation, selection of treated seed, early sowing, sowing thicker on the edges of fields, weed control, irrigation fields in April and May, and inter row cultivation) contribute to the protection of crops from pests.

c) Mechanical-chemical suppression measures

If the examinations of the soil in the autumn establish a greater number of populations, digging the catching channels around last year's sugar beet fields is recommended. Žitkevič (1959) points to the ability to control 80-90% of the adult population of insects with timely trenches. The channels are dug to depths of 25-30 cm and a width of 3-18 cm, depending on the tool that performs the action. The walls of the canal have to be strictly vertical, as imago cannot escape by climbing the edges. In the area of Eastern Europe, trenching was mainly performed using ploughs with manual finishing canal walls.

Figure 7. Catching channels (source: Portal Prognozno-izveštajne službe zaštite bilja, 2015)

The bottom of the channel was repeatedly treated with dust compositions (based fenitrothion, matilparationa or lindane), granular insecticides (based phorate, fenitrothion, fenitrothion combination with lindane), and setting the straw to be treated with contact insecticides (Čamprag, 1984). The described measure only has an effect in colder periods, before the pest has started flying.

d) Chemical suppression measures

Suppression of sugar beet pests with insecticides is carried out through the seed treatment of sugar beet, the incorporation of insecticides into the soil and treating the sugar beet crop at the time of pest attack (usually in the cotyledon stage to the stage of first leaves). According to Čamprag (1984), by sowing sugar beet in the fall (if a winter crop preceded sugar beet), manufacturers will get spring-developed plants that will play the role of catching plants. The catching plants (with increased leaf surface) can be treated with chemical insecticides. Pest control is mainly based on the use of insecticides (Sekulić et al., 1997). Chlorinated hydrocarbons (Čamprag 1984), organic phosphorus (OP) insecticides (Radin, 1983) and pyrethroids (P) in combination with organophosphorus (OP) insecticides (Bažok et al., 2012) have been used with varying degrees of success. Due to the acceptance of EU pesticide legislation, the number of active ingredients allowed for sugar beet weevil control in Croatia has been reduced in the past ten years. Currently, for sugar beet weevil control, three insecticides based on four active ingredients are allowed: lambdacyhalothrin (Karate Zeon, Syngenta), the combination of chlorpyriphos and cypermethrin (Chromorel D, Agriphar) and acetamyprid (Mospilan, Nippon) (Bažok, 2016a). Allowed active ingredients belong to the group of OP insecticides (chlorpyriphos), pyrethroids (lambdacyhalothrin and cypermethrin) and neonicotinoids (acetamyprid). There is an intention in the European Union to limit the use of all of these insecticides in the future. Due to the specific morphological structure of weevils, their large feeding capacity and the small leaf area of plants at the time of insecticide application, even the permitted insecticides can give very poor results and require repeat treatments (Bažok et al., 2012). Such practice is not in accordance with the principles of integrated pest management (IPM) nor with the rational use of pesticides in modern agriculture. Due to the low efficacy and small number of available active ingredients for sugar beet weevil control, this pest could become a limiting factor for the production of sugar beet in Croatia. These facts imposed a need for the elaboration of a system of measures which would ensure optimal crop protection according to the principles of integrated pest management (IPM). When developing such a system, chemical control remains the main control measure (Inđić et al., 1998; Vuković, 2003) and will probably remain this way in the near future. It is therefore important to find new insecticide compounds which can be used for beet weevil control. The study carried out by Bažok et al. (2016b) proved that spinosad is a good candidate and should be introduced in sugar beet weevil control. In laboratory trials, good efficacy was obtained with a dose of 96 g a.i./ha, but, for determining the recommended dose, further field trials are needed. The use of spinosad against beet weevil would be in line with IPM principles because spinosad has a unique mode of action and low toxicity to non-target organisms (including many beneficial arthropods) which makes it an excellent tool for the management of various insect pests (Thompson et al., 2000). Many authors mentioned binary mixtures of insecticides as a strategic measure in sugar beet weevil control (Inđić et al., 1998; 2000, Vuković et al., 2004). Based on the results of Vuković et al. (2004), good candidates for mixtures could be chlorpyriphos or cypermethrin. Research conducted by Bažok et al. (2016b) showed that spinosad may be also a very good candidate for use in the mixtures and this possibility shall be further investigated.

3.2.5. Biological control and biotechnical measures

Previously, for the collection of weevils, domestic animals, such as turkeys and chickens, were used. Today, these mark the pest’s natural enemies. The research of Bogdanov (1961) showed the 56-85% mortality of larvae and pupae after the use of microbial insecticides based on entomopathogenic fungi. According to Beratlief (1979), continued research with microbial insecticides (also based on *Beauveria bassiana* (Bals.-Criv.) Vuill., 1912 strains) has contributed to 92-100% mortality after 12 days of treatment in the lab. In field conditions, mortality of 74% has been achieved. In the years of strong attack, pest entomopathogenic fungi belonging to the genus *Metarhizium* Sorokīn 1879, *Beauveria* and *Tarichium* Cohn, 1875 may increase the mortality of sensitive stages of pests (Čamprag et al., 2006). By applying entomopathogenic fungi with the addition of sub lethal doses of insecticides, it is possible to achieve larval reduction of up to 85%.

The nematode of the genera *Steinernema* and *Heterorhabditis* (Rhabditida) are pathogenic to many insect species (Poinar et al., 1990). Together with symbiotic bacteria of genera *Xenorhabdus* Thomas and Poinar, 1979 and *Photorhabdus* (Boemare et al. 1993) nematodes were used to research sugar beet weevil control. These organisms have a short life cycle, a broad spectrum of action (local variety) and can survive unfavourable conditions, including the temperature of 30°C (Hassan, 2010). In the area of Ankara, Turkey, the nematodes *Steinernema feltiae* Filipjev*,* 1934, *Steinernema weiseri* Mracek, Sturhan and Reid 2003 and *H. bacteriophora* Poinar, 1975 were isolated. Nematodes grown in symbiosis with the bacteria *Xenorhabdus* and *Photorhabdus*, in the third developmental stage, were infected with the larvae of pests. After penetration into pests, nematodes release bacteria. Two days after infection, the larvae had increased mortality under the influence of metabolic toxins released by the bacteria. The authors concluded that it is possible to use this model to control insects at different soil depths (5-20cm) and at different soil temperatures (5-25°C) (Susurluk, 2008). Although all researches were conducted in the laboratory, these and similar methods of biological control for pests has and will have in the future a great importance in the field.

Scientists from Hungary (Toth et al., 2003) have developed aggregation pheromone for sugar beet weevil. In their further work (Toth et al., 2007) they established the efficacy of the developed traps and determined the exact mixture of the components to be used as sensitive and powerful trapping tools in the control of the sugar-beet weevil. With pheromone-baited traps, pests climb into boxes and are physically exported from the fields. Baits are placed on “old” sugar beet field at a distance of 20-30 m, at a concentration of 30 traps/ha (Tomašev et al., 2007).

3.3. AREA-WIDE PEST CONTROL

Classical integrated pest management (IPM), which aims at managing pests by the integration of biological, cultural, physical, and chemical tools in a way that minimises economic, health, and environmental risks (National IPM Network, 2001), has remained a dominant paradigm of pest control for the last 50 years (Barclay et al., 2011). Area-wide is a form of IPM that aims to reduce pests in a particular area to numbers below those that can cause damage. According to Klassen (2005), area-wide pest management entails the integration of various control tactics against an entire insect pest population within a delimited geographical area. The goal of this program is a long-term solution, as opposed to individual pest control, which aims to cover a substantially smaller area with the short-term elimination of damage (Vreysen et al., 2007a). It is an organised system of pest control in which producers of similar or identical crops team up and operate on wide-growing areas. The concept of area in the term area-wide refers to the area in which the pest inhabits. This kind of pest control approach is proactive, as action is taken before a pest population reaches damaging levels and is aimed at protecting agriculture and/or human health over an entire area (Vreysen et al., 2007b). Area is not limited solely to the protection of the major crops produced. Most of the costs of the program are used to divert pests from plants grown on wild hosts, abandoned orchards, gardens and similar. Control is often conditioned by a separate organisation that combines planning and program execution. Such an organisation should agree on a plan to use highly specialised technology in order to obtain accurate information regarding the exact number and distribution of potential wild hosts, speed and direction of movement of pests, computer programs that predict changes in insect populations on the basis of biological parameters, systematic approach for identification and activation of natural enemies, genetic analysis, and the development of resistance, which is a choice that will prevent the development of resistance to pests. Furthermore, AW encourages the use of methods such as sterile insect technologies which are not effective in certain control measures (field-by-field), to solve the problem as a whole. Although there are numerous examples of "area-wide pest management" (AW) (Klassen, 2000; 2005; Hendrichs et al., 2007), the scientific basis of this approach has been ascribed to Knipling (1972, 1979).

3.3.1. Scientific foundations and principles

IPM offers a strategic approach to solving pest problems in an ecosystem context while guarding human health and the environment (Brader, 1979). IPM is a pest management system that has a strategic approach to solving pest damage while at the same time protecting human health and the environment. More than half of the world's countries with stable agricultural production have a national policy of IPM. The principles of IPM include production of agricultural products by the maximum possible biological approach. Chemical agents are justified if their use is unavoidable and economically and environmentally justified. AW provides long-term solutions in the entire area affected by the plan, which aims to reduce pest populations. Very early initiative programs are focused on key pests over a wide area, such as programs to control *Phylloxera* Boyer de Fonscolombe 1834 (placed under the control in 1890), *Daktulosphaira vitifoliae* Fitch 1855 (Kogan, 1986); the example of cotton cushion scale, *Icerya purchasi* Maskell 1878, regarding pests that seriously affected the California citrus industry in the 1880s, two biological agents from Australia, Vedalia ladybeetle, *Rodolia cardinalis* Mulsant 1850, and parasitic flies, *Cryptochaetum iceryae* Williston 1888. Successful programs have focused on bringing the population closer to zero: for example the cattle tick, *Boophilus annulatus* Stiles and Hassall, 1901 and *Boophilus annulatus microdus* Arnold, 1935 and screwworm, *Cochliomyia hominivorax* Coquerel 1858. Two species of cattle ticks have been mainly eradicated in the field in the US since 1950 (Cole and MacKellar, 1956) using the sterile male technique (SIT), and since 1991, the screwworm was also eliminated from Belize (1994), Guatemala (1994), El Salvador (1995), and Honduras (1995) (USDA APHIS, 1998). SIT has also been used to eradicate melon fruit fly from Okinawa and the southern islands of Japan, as well as against tsetse flies on the island of Unguja, Zanzibar (Vreysen et al., 2000). Area-wide, as a method of pest control, is very successful, except in the case of crop farming, where the possibility of application is much lower.

In September 1992, Knipling presented a proposal for the North American Plant Protection Organization (Nappo) called "Area-Wide Pest Management". Their vision was a process by which AWPM programs must be: (i) conducted over a large geographical area, (ii) coordinated by the organisation, not by individual producers (iii) may include eradication, if it is practical and affordable, but which should be focused on reducing and maintaining pest populations at an acceptably low abundance; and (iv) include a mandatory component to ensure the success of the project within large geographical area, because voluntary programs have not historically provided the desired level of pest population control. In AW

Pest Management, from semi-chemical ways to controlling pests with mating disruption, the sterile insect technique (SIT) lures and kills target system and manipulating natural enemies, has been repeatedly applied for mass trapping. This method involves placing traps at a high density in a given area in order to physically remove as many pests before they can reproduce. At mass trapping using special synthetic chemical baits, by gender and crowding, pheromones and food/host attractants lure insects into the trap where they remain trapped and die. Mass trapping using odour-baited traps is one of the older approaches for the direct control of insects for population suppression and eradication (Steiner, 1952).

3.3.2. Area-wide suppression methods

The approach of IPM involves a series of pest management evaluations, decisions and controls. In practicing IPM, a four-tiered admission is required. The first step is a request the establishment of an action threshold, a point at which pest populations or environmental conditions indicate that pest control should be taken. The emergence of a pest does not necessarily mean that there is a need for suppression. The level at which the pest causes economic damage represents a critical point from which the grower begins to make future decisions in pest control. The importance of this "total pest population management" approach has significantly increased for many pests in the past decades, and it is now generally accepted that AW-IPM leads, in many cases, to more sustainable pest control, especially for mobile insects (Klassen, 2005). After tracking and identification, when the action thresholds indicate that pest control is required, and preventive methods are no longer effective or available, IPM programs then evaluate the proper control method both for effectiveness and risk. Less risky, effective pest control is chosen first. Initially, action is taken with highly targeted chemicals (pheromones to disrupt pest mating) or with mechanical control (trapping or weeding). If the measures do not achieve the goal, and the damage still occurs, the IPM program continues to spray insecticides. Broadcast spraying of non-specific pesticides is a last resort. Depending on the nature of the pests, the AWPM approach includes several technologies (Faust et al., 2008):

1. Traditional biological control – the use of parasites, parasitoids, predators, pathogens, competitors and other beneficial organisms to reduce the harmful effects of pests, which may embody augmentation and biological conservation tactics;

2. Biologically-based (bio-rational) control – the use and application of biological base methods (hormones, antimetabolites, feeding deterrents, repellents, pheromone and

allelochemical (semi chemical) and other naturally produced chemicals, attracidal compound, traps, autocidal methods, SIT);

3. Host resistance – the use and application of pest-resistant crop cultivars and animal breeds, including genetically engineered plants and animals resistant to pests;

4. Cultural practices – the use and application of tactics such as crop rotation, intercropping, tillage approaches, cover crops or mulches, managing irrigation and drainage, fertilisation, removal of crop residues and other field sanitation procedures, altering planting and harvesting schedules;

5. Physical and mechanical control – the use of physical and mechanical methodology, thereby exerting economic control or reducing the rates of pest contamination and damage (vacuum collection, screening, trapping)

6. Chemical control – the use of broad-spectrum synthetic organic (non-naturally occurring), or analogues of natural chemicals (pyrethroids, insect grow regulators) or inorganic chemicals for the control of animal and plant pests, including fumigation, the use of improved chemical pesticide formulations and the proved insecticide application technologies.

The monitoring and control of harmful insects can be done in two ways: individually (Figure 8), on each surface (field by field), and over the wider area, as an AW program (Figure 9). The broad form of control is certainly a field by field case; such an approach only has control of a small part of the population over a given period of time. With such an approach, it is not possible to determine the true presence of pests. The actual number of insects must include information about their migration to adjacent fields or alternative wild hosts. Access in this way gives the farmer limited data; those data depend on the surrounding producers. In such circumstances, the need arises for pest control over the whole area.

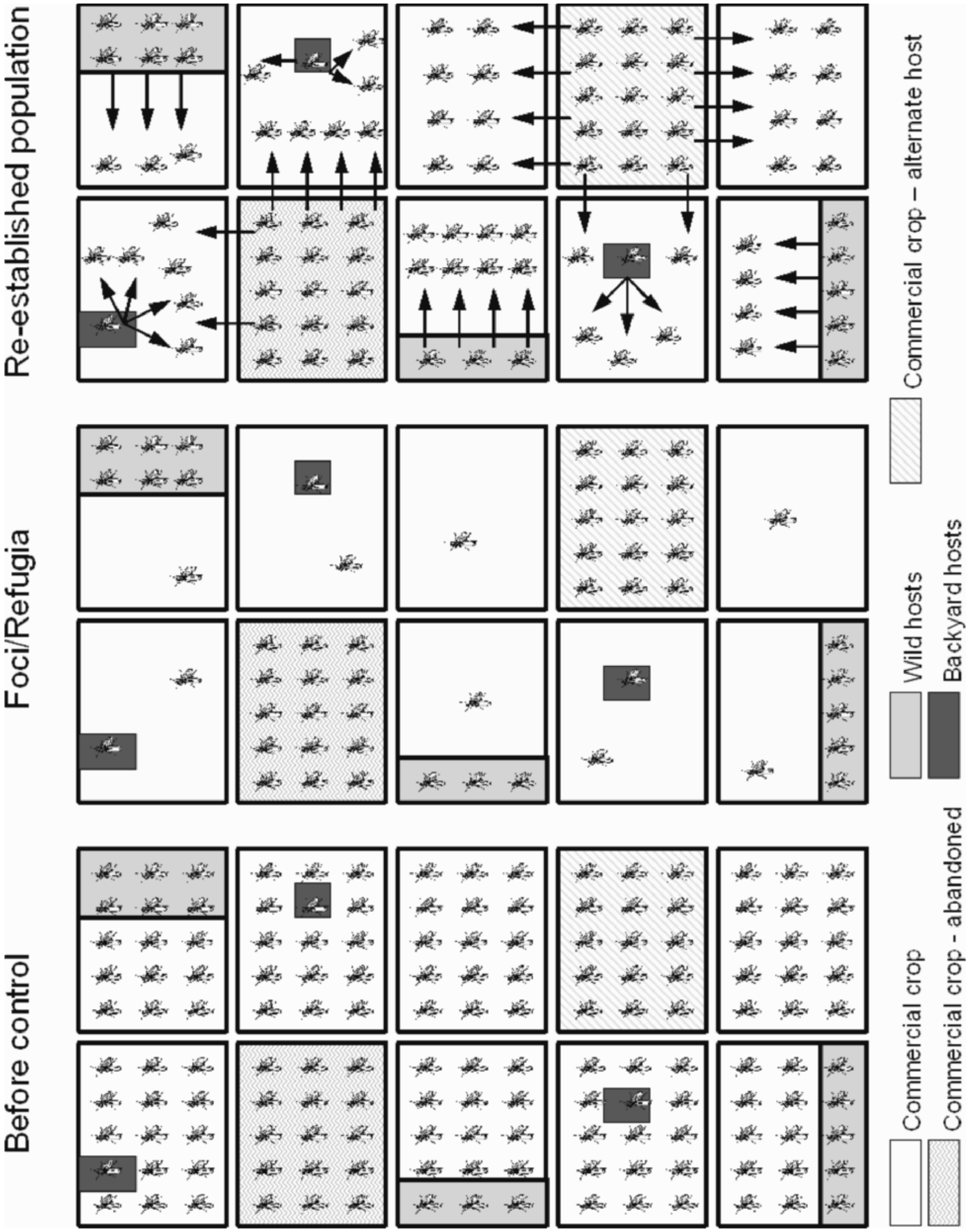


Figure 8. Graphic display of field-by-field IPM. (The pest is suppressed below an economic threshold in areas of commercial interest, but often not in abandoned crops, alternate hosts, backyard hosts or on wild hosts). As a result, significant untreated refugia of the pest remain, from which recruits re-establish damaging densities of the pest population (Hendrichs et al., 2007)

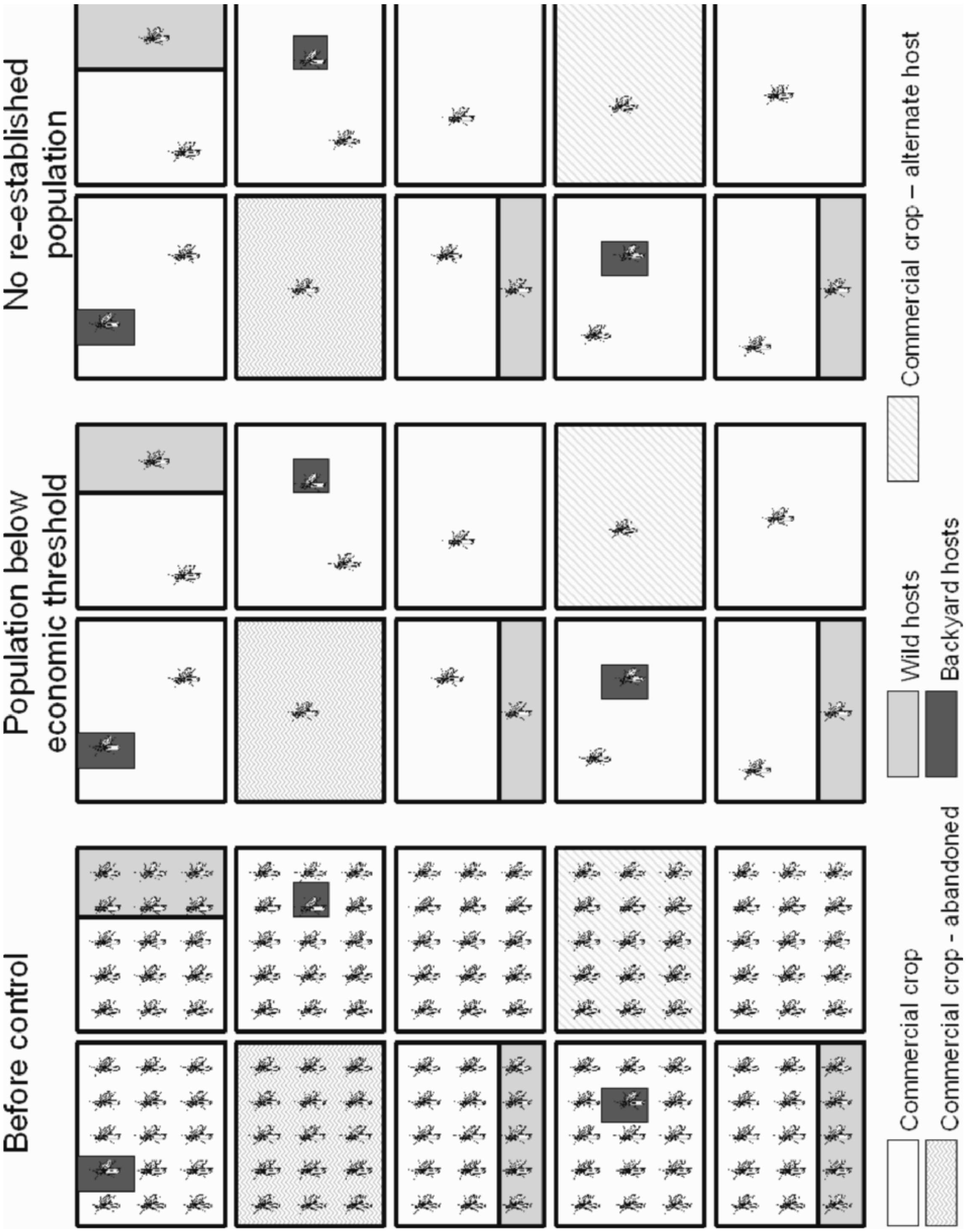


Figure 9. Graphic display of AW-IPM. (The pest is suppressed below an economic threshold level in all areas, including abandoned crops, alternate hosts, backyard hosts or on wild host). As a result, no significant untreated refugia of the pest remain from which recruits can re-establish damaging densities of the pest population (Hendrichs et al., 2007).

A powerful AW control tactic is the sterile insect technique (SIT), which has become accepted over the past decades as an efficient and cost-effective part of the AW-IPM programs aimed against a selected number of insect pests of veterinary, human health, and agricultural importance (Dyck and Zingales, 2004). The sterile insect technique, as such, is the first involving genetics to control insect populations, which can be applied only to those pest species that reproduce sexually. Effective technologies provide sterile males that are sexually aggressive and successfully compete with wild males looking for and mate with native females. The SIT can be considered a form of contraception, and is carried out through several forms: the destruction of males, parasites and predators, hunting for plants, resistant varieties and hybrids, disrupting copulation by chemical and biological insecticides and physical control measures.

Mass trapping

The objective of mass trapping pest control or eradication is to capture a sufficient number of insects in the treated area before they are able to reproduce or damage crops. Controlling a pest by using a synthetic-sex pheromone in conjunction with mass trapping and communication disruption is less likely to damage the natural environment than insecticides (Yamanaka et al., 2011). Deploying traps that release pheromone/attractants to perceive a high proportion of target insects in a specialised field is the first step. Then, the baits are able to attract insects more effectively than natural attractions, like calling virgin females, mating aggregates, or food sources. Traps are effective in keeping hunting and attracted insects before they can steam or oviposit, during the entire period of the growing and breeding of pests. Also, the costs of this kind of pest control are lower than alternative treatments used in the commercial realisation of yield/quality. Mass trapping as a method of pest control has mainly been effective against several insects to date (Table 1). Mass trapping is very difficult to apply in crop production, mainly due to large surfaces which require substantially greater organisation by setting research, and the problems that occur with insulation. According to Howse et al. (1998), there are a number of difficulties in achieving efficacy in mass trapping using sex pheromones: (1) for most pest moth species, the trap target includes males only; (2) there is a lack of highly efficient traps; (3) there are problems of high density of insect populations and the saturation of traps with male moths, and so on. Each of these methods has advantages and disadvantages, like IPM. The implementation of IPM as an ecological approach is not questioned, and neither are long-term solutions for pests resulting from such programs. The biggest problem in farming is the size of the land. Large areas are very difficult to limit, partly due to the surrounding areas, such as raw wood, and partly due to the fragmentation of land. There is considerably more training required, as well as workshops and the practical view of how farmers would adopt the principles of IPM. AWPM, as shown in our areas, should explore the cultures of sugar beet (for the suppression of sugar beet weevil *Bothynoderes punctiventris*, small beet weevil *Lixus scrabricollis* Boheman 1842, maize leaf weevil *Tanymecus dilaticollis* Gyllenhal 1834, beet leaf weevil *Tanymecus palliatus* Germar, 1817) and soybean crops for the control of red spider mite (*Tetranychus urticae* Koch 1835).

Table 1. Overview of area wide programs

Crop	Pest	Area	Used/ Aim of	Literature
Maize (*Zea mays* L.)	Corn rootworm *Diabrotica spp.*	Midwest USA	Semio chemical-based bait to attract and kill	Chandler, 1998
Lodgepole pine, (*Pinuscont-orta* var. *latifolia* Engelm)	Mountain pine beetle *Dendroctonusp-onderosae* Hopkins	Canada	Aggregation pheromone for mass-trapping	Borden, et al., 1993
Tobacco store	Cigarette beetle *Lasioderma serricorne* (F.)	Greece	Pheromone baited multi-surface traps for male mass trapping	Buchelosand Levinson, 1993
Cherry and Apple orchards	scarab beetle *Anomala solida* Erichson 1847	Bulgaria	Attractant-baited traps to attract male	Tóth, et al., 2003
Isolated area	Japanese beetle *Popillia japonica* Newman	Minnesota	Pheromone-baited mass trapping	Wawrzynski and Ascerno, 1998
Stone fruit orchards	*Carpophilus mutilatus* Erichson *Carpophilus davidsoni* Dobson	Wales	Aggregation pheromone mass-trapping	James et al., 1998
Welsh onion *(Allium fistulosum* L.)	Beet armyworm *Spodoptera exigua* (Hübner)	Korea	Sex pheromones for mass trapping	Park and Goh, 1992
Peach orchard (*Prunus persica* (L.) Batsch)	Peachtree borer *Synanthedon exitiosa* Say Peachtree borer *Synanthedon pictipes*	Michigan	Pheromone dispensers for mating disruption	Teixeira, et al., 2010
Tea *(Thea sinensis* L.)	Tea tussock moth *Euproctis pseudoconspersa (Strand)*	China	Sex pheromone for mass trapping	Yongmo, et al., 2005

Crop	Pest	Area	Used/ Aim of	Literature
Coconut palms (*Cocos nucifera* L.)	Cocoa pod borer *Conopomorpha cramerella*	Sabah, East Malaysia	Pheromone-baited mass trapping	Beevor et al., 1993
Chinese scholar-tree (*Sophora japonica* L.)	Chinese tortrix *Cydia trasias* Meyrick 1928	Beijing, China,	Sex pheromone baited traps for mating disruption	Zhang et al., 2003
Apple (*Malus domestica* Borkh*)*, Pear (*Pyrus communis L.)*	Codling moth *Cydia pomonella* L.	Washington Oregon, California	Sex pheromone mating disruption	Knight, 2008
Data palm (*Phoenix dactylifera* L.)	Red palm weevil *Rynhophorus ferrugineus* Olivier	Al-Hassa, Saudi Arabia	Bait free attract and kill mass trapping	El-Shafie, et al., 2011
Erect prickelpear *Opuntia stricta* (Haw.) Haw.	Cactus Moth *Cactoblastis cactorum* (Berg)	Georgia	SIT (sterile males alone or in combination with fully sterile females)	Hight et al., 2005
Citrus and stone fruit	False Codling Moth *Thaumatotibia leucotreta* (Meyrick)	South Africa	SIT	Bloem et al., 2007
***Asimina triloba* (L.) Dunal**	Mediterranean fruit fly *Ceratitis capitata* (Wiedemann).	Central America	Aggregation pheromone mass trapping	Podleckis, 2007
Citrus	Mexican fruit fly *Anastrepha ludens* (Loew)	River Valley, Rio Grande Texas	Bait spray treatments with a preventive sterile fly release programme	Steck, 1998
Rice	Rice striped stem borer *Chilosuppressalis* (Walker) Yellow stem borer *Scirpophaga incertulas* (Walker) Pink stem borer *Sesamia inferens* (Walker)	Yangtze Delta, China	Pheromone trapping to trap and kill moths	Zhu et al., 2007
Olive (*Olea europaea* L.)	Olive fruit fly *Bactocera olea* (Gmel.*)*	Tuscany and Liguria, Italy	Aggregation and sex pheromones mass trapping, to lure and kill	Petacchi et al., 2003

Crop	Pest	Area	Used/ Aim of	Literature
Cotton (*Gossypium hirsutum* L.)	Pink bollworm, *Pectinophora gossypiella* Saunders	Safford, Arizona	Sex attractant pheromone for mass trapping male moths	Huber and Hoffmann, 1979
Persimmon (*Diospyros kaki* Thunb.)	Stinkbug *Plautia stali*	Japan	Aggregation pheromones lure Attract and kill	Yamanaka et al., 2011
Coconut palms (*Cocos nucifera* L.)	Red palm weevil *Rhynchophorus ferrugineus* Olivier	India	SIT to target populations at low densities	Krishnakumar and Maheswari, 2007
Urban, Suburban and forested areas	Emerald ash borer *Agrilus planipennis* Fairmaire	North America	Insecticide Baited pheromones to lure-kill	Herms and McCullough, 2014
Pastures	Fire ants *Solenopsis invicta* Buren and *Solenopsis richteri* Forel	Florida, Mississippi, Oklahoma, South Carolina and Texas	Insecticide Baited pheromones with pathogen *T. solenopsae*.	Vander Meer et al., 2008

4. MATERIALS AND METHODS

4.1. RESEARCH AREA

Investigations of sugar beet weevil have been carried out over four years (2012, 2013, 2014 and 2015) within the vicinity of the Municipality of Tovarnik (Figure 10). Geographic characteristics of the study area were determined by the coordinates 45°09'50"N/19°09'09"E.

Figure 10. Geographical location of the study area

Tovarnik is located in the East of the Republic of Croatia, in Vukovar-Sirmium County. More than 180 subjects dealt with agricultural production, out of which 117 dealt with arable crop production. Out of the 4,007 ha of agricultural arable land 2,400 ha are cultivated by medium local agricultural enterprises, 305 ha by small agricultural enterprises and 1,302 ha by family farms. In sowing structure, sugar beets represented 26%, cereals accounted for 32% and sunflower, soybean and corn each account for 14% (Vojvodić et al., 2015). The field area in Tovarnik is represented as chernozem soils with lower rates of humogley (Bogunović, 1987). According to the composition, the area belongs to the loess soils of chernozem (Bogunović et al., 1996).

4.2. CLIMATIC CONDITIONS

Analysis of climate data for the area of Tovarnik, all years was performed with help from the closest weather stations, from the Croatian Meteorological and Hydrological Service (Gradište; 45°52' N/18°58' E, Vukovar; 45°21' N/19°01' E). Out of the climate data, average air temperature (ºC), average daily temperature of the soil at 10 cm and sum of precipitation (mm) collected from the meteorological stations were used in the period from 1st January to 31st December. In attachment A, average monthly temperatures and monthly precipitations during 2011, 2012, 2013, 2014 and 2015 are presented in climate diagrams according to Walter (10 items). Climate diagrams according to Walter show a relation of average monthly temperature and monthly precipitation in a ratio of 1:4 (temperature of 10 ºC = 40 mm precipitation). The Ddiagram also shows the onset of drought and dried dry or wet conditions during the year. Also, the climate diagram quotes values of the absolute maximum and minimum temperatures during the years of research, like the average annual temperature and total annual precipitation.

Data on average air and soil temperatures and the sum of precipitation between among years were analyzed analysed by ANOVA (ARM 2016 GDM® software, Revision 2016.2 May 6, 2016) with mean separation estimated using Tukey's HSD mean separation on a standardized standardised summary.

4.3. LIFE TABLE PARAMETERS AND POPULATION CHARACTERISTICS

The population dynamics study for sugar beet weevil was carried out between 2012 and 2015 in infested fields under the climatic climate conditions of eastern Croatia. The study included the emergence and dynamics of adults, eggs larvae and pupae appearance and sexual index as the main indicators of dynamic changes in insect populations.

4.3.1. Dynamic of adults' emergence

Baited pheromone traps (Plant Protection Institute, CAR HAS, Budapest, Hungary) (Figure 11) were used to catch adult insects. Modified pitfall trap (TAL) is a trapping tool suitable to detect and to monitor adult sugar-beet weevils (Toth, et al., 2002).

Figure 11. Pitfall trap (TAL)

(Photo: Drmić, 2012.)

Detection and monitoring is achieved using plastic buckets which are dug into soil in old sugar beet fields. The traps were placed in the spring immediately after the conditions become favourable for pests to emergence. Beetles attracted by pheromone fall in and get caught. Traps were set up in old sugar beet fields at a rate of 15 traps/ha. The traps were installed in the second decade of March in 2012, 2014, 2015, and in the first decade of April during 2013. Traps were set each year for a period of 5-7 weeks and emptied once a week andtrapped weevils were counted. The last check was conducted at the beginning of May. The same traps were used for mass trapping of sugar beet weevils as described in chapter 4.4. The number of involved fields and traps involved varied between among the years. The data on the monitoring of adult emergence are shown in Table 2.

Table 2. The data on sugar beet weevil trapping, Tovarnik, 2012-2015

Year of trapping	2012	2013	2014	2015
Number of fields on which traps were set up	14	15	19	7
Number of set traps	929	3518	614	191
Week of first trap setting	12th week	14th week	11th week	12th week
Weeks last trap removing	18th week	19th week	18th week	18th week
Number of days on observations	46	38	42	41

4.3.2. Appearance of the eggs, larvae and pupal stages

To monitor the dynamics of pest development, the surveys of the old sugar beet fields and sugar beet plants on the newly sown fields were conducted periodically, every 10 days. For overwintering beetles the data collected in the previous section were used. For further development of the pest, ten sugar beet plants on newly sown sugar beet fields were dug together with the surrounding soil and carefully inspected for different life stages. Each observation was replicated four times. The number of surveys depended on the year (Table 3). The number of eggs, larvae, pupae and adults per plant were established.

Table 3. Data on soil survey for different life stages

Year of inspection	2012	2013	2014	2015
Number of inspections	21	22	22	20
Date of first inspection	11th March	1st March	1st March	1st March
Date of last inspection	2nd October	2nd October	2nd October	12th September

4.3.3. Sexual index

Out of each weekly trap emptying four samples containing 100 adults were separated,from which gender was determined. Collected weevils were kept in 96% ethanol with the help of binoculars gender was determinated with the use of binoculars. For gender determination, the characteristics (on the basis of the end of the abdominal ventral side) of males and females described by Čamprag (1984) were used. Usually, males are smaller, longer and lighter, and the third foot segment of the front leg is larger and longer than in females. The final tentacles segment is longer and thinner and there is a longitudinal cavity (Figure 12). As a noteworthy sign of recognition equality, Tielecke (1952) lists the characteristics of the dorsal end of the abdomen (Figure 13), which are observed when the needle for the preparation of insects raises the elytra. Females is are known by for the more chitinized chitinised back plate at the end of the abdomen, and the last interior segment externally is not visible externally, while two plates in males can be seen in malestwo plates.

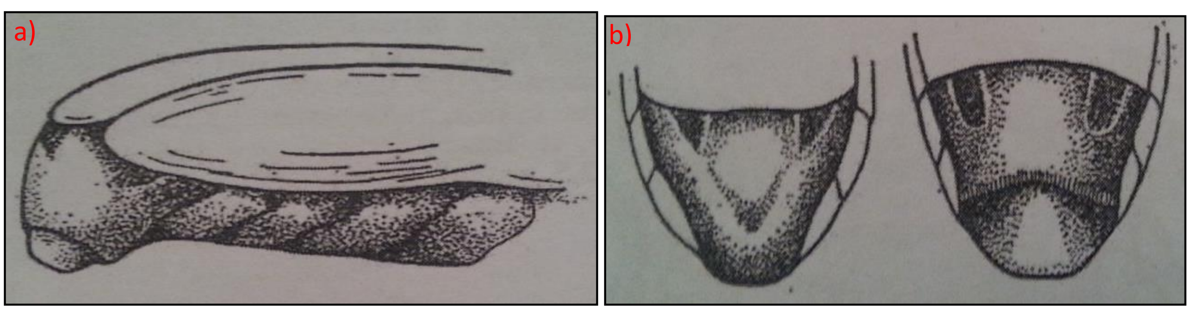


Figure 12. The last segment of the abdomen in adult's weevils: a) in females; b) in females and males in on the right as seen from the dorsal side (Auersch, 1954; Čamprag, 1984).

Figure 13. The final part of the last segment of the weevils abdomen viewed from the ventral side: a) up - in males (E - small dent); b) down - in females (Auersch, 1954; Čamprag, 1984) (Photo: Drmić, 2012)

4.3.4. Population fluctuation

To establish population fluctuation and the impact of winter conditions on sugar beet weevil overwintering ability, soil surveys were conducted twice, in the spring and autumn. The soil examination in the spring was carried out on fields where sugar beet was sown the previous year. Depending on the year, the soil survey in the spring was conducted between the 10th and 12th week of the year (Table 4). The soil survey in the autumn was carried out after sugar beet was harvested; in October (and September in 2015) in all sugar beet fields that were harvested in particular area in which mass trapping had been conducted (see chapter 4.4.) (Table 4). The same fields were inspected in the spring again. For soil inspection holes were dug with the dimensions 0.5 m x 0.5 m (0.25 m^2) up to a depth of 0.5 m. The number of samples (pit) is determined regarding the size of the field surveyed. In fields up to five hectares four holes were inspected, while 8 holes were inspected in areas of 5-10 ha and 12-15 holes were made in fields larger than 10 hectares were made 12-15 hole. Most of Tthe fields were are mostly about size up to 5 hectares in size and are inspected using with 4 samples on the field. Exceptionally, some fields that were over 5 ha; in 2012, one a field of was 36 ha (8 samples), and in 2013 there were two the fields of of 130 and 60 ha (with 16 samples each).

Soil inspections were conducted at selected moments when soil had a medium moistness. During the soil examination data of pest number crossing the surface were recorded. The excavated soil was inspected for the presence of adults. Since one hole covers 0.25 m^2, the average number of adults per/m^2 was calculated by multiplying the average number of adults/hole by with four. The average infestation of fields in the inspected area with sugar beet weevils in the autumn and spring was established. Based on the data of average infestation with sugar beet weevils/m^2 we calculated the population growth index as it is explained in the section.

Table 4. Data on soil surveys

Data	Years of the survey (autumn/spring)			
	2011/2012	2012/2013	2013/2014	2014/2015
Week of autumn soil survey	x	41^{st} – 43^{rd}	44^{th}	36^{th} -37^{th}
Number of fields examined in autumn	x	15	2	7
Number of holes dug in autumn	x	82	8	40
Week of spring soil survey	11^{th} – 12^{th}	10^{th}	7^{th}-10^{th}	11^{th}
Number of fields examined in spring	14	15	2	7
Number of holes dug in spring	56	68	80	36

4.3.5. Data analysis

a) Dynamics of adults' emergence

Data on weevils' emergence were expressed as the percentage of total weevil abundance for each week. The percentages's of weevil abundance in different weeks were compared between years by ANOVA (ARM 2016 GDM® software, Revision 2016.2 May 6, 2016), and was estimated using the Tukey HSD test and the mean values. Where appropriate, data were $\sqrt{(x+0.5)}$ transformed. All values were determined from 12^{th} to 19^{th} of the year. In order to establish the timing of the first weevil emergence we calculated degree day accumulation (DDA) for each period of beetle emergence. The threshold of 5 °C for soil temperature at 10 cm depth has been used. The statistical software ARM 2016 (GDM® software, Revision 2016.2 May 6, 2016) was used to calculate correlation coefficients and to conduct regression analysis between the independent variables: the degree day accumulation (°C) for air and for soil temperatures vs. the percent of beetles emerged from the soil as dependent variable. The value of the correlation coefficient was ranked using the very precise Roemer-Orphal scale (0.0-0.10, no correlation; 0.10-0.25, very weak; 0.25-0.40, weak; 0.40-0.50, modest; 0.50-0.75, strong; 0.75-0.90, very strong; 0.90-1.0, full correlation) at the 95% confidence level (Vasilj, 2000).

b) Appearance of the eggs, larval larvae and pupal stages

For each inspection date in every year of investigation, the data on the share of each particular developmental stages were compared among the stages by ANOVA (ARM 2016 GDM® software, Revision 2016.2 May 6, 2016), and with the mean separation was estimated using the Tukey's HSD test. Based on the specimen findings in different stages (imago, egg, larvae, and pupae) of the pest, life table of the sugar beet weevil has been composed.

c) Sexual index

For each inspection date (week) sexual indexes has been established. Established sexual index in each week was compared between genders by ANOVA (ARM 2016 GDM® software, Revision 2016.2 May 6, 2016), and the mean separation was estimated using Tukey HSD test. Where appropriate, data were $\sqrt{(x+0.5)}$ transformed.

d) Population fluctuation

Based on the number of weevils established by soil survey in the autumn and spring the overwintering success rate was calculated for each field (0-100%). The data on overwintering success rate were compared between years by ANOVA (ARM 2016 GDM® software, Revision 2016.2 May 6, 2016), the mean separation was estimated using the Tukey HSD test.

The population growth rate in marked areas has been calculated based on average infestation of old sugar beet fields in marked area established in the spring and the number of weevils/m^2 established on the sugar beet fields before their overwintering, in the autumn. The statistical software ARM 2016 (GDM® software, Revision 2016.2 May 6, 2016) was used to calculate correlation coefficients and conduct regression analysis between the independent variables: average air temperatures (°C), total precipitation (mm) average soil temperature at a depth of 10 cm during the vegetation period (from March to September), average air and soil temperatures in May, total amount of rainfall in May and ratio between new and old sugar beet fields in marked area vs. the population growth rate as a dependent variable. The value of the correlation coefficient was ranked using the very precise Roemer-Orphal scale (0.0-0.10, no correlation; 0.10-0.25, very weak; 0.25-0.40, weak; 0.40-0.50, modest; 0.50-0.75, strong; 0.75-0.90, very strong; 0.90-1.0, full correlation) at the 95% confidence level (Vasilj, 2000).The overwintering success rate was correlated with the average air temperatures (°C), total precipitation (mm) and average soil temperature at a depth of 10 cm during the overwintering period (from October to February).

4.4. AREA WIDE CONTROL OF SUGAR BEET WEEVIL BY MASS TRAPPING

4.4.1. Mass trapping area

The mass trapping of pests in large areas was implemented over four years (2012, 2013, 2014 and 2015) within the vicinity of the Municipality of Tovarnik using an aggregation pheromone manufactured in Hungary (Csalomon).

In the spring of 2012, the borders of the mass trapping area, including 111 fields and covering a total area of 537 ha, were determined (Figure 14). Mass trapping was implemented within a total area of 6 km^2. All fields were identified in ARKOD. Information regarding the land owners was obtained, and owners were asked about the crops sown in 2011.

Figure 14. Map of area wide mass trapping in 2012

In 2013 the mass trapping was conducted on all old sugar beet fields within the same area (Figure 15).

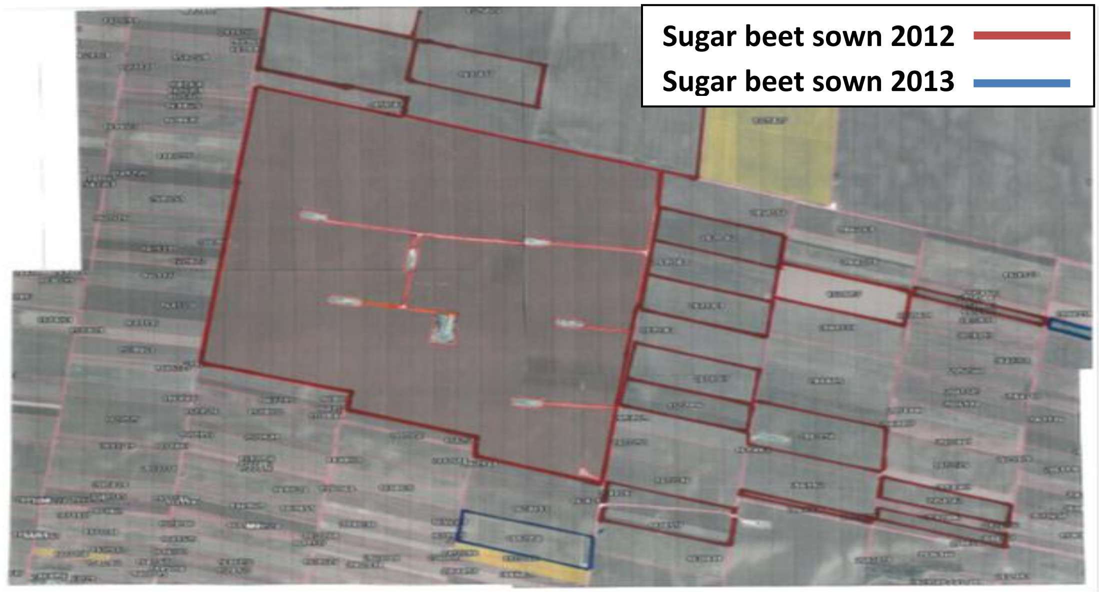


Figure 15. Map of area wide mass trapping in 2013

After identifying any fields sown with sugar beet by agricultural producers in 2013 (Figure 15), the area of mass trapping in 2014 was extended to an additional 576 fields (Figure 16). The total area of research in that year amounted to 1,326 ha with 687 fields. The total mass trapping area therefore was expanded by an additional 8.8 km^2, for a total area of 14.8 km^2 (Figure 16).

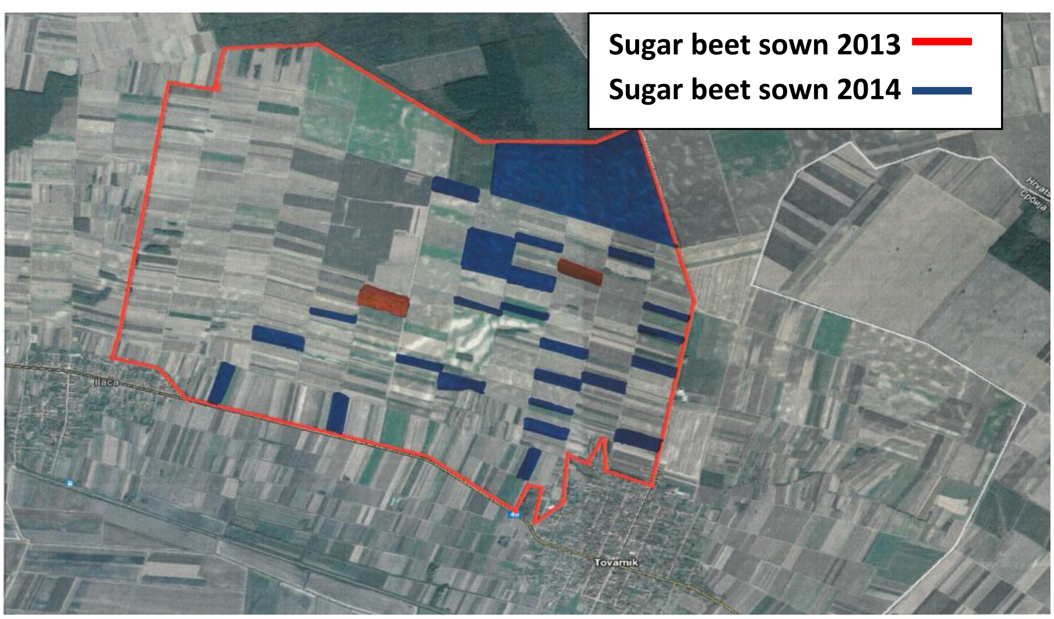


Figure 16. Map of area wide mass trapping in 2014

In 2015, the research area was reduced to the same area as in 2012 and 2013 (Figure 17).

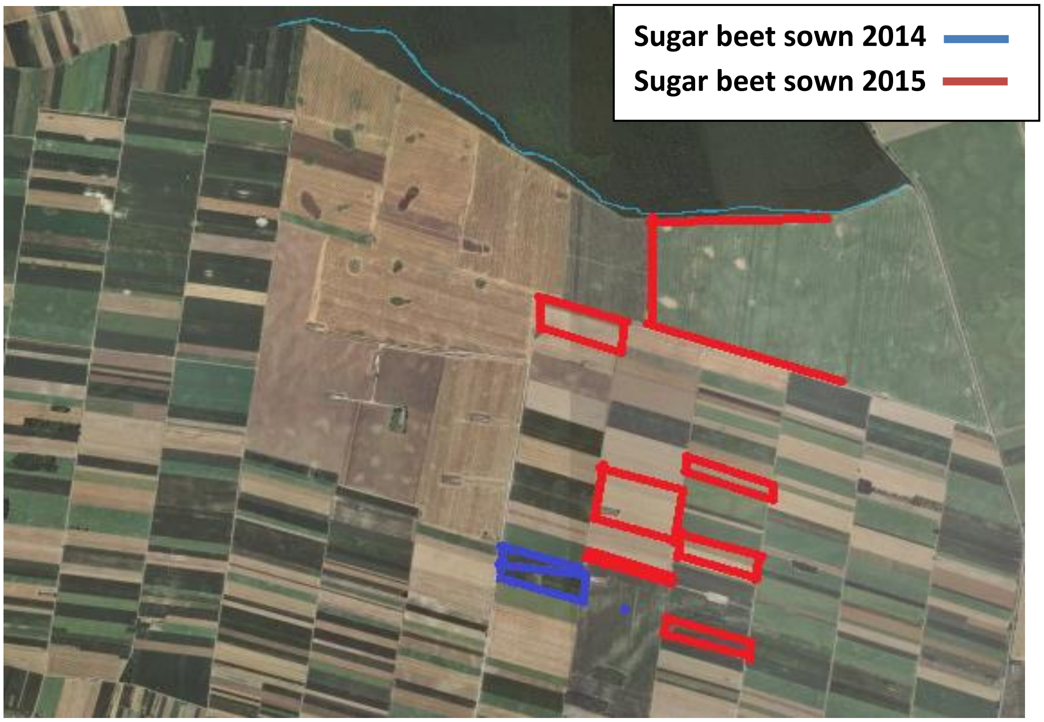


Figure 17. Map of area wide mass trapping in 2015

The overview of the research area in which mass trapping of sugar beet weevil has been implemented is shown in Figure 18.

Figure 18. Map of the mass trapping area from 2012-2015

4.4.2. Pheromones and methods of implementation

For the mass trapping of sugar beet weevils the aggregation pheromones described by Toth et al. (2002) were used. The commercially available pheromone traps produced by Plant Protection Institute, CAR HAS, Budapest, Hungary were used. Tall traps are designed as plastic buckets (17.5 cm long, 12 cm wide and 8 cm high at opening, 16.5 cm long and 11 cm wide at bottom, 1.7 l capacity) and were used as pitfall traps (Figure 11). Also, there were 5 holes (2–3 mm diameter) bored ino the bottom part serving as outlets for the water from rain. Synthetic attractant in bait dispensers was a 1:1 mixture of (Z) - and (E)-2-ochtodenal [¼Grandlure III–IV; (Z) - and (E)-(3, 3-dimethyl) cyclohexylidene acetaldehyde] (Tomašev et al., 2007). The overall purity of the sample was 99% by GC. Rubber dispensers were prepared by using pieces of rubber tubing (Taurus, Budapest, HG; No. MSZ 9691/6; extracted 3 times in boiling ethanol for 10 min, then also 3 times in methylene chloride overnight). The rubber dispensers were attached to 8x1 cm pieces of plastic sheet for easier handling when assembling the traps. For making up the baits, 500 mg of the 1:1 mixture of (Z)- and (E)-2-ochtodenal (Grandlure III–IV) was administered to the surface of the rubber dispensers in hexane solutions.

The traps (Figure 19) were installed on all old sugar beet fields in a marked area in the second decade of March in 2012, 2014 and 2015, and in the third decade during 2013. The last check was conducted in the beginning of May. Traps were set each year for a period of 5-7 weeks, and were inspected and emptied once a week. Every year we established a net of baits, so that within the area of research traps covered all fields of sugar beet sown in the previous year.

Figure 19. Setting traps for mass trapping (Photo: Drmić, 2012)

The number of traps per ha was 15, for each field, depending on the field size and shape, the schematic arrangements of the traps was prepared in advance. In 2012 traps were distributed on the whole field surface and in 2013-2015 traps were set up 15-20 m from the field edge distributed in one or two lines following the field edges. The distance between traps was 15-20 m, as shown in Figure 20.

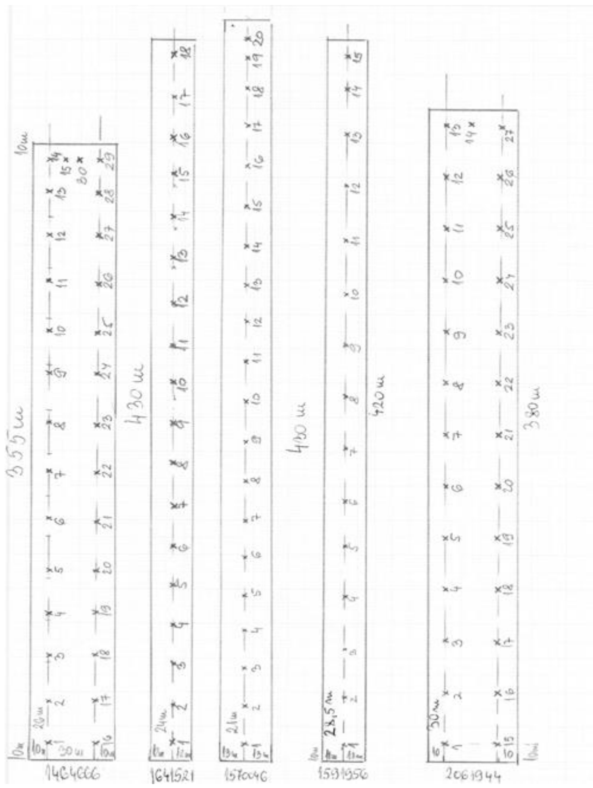


Figure 20. Distribution of the traps (x) on one field

The mass trapping was conducted on all sugar beet fields within the marked area. The number in fields on which mass trapping was conducted varied from 7 (in 2015) to 19 in 2014 (Table 5). The surface of the fields on which area wide mass trapping was employed varied from 41.24 ha in 2014 to 237.19 ha in 2013. The number of traps employed for mass trapping varied from 191 in 2015 to 3,558 in 2013.

Table 5. Number and surface of fields (ha) on which mass trapping was carried out

Year of research	Previous year sugar beet		Number of traps	Newly sown sugar beet	
	Number of fields	Ha		Number of fields	ha
2012	14	64.98	929	17	237.19
2013	15	241.42	3518	2	5.54
2014	19	440.87	614	23	157.66
2015	7	12.93	191	4	170.19

4.4.3. Determining the infestation and damage in newly sown sugar beet fields

All fields sown with sugar beet in the marked area in each year of investigation were regularly inspected once a week on an average number of weevils and leaf area weevil damage. The same was done in each year on two control fields outside the marked area where no mass trapping had been conducted. Inspections were made so that a wooden square (area of 1 m^2) was randomly cast on the surface and then all individuals inside the square were counted. Within, in the same square, was counted all emerged beet were counted. Out of counted number of plants, damaged ones were classified into categories based on the percentage of damaged leaf area. Based on the frequency of plants with a certain percentage of damage calculated % of damage was calculated.

In the results we worked with TH-values (Townsend-Heuberger) (Townsend & Heuberger, 1943).

These values are used when the result of an observation is divided into 5 different classes.

$$\% \ \mathbf{damage} = \frac{\mathbf{\Sigma(f \ x \ n)}}{\mathbf{a \ x \ N}} \ \boldsymbol{x} \ \mathbf{100}$$

Where, f is the number of plants in the group;

n = score group (0-4);

a = the number of groups (in this case 5);

N = total number of plants in the sample examined.

Damage was Classes in which were classified as follows damage (Čamprag, 1984):

0. No damage;
1. 1-25% of plant parts damaged;
2. 26-50% of plant parts damaged;
3. 51-75% of plant parts damaged;
4. Over 76% of plant parts damaged.

On a large plot, - the central field (Figure 19) surveys were done performed on all four edges (edge considers 20 m from entering). The square was cast 4 times on the edges and once time in the interior of the field. Each square cast consisted of 4 repetitions, out of which is an averaged number was recordedin results.

The use of insecticides on all newly sown sugar beet fields inside the marked area was recorded regularly in order to establish average amounts of insecticides used for sugar beet weevil control within the areas where mass trapping has been was conducted.

In Aadditionally to two fields outside the marked area on in which sugar beet weevil infestation and damages were recorded, three fields were recorded regarding for the use of insecticides for sugar beet weevil control. Therefore, five yields outside the marked area were monitored for the use of insecticides for sugar beet weevil control.

4.4.4. Data analysis and determining the success of area wide mass trapping

As described in the chapter 4.3.4., soil samples were dug in the spring on all "old" sugar beet fields in the marked area and in the autumn on all "new" sugar beet fields. For each field average infestation/m^2 was calculated based on the average number of weevils found in soil samples multiplied by four (the number of adults found on the soil surface was added to those found in soil samples). Based on the average infestation/m^2 and the field size, the total population for each field and the total population in the entire marked area were estimated. For each field the number of beetles caught in pheromone traps at each inspection was recorded and the total caught population has been estimated. The population reduction was expressed as the percent of caught beetles comparing to the spring population.

Pests found in fall soil survey can be classified into 4 groups of infection; (a) poor; up to 0.5 weevils/m^2, (b) mean; 0.6-3.0 weevils/ m^2, (c) strong; 3.1 - 10 and over 10 weevils/ m^2 is very strong infection (Popov, 1965). At presence over 1 weevil/ m^2 strong pest's offense has to be considered in the next growing year in sugar beet fields (Stojanović et al., 1971).

The data on field infestation with sugar beet weevil established in autumn and is spring soil surveys as well as the data on the weevil capture in pheromone traps were compared among fields and years by ANOVA (ARM 2016 GDM® software, Revision 2016.2 May 6, 2016) and with the mean separation was estimated using the Duncan's multiple range test. Where appropriate, data were log x+1 or arc. sin √x transformed.

In order to determine the success of AW mass trapping, the four basic parameters were used:

a) comparison of the number of weevils in the marked area estimated based on the soil samples taken from old sugar beet fields in the spring and number of weevils caught in pheromone traps;

b) average infestation of sugar beet fields in the marked area expressed as number of adults/m^2, and damage established on sugar beet plants;

c) average infestation of sugar beet fields outside the marked area expressed as number of adults/m^2, and damage established on sugar beet plants on two fields in each year of investigation;

d) average number of insecticide treatments and amount of insecticide applied/ha in the fields inside the marked area and the fields outside marked area.

4.5. ESTABLISHING EFFICACY OF ENTOMOPATHOGENIC NEMATODES (EPN) ON SUGAR BEET WEEVIL

In order to establish potential of the entomopathogenic nematode *Heterorhabditis bacteriophora* to reduce the sugar beet weevil population, field and laboratory trials with the commercial product Nematop (Figure 21) (manufacturer e-Nema, Croatian representative ProEco) were carried out. In all trials the efficacy of three different doses (3, 5 and 7 million of nematodes /10 m^2) was established.

4.5.1. Laboratory experiments

Laboratory experiment in 2014

Research was carried out on plants of sugar beet sown in greenhouses in Zagreb (Faculty of Agriculture, Department of Agricultural Zoology). Seeds (in pairs) were sown in plastic containers with a diameter of 8 cm and a depth of 10 cm. Pots were watered daily. In April 2014, in the area of Tovarnik, specimens of sugar beet weevil were collected and transported to the Laboratory of Entomology of the Faculty of Agriculture in Zagreb. After sex determination (under the stereomicroscope), couples of weevils were released into entomological cages in which containers with sugar beet were also placed. Examination of soil was performed every day and eggs were isolated. Through 20 days of examinations of the soil, a total of 56 eggs were determined. After the larvae had begun to emerge from the eggs, trial was set up.

Figure 21. Nematop: a) weighed quantity of Nematop; b) Nematop solution in water, c) watering sugar beet plants with nematodes (Photo: Šatvar, 2014.)

Three variants were set with Nematop solution containing 3, 5 and 7 million nematodes, along with a control. The experiment included three replicates per variant. Five eggs (larvae) were placed in two bowls with sugar beet and 4 eggs (larvae) were placed in the third container. The required amount of Nematop solution was calculated based on the amount required in field conditions. The average number of sugar beet plants in the field was 100,000 plants/ha, which is one plant per 0.1 m^2. For the required quantity of 0.1 m^2, 30,000, 50,000 and 70,000 nematodes per plant were applied by watering, in a water solution of 50 ml.

Figure 21. Nematop: a) weighed quantity of Nematop; b) Nematop solution in water, c) watering sugar beet plants with nematodes (Photo: Šatvar, 2014.)

Three variants were set with Nematop solution containing 3, 5 and 7 million nematodes, along with a control. The experiment included three replicates per variant. Five eggs (larvae) were placed in two bowls with sugar beet and 4 eggs (larvae) were placed in the third container. The required amount of Nematop solution was calculated based on the amount required in field conditions. The average number of sugar beet plants in the field was 100,000 plants/ha, which is one plant per 0.1 m^2. For the required quantity of 0.1 m^2, 30,000, 50,000 and 70,000 nematodes per plant were applied by watering, in a water solution of 50 ml.

Figure 21. Nematop: a) weighed quantity of Nematop; b) Nematop solution in water, c) watering sugar beet plants with nematodes (Photo: Šatvar, 2014.)

Laboratory experiment in 2015

In mid-March 2015, 5 seeds of sugar beet (KWS Jadranka) were sown in containers. After the emergence of sugar beet, pots with seedlings were moved out of the greenhouse. (Faculty of Agriculture, Department of Agricultural Zoology) As in 2014, adult sugar beet weevils were collected in 2015 in the area of Tovarnik and transported to the Laboratory of Entomology of the Faculty of Agriculture in Zagreb. After sex determination (under the stereomicroscope), couples of weevils were released into entomological cages in which containers with sugar beet were also placed. Examination of the soil was performed every day, but the eggs were not isolated from the soil. Therefore, the number of eggs in containers was not known. On May 29th, 2015, four variants of ten repetitions were setup. There were 3 plants in each repetition there was 3 plants, or 30 plants per treatment. The calculated quantity of Nematop was dissolved in 1 l of water and an amount of 100 ml/pot was applied as the treatment.

4.5.2. Field experiments

Field experiments were conducted with the entomopathogenic agent Nematop during 2014 and 2015. Field trials in both years were setup in the area of Tovarnik. In the first year, a plot of 105 ha was selected, while a field with an area of 4.06 ha was selected in the second year. The selection of fields was affected by the unevenness of the soil surface. Sugar beet weevils lay their eggs on elevated spots in fields (Maceljski, 2002).

Before setting up the experiment, visual inspection was conducted to determine the number of pests. The number of sugar beet weevils was established by randomly throwing a wooden square (m^2) into the sugar beet field, and weevils in the square were counted. In 2014, the number of pests was determined on 22nd April and amounted to 0.75 weevils/m^2 or 7,500 weevils/ha. The next year, 2015, the number of pests was established in the same way. On May 27th, a pest amount of 0.5 weevils/m^2 or 5,000 weevil/ha was visually found.

The basic data on trial treatments are shown in Table 6.

Table 6. Number and amount (g) of the applied product in 2014 and 2015

Trial tretament		Number of nematodes / 10 m^2	Amount (g) of nematode producton 10 m^2	Number of nematodes in 100 l/270 m^2	Amount (g) of nematode product in 100 l/270 m^2
1	Nematop	3 million	27	81 million	72.9
2	Nematop	5 million	39	135 million	105.3
3	Nematop	7 million	45	189 million	121.5
4	Control	0	0	0	0

The experimental area in the plot in both years amounted to 1,080 m^2; it encompasses 36 rows of sugar beet (18 m) with a length of 60 m. Nematode products were applied by spraying according to the date established based on the observation of sugar beet weevil copulation. It was performed ten days after weevil copulation. In 2014, this was on 10th May and in 2015 it was 1st June. Before nematode application, pure water in a quantity of 3,700 l/ha (i.e. 400 l/1,080 m^2) was applied on the whole trial surface using a trailed sprayer, Amazone UG 3000 Special, with a working width of 18 m (Figure 22). The amount of nematode product was calculated in order to achieve the optimal dose per ha (Table 6). Applied treatments were Nematop in doses of 3, 5 and 7 million nematodes, along with an untreated control. Each treatment was applied on a plot of 270 m^2 (i.e. 36 rows 15 m long) with 100 l of water (i.e. 3,700 l of water/ha). For the untreated control, pure water was applied. After the nematode application, pure water was again applied in a quantity of 400 l (i.e.3,700 l/ha) over the whole trial surface (Figure 23).

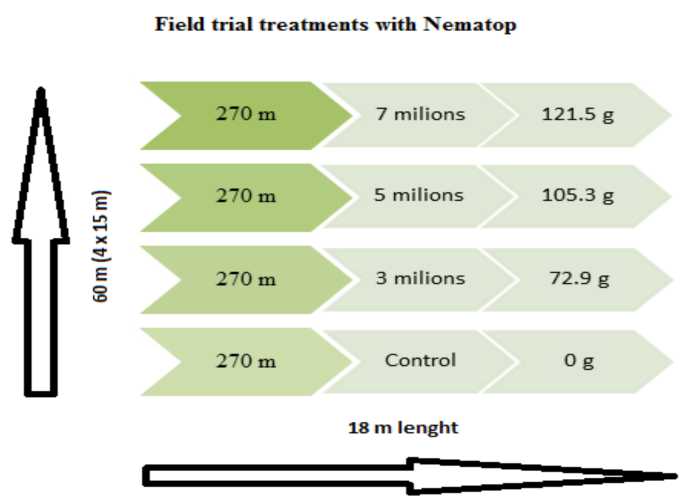


Figure 22. Experiment field area

Figure 23. Application of entomopathogenic nematodes in 2014 (Photo: Drmić) and in 2015 (Photo: Šatvar)

4.5.3. Efficacy assessment

Laboratory trials

In 2014, on two occasions (June 10th and June 25th), sugar beet plants in containers were inspected. In the first examination, only the soil around the plants was carefully inspected, while the second plant check was conducted by removing soil. With the help of a stereo loupe, roots and all of the soil from the jar were reviewed. Also in 2015, on two occasions (July 3rd and July 7th), plants and soil in jars were inspected.

Field trials

Examinations of sugar beet roots were conducted every two weeks in both years. It started four weeks after the application of treatment. The first examination was on 7th June and lasted until 1st September 2014 (a total of 7 inspections), while the examination lasted until 31st August in 2015. Five samples containing 5 plants (roots) per treatment were collected at each inspection. Altogether, every two weeks 100 roots of plants were removed and examined. On root inspection, the number of infected roots, the number of larvae on the roots and the development stage of the larvae were determined. After thickening, root dissection was performed in order to determine the number of larvae on the surface and in the interior of sugar beet root.

4.5.4. Data analysis

Based on the number of larvae/sample established in the treatment and in the untreated control, the efficacy of the trial treatment was determined according to Abbott's formula (1925). Results were analysed using analysis of variance procedures with the ARM 9® software (Gylling Data Management, 2014), with mean separation estimated using the Duncan's multiple range test. Where appropriate, data were *arc.sin*√*(x* transformed.

5. RESULTS

5.1. CLIMATIC CONDITIONS

Although the investigations started in 2012, the data from 2011 were included in all analyses because it could be assumed that the population developed in 2012 was under the strong influence of climatic data which prevailed in 2011. In attachment (A), average monthly temperatures and monthly precipitation during 2011, 2012, 2013, 2014 and 2015 are presented in a climate diagram according to Walter. The climate diagram according to Walter shows a relation of the average monthly temperature and monthly precipitation in a ratio of 1:4 (temperature of 10ºC = 40 mm precipitation). The diagram also shows the onset of drought and dried or wet conditions during the year. Also, the climate diagram quotes values of the absolute maximum and minimum temperatures during the years of research, like the average annual temperature and total annual precipitation.

The comparison of average monthly air and soil temperatures and total amount of precipitation is shown in Figures 24-26.

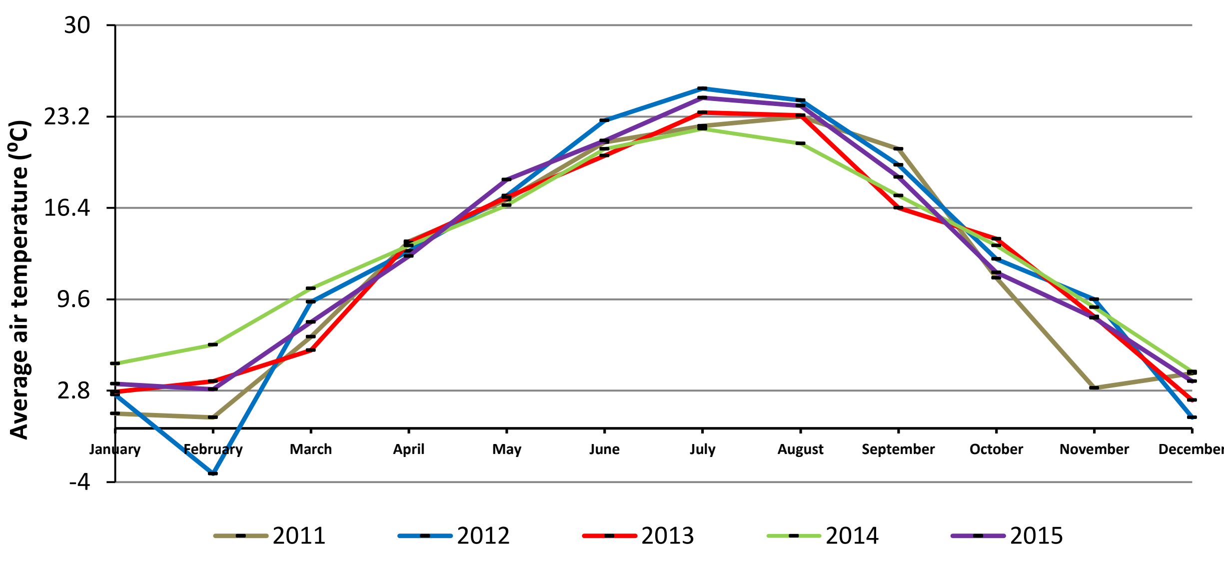


Figure 24. Average monthly air temperatures in investigated areas in 2011, 2012, 2013, 2014 and 2015

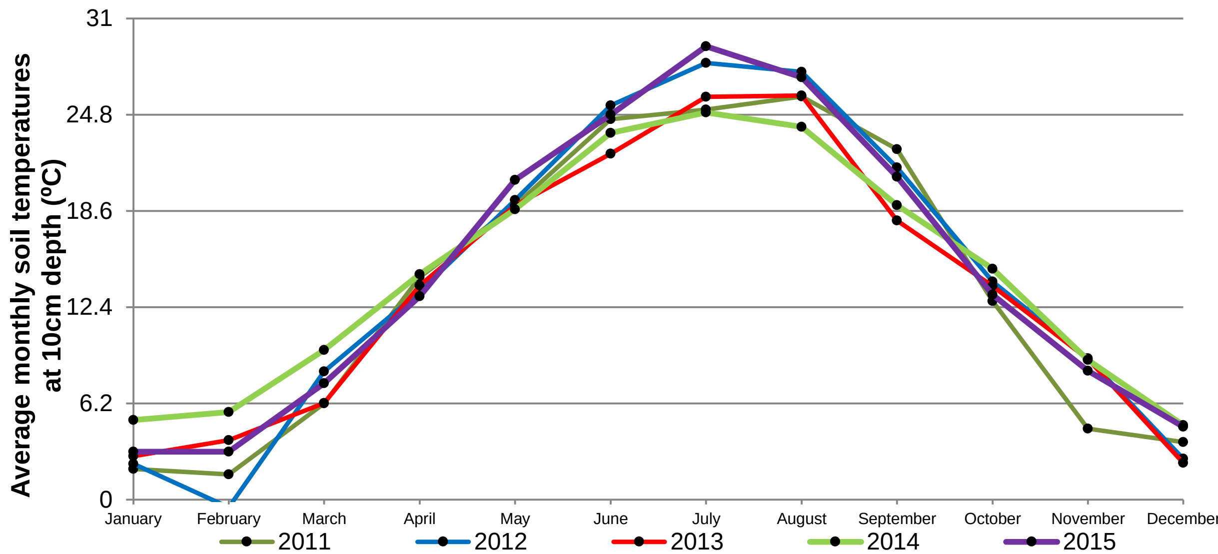


Figure 25. Average monthly soil temperatures (10 cm depth) in investigated areas in 2011, 2012, 2013, 2014 and 2015

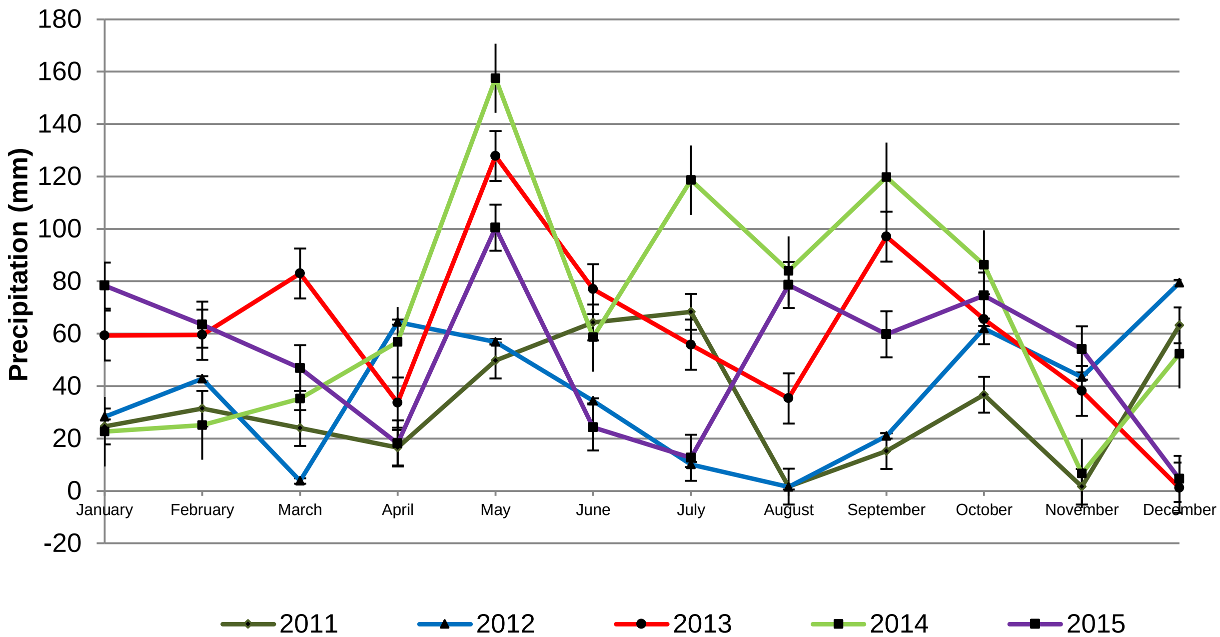


Figure 26. Total monthly amount of precipitation in investigated areas in 2011, 2012, 2013, 2014 and 2015

The comparison of the climate condition among five years (2011-2015) is shown in Table 7.

Table 7. The comparison of the average climatic data in the investigated area of Tovarnik, 2011-2015

Year	Average air temperature (°C)	Average soil temperature (°C)	Total amount of precipitation (mm)
2011	12.11 d	13.74	395.45 c
2012	12.82 b	14.47	487.10 c
2013	12.49 c	13.8	721.35 ab
2014	13.19 a	14.56	823.10 a
2015	13.05 ab	14.44	629.65 b
LSD P=0.05	0.296	ns	124.5

As the overwintering period of sugar beet weevil is five months, October, November, December, January and February could be considered. The climate data (average air and soil temperature and total amount of precipitations) prevailing during this period for four overwintering seasons (2012/2013, 2013/2014, 2014/2015 and 2015/2016) were analysed and the results are shown in Table 8.

Table 8. A Comparison of the average climatic data during the overwintering period (October-February) in the investigated area Tovarnik, 2011-2016

Overwintering season	Average air temperature (°C)	Average soil temperature (°C)	Total amount of precipitation (mm)
2011/2012	3.38 d	4.79 d	176.70 c
2012/2013	5.75 c	6.63 c	312.75 a
2013/2014	6.96 a	7.31 a	169.70 c
2014/2015	6.49 b	7.01 b	286.70 a
2015/2016	5.88 c	6.33 b	219.35 b
LSD P=0.05	0.229	0.813	42.247

The period of sugar beet weevil development from juvenile to adult lasts seven months, from March until September. Therefore, the climate data (average air and soil temperature and total amount of precipitations) which prevailed during this period of seven months in four vegetation seasons (2012, 2013, 2014, and 2015) were analysed; the results are shown in Table 9.

Table 9. The comparison of the average climatic data during the vegetation period (March-September) in investigated area Tovarnik, 2012-2015

Vegetation season	Average air temperature (°C)	Average soil temperature (°C)	Total amount of precipitation (mm)
2012	18.86 a	20.78 a	220.35 c
2013	17.14 c	19.03 b	484.15 ab
2014	17.31 c	19.26 ab	621.15 a
2015	18.20 b	20.22 ab	348.15 bc
LSD P=0.05	0.409	1.682	157.18

Some authors (Maceljski, 2002) stated that for sugar beet population growth the most critical period is May when the weevils are laying eggs and larvae started to emerge. We analysed the climate data (average air and soil temperature and total amount of precipitations) prevailed during this month in four vegetation seasons (2012, 2013, 2014, and 2015) and results are shown in the table 10.

Table 10. The comparison of the climate data in May in the investigated area Tovarnik, 2012-2015

Year	Average air temperature in May (°C)	Average soil temperature in May (°C)	Total amount of precipitation in May (mm)
2011	16.9 b	18.88 b	48.8 d
2012	17.10 b	19.1 b	66.4 cd
2013	17.05 b	19.02 b	122.85 b
2014	16.4 c	18.61 b	161.25 a
2015	18.35 a	20.3 a	99.55 bc
LSD P=0.05	0.263	0.804	34.767

5.2. LIFE TABLE PARAMETERS AND POPULATION CHARACTERISTICS

5.2.1. Dynamic of adults' emergence

The dynamic of adults' emergence, expressed as the share of collected weevils in the total capture among the four years of investigation, is shown in Figure 27.

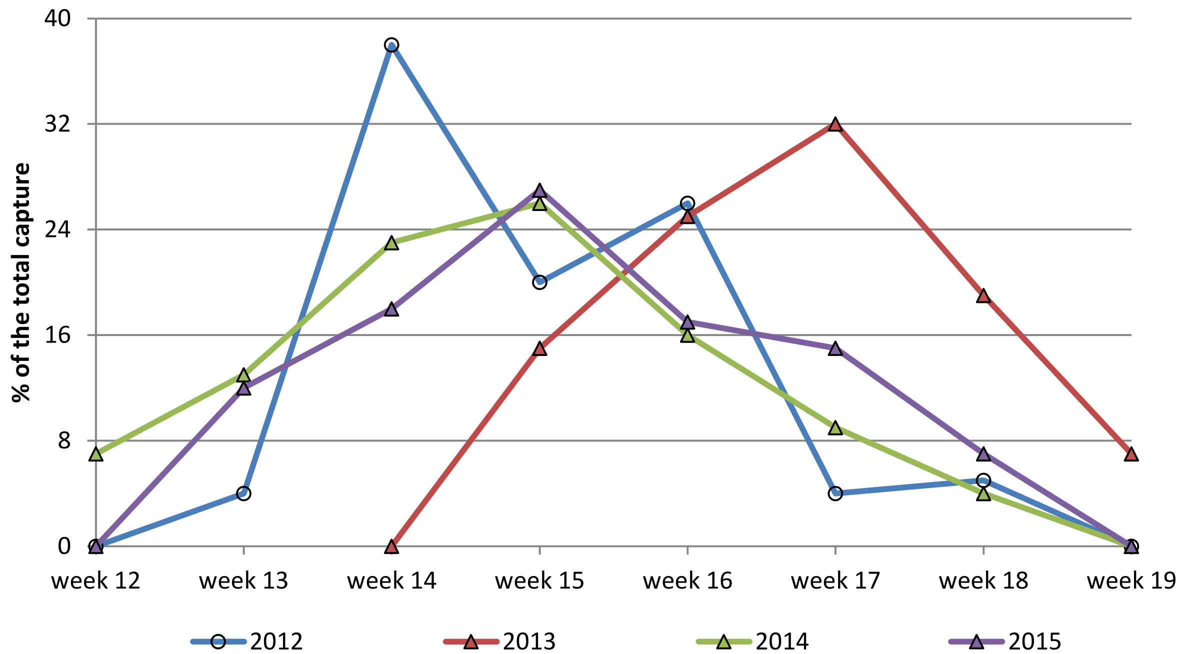


Figure 27. The dynamic of sugar beet adult emergence from overwintering sites expressed as the share of the number of collected weevils in the total capture

In order to establish the time period when the beetle emergence is the most abundant, we analysed the data on adult capture among eight weeks (from 12th to 19th week). The results are presented in Figure 28.

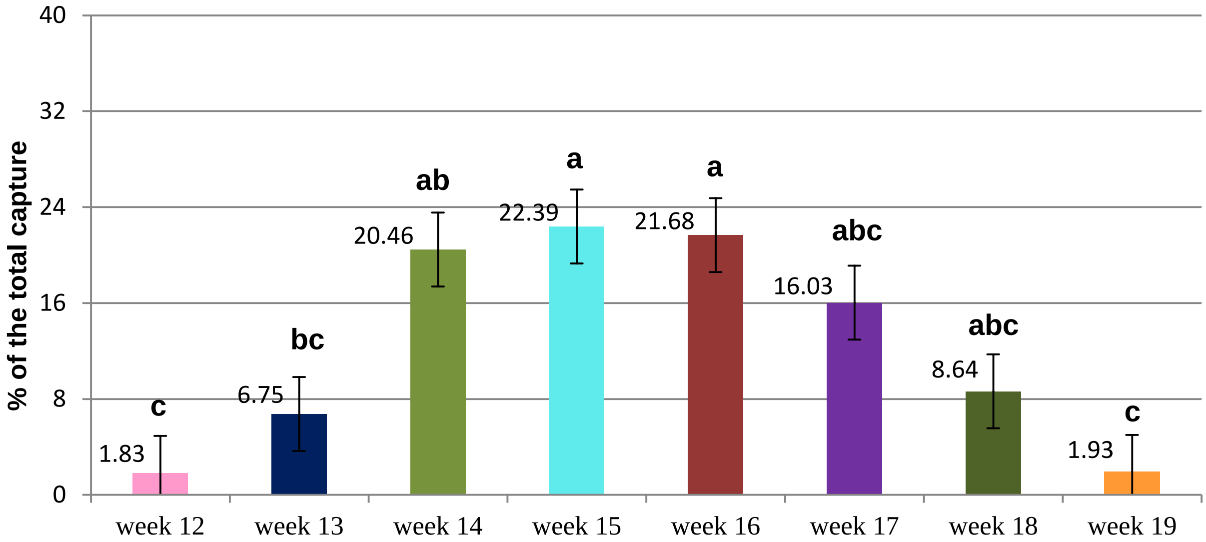


Figure 28. Average adult capture of sugar beet weevil (% of the total capture) during the eight weeks of survey on overwintering places (2012-2015)

Degree Day Accumulations (DDA) was calculated for the first 130 Julian days for soil temperature at 10 cm depth. As thermal threshold 5°C has been used. Results are shown in the Figure 29.

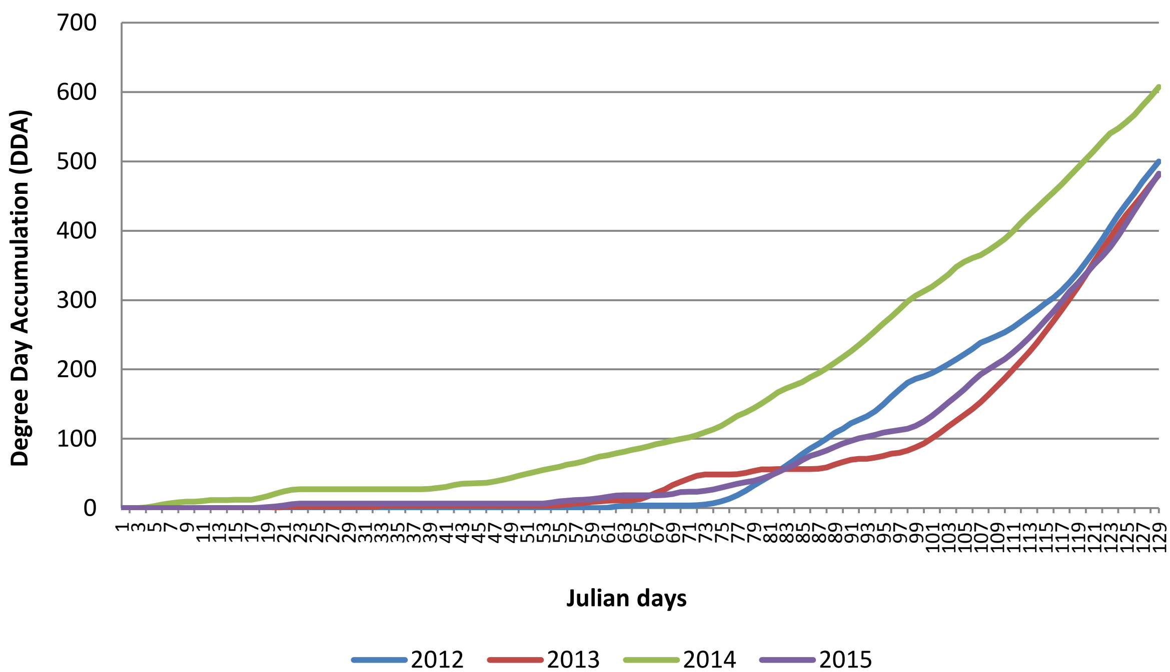


Figure 29. Degree Day Accumulations (DDA) in °C for soil temperature at 10 cm depth in the years 2012–2014 (the thermal threshold of 5°C has been used)

The percent of beetle emergence in each week was correlated with average air and soil temperature and the amount of precipitation in the week when the emergence occurred. Results are shown in Table 11.

Table 11. Correlation coefficients and coefficients of determination for the dynamics of the emergence of sugar beet weevil (y) on mean degree day accumulation (DDA) (x) for soil temperatures at 10 cm depth established for each year of investigation

Year	n	Correlation coefficient r	Coefficient of determination r^2	Probability P[1]
2012	8	0.8779	0.7707	0.0041**
2013	8	0.9774	0.9552	0.0001**
2014	8	0.9287	0.8624	0.0009**
2015	8	0.8937	0.7988	0.0028**

[1]**significant at the level of 99%

The established correlation coefficients in all four years were significant at the confidence level of 99%. Therefore the regression analysis carried out jointly and presented in the Figure 30.

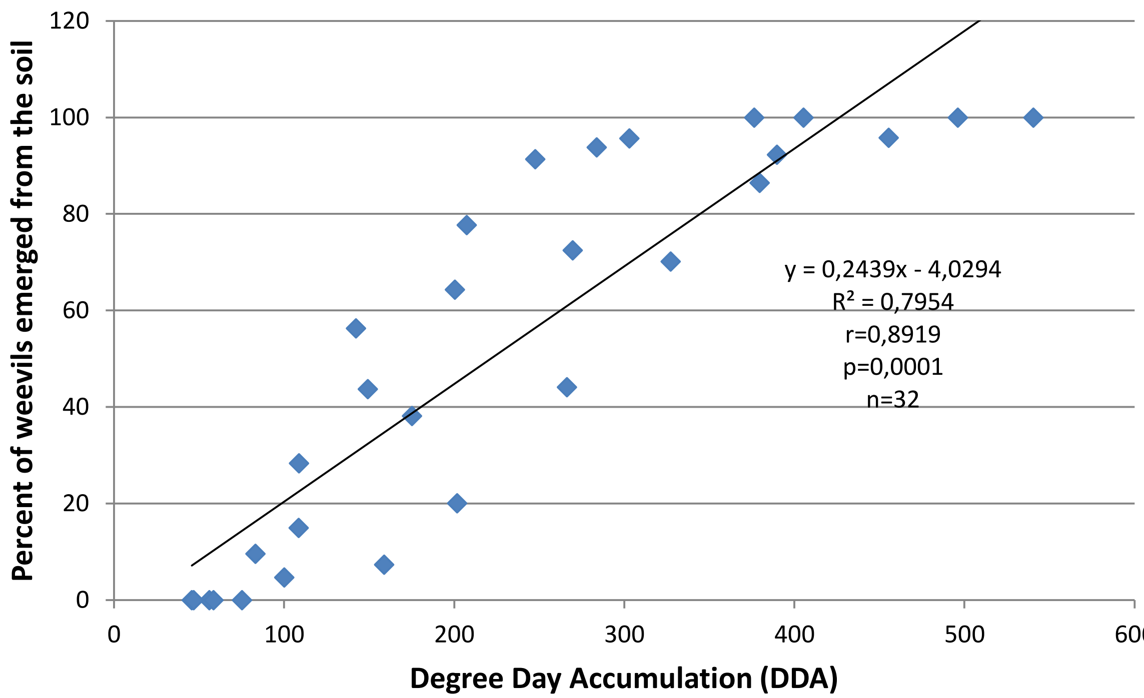


Figure 30. Regression analysis of the dynamic of weevil's emergence from the soil (y) versus degree day accumulation (DDA) for soil temperatures at 10 cm depth (x), Tovarnik, 2012-2015.

5.2.2. Appearance of adults', eggs, larvae and pupae

The average number of each insect stage found in visual inspection of 40 plants per inspection point in the four years of investigations is shown in Figures 31-35.

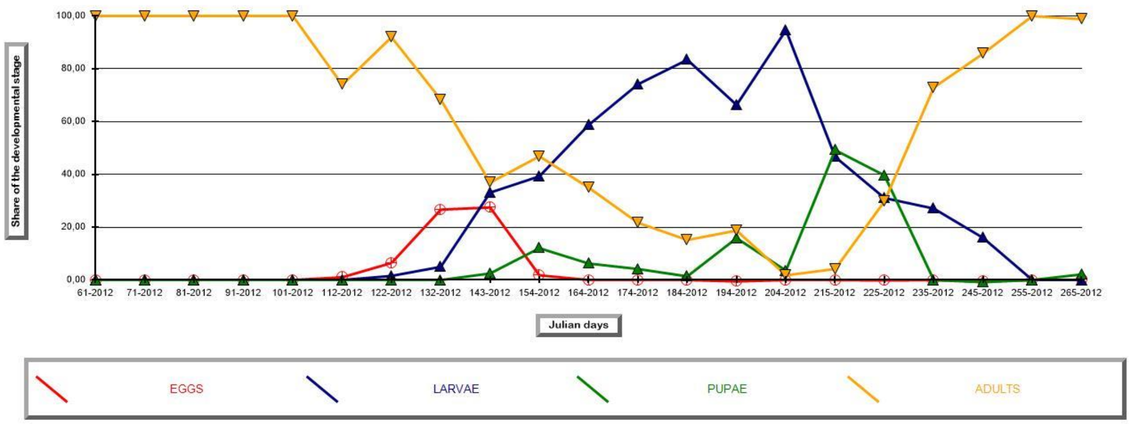


Figure 31. The dynamic of the appearance of different developmental stages of sugar beet weevil (adults, eggs, larvae and pupae) found in 21 surveys in 2012

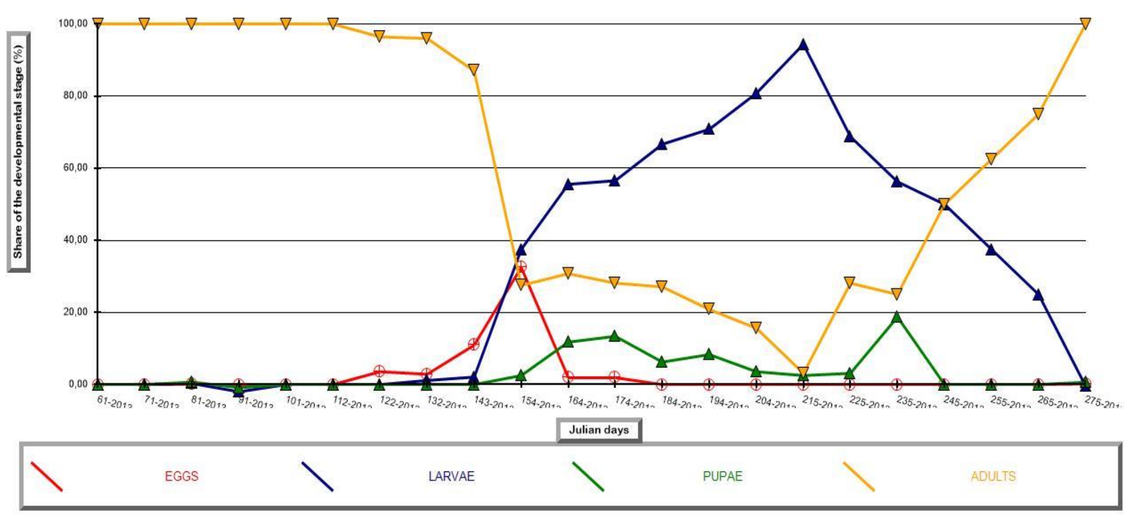


Figure 32. The dynamic of the appearance of different developmental stages of sugar beet weevil (adults, eggs, larvae and pupae) found in 21 surveys in 2013

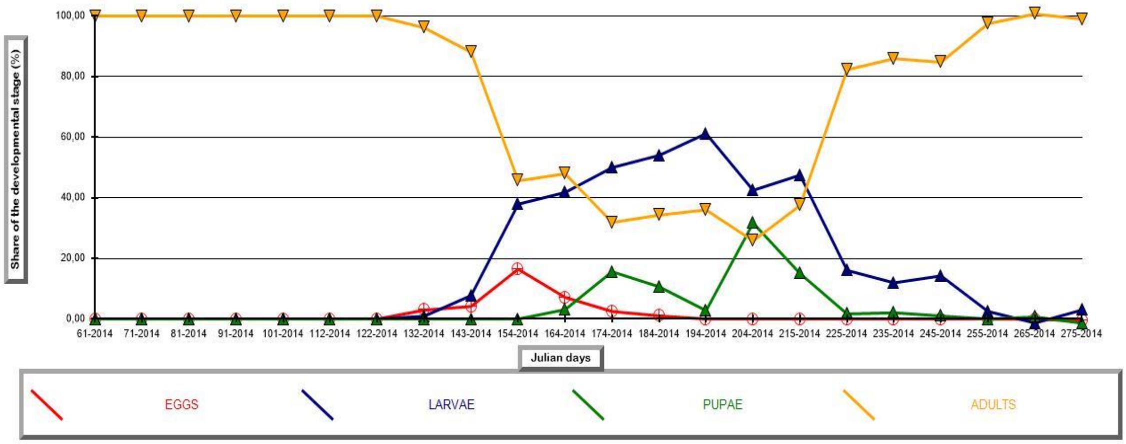


Figure 33. The dynamic of the appearance of different developmental stages of sugar beet weevil (adults, eggs, larvae and pupae) found in 21 surveys in 2014

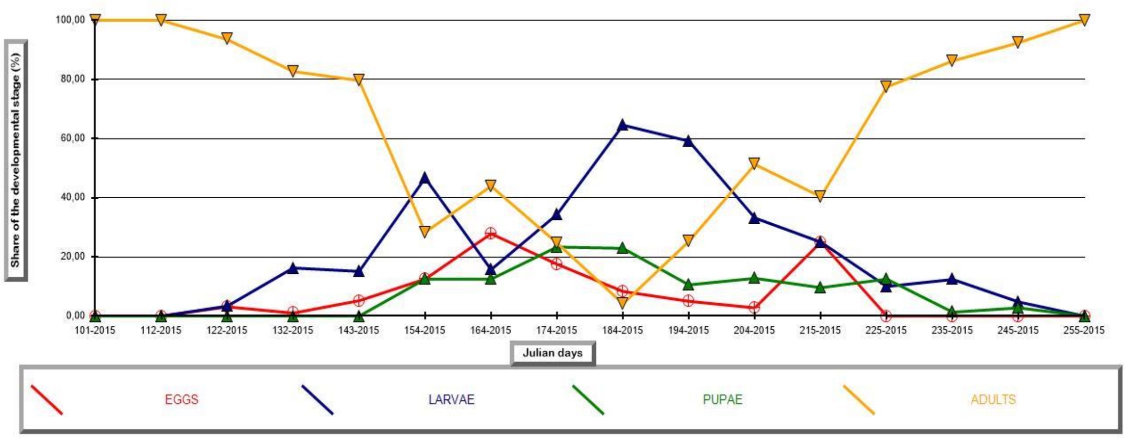


Figure 34. The dynamic of the appearance of different developmental stages of sugar beet weevil (adults, eggs, larvae and pupae) found in 21 surveys in 2015

The average share of different developmental stages in the total population of sugar beet weevil established in each of the 22 surveys in the period 2012-2015 is shown in Figure 35.

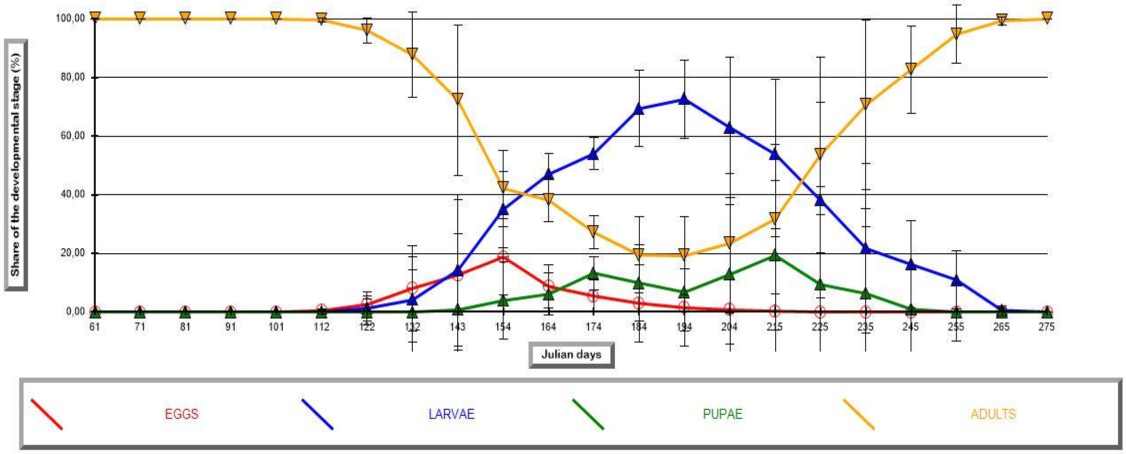


Figure 35. Average share of different developmental stages of sugar beet weevils (adults, eggs, larvae and pupa) established in soil surveys (2012-2015)

Based on the collected results, the phenogram of the development of sugar beet weevil in east Croatia is composed and presented in Figure 36.

No. of gen.	Months of the year											
	1	2	3	4	5	6	7	8	9	10	11	12
	++++	++++	++++	++++	++++	++++	+++					
					•••	•••						
1					o	oooo	oooo	ooo				
							**	****	**			
							+	++++	++++	++++	++++	++++

+ adult • egg o larvae * pupae —— period of harmfulness

Figure 36. Phenogram of sugar beet weevil development

5.2.3. Sexual index

The sexual index of the emerged sugar beet weevils established on the samples of weevils collected on overwintering sites during the four years of investigation is presented in Figure 37.

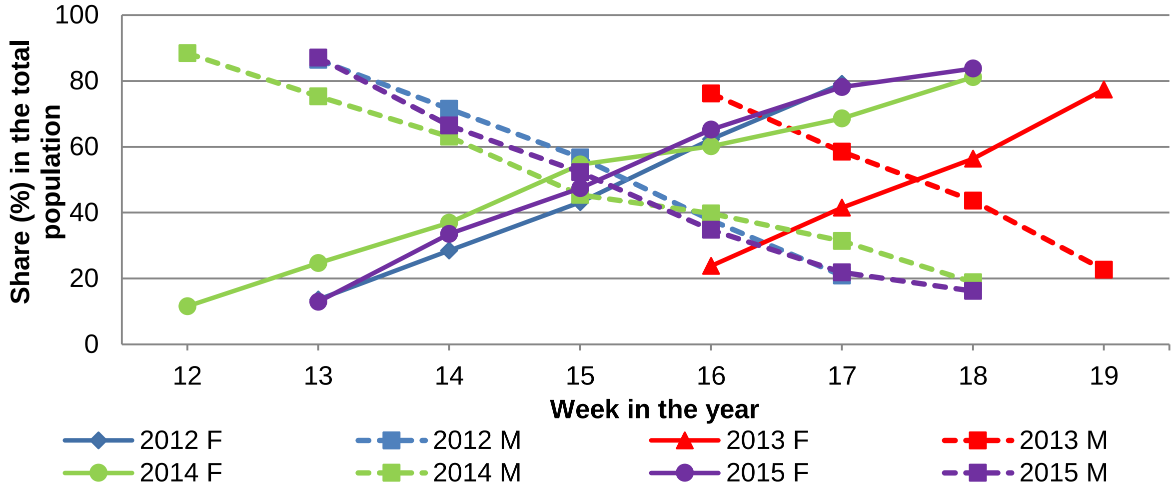


Figure 37. The share of females (F) and males (M) (dotted lines) in the total population of weevils established on the sample of collected beetles in 2012-2015

Since the emergence of adults in 2013 started four weeks later, than in the other three years, the data from three years of investigation were submitted to ANOVA. Results are shown in Figure 38.

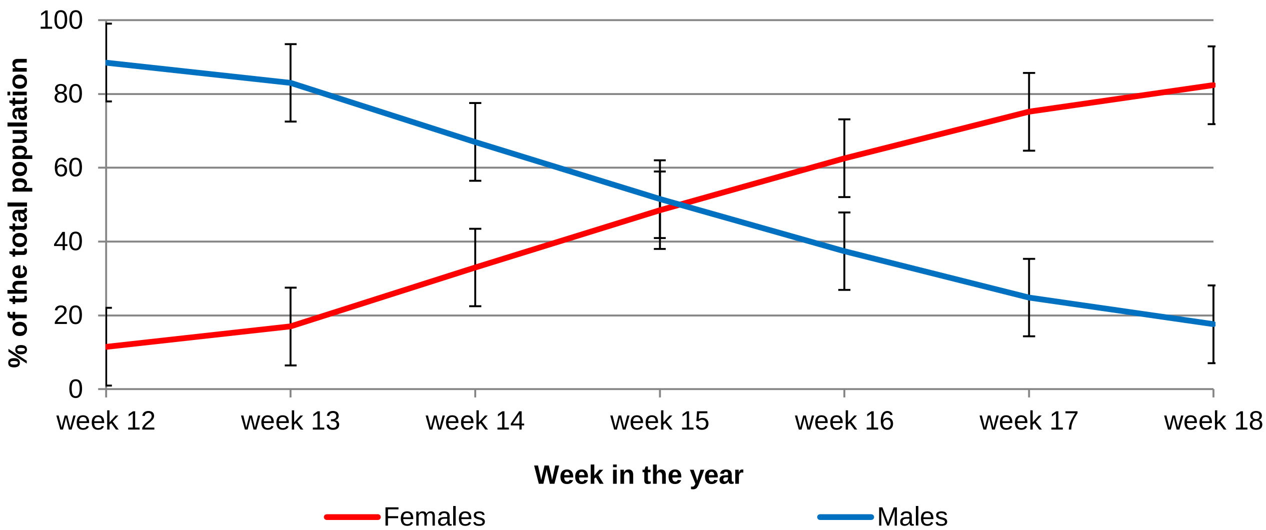


Figure 38. The average ratio of different sexes established between the 12th and 18th weeks of the year (based on the data collected from three years of investigation)

5.2.4. Population fluctuation

The biotic capacity of one species is determined by its capacity for overwintering and its capacity to build up the population in one season.

For each year of investigation, based on the average adult infestation (No of adults/m^2) on the same fields established in the autumn (October, November) and the spring, we estimated the success of overwintering. The population growth was calculated based on the average infestation of old sugar beet fields (expressed as number of adults/m^2) before the beetle emerged and on the average infestation of sugar beet fields before overwintering in the same area. The data on overwintering success and population growth are shown in Table 12

Table 12. The population growth rate in vegetation period and overwintering success during the following winter (2012-2016)

The vegetation and overwintering period	Population growth rate	Overwintering success (0-1)
2012 – 2012/2013	1.92	0.42 b*
2013 – 2013/2014	0.26	1.00 a
2014 - 2014/2015	0.85	1.00 a
2015 – 2015/2016	1,13	0.66 ab
LSD p=5%	Ns	0.289

* Means followed by the same letter are not significantly different according to Duncans' multiple range test (P=0.05)

**Population growth rate was calculated based on average infestation on the whole marked area, therefore means are not compared between years

Population growth rate varied between years and it is clear that the population growth rate was the lowest in 2013, and highest in 2012. Overwintering success was the highest in the winters of 2013/2014 and 2014/2015 (100%). The summarised results of the analysis of the correlations between different climate data (independent variables) and population growth rate as dependent variables are shown in Table 13.

Table 13. Correlation coefficients, coefficients of determination and probability for population growth rate of sugar beet weevil (y) on mean average air (x_1) and soil temperature (x_2) and total amount of rainfall in vegetation season (March-September) (x_3) as well as mean average air (x_4) and soil temperature (x_5), total amount of rainfall in May (x_6) and the ratio between new and old sugar beet fields in the marked area (x_7)

Dependent variable (y)	Independent variable x	n	Correlation coefficient r	Coefficient of determination r^2	Probability P^1
Population growth index	Average air temperature in vegetation period (March-September)	4	0.9409	0.8853	0.0001**
	Average soil temperature in vegetation period (March-September)	4	0.9307	0.8662	0.0001**
	Total amount of precipitation in vegetation period (March-September)	4	-0.7971	0.6354	0.0001**
	Average air temperature in May	4	0.1342	0.0180	0.6077
	Average soil temperature in May	4	0.1016	0.0103	0.6980
	Total amount of precipitation in May	4	-0.7794	0.6074	0.0002**
	The ratio between new and old sugar beet fields in marked area	4	0.7813	0.6104	0.001**

[1]*significant at the level of 95%
[1]**significant at the level of 99%

Regression analysis of the population growth index (y) versus average air (x_1) and soil (x_2) temperature (Figure 39, Table 13) showed that the regression curves are linear and correlations (measured by Pearson's coefficient of correlations) between those variables are positive and full according to Roemer and Orphal (Vasilj, 2000).

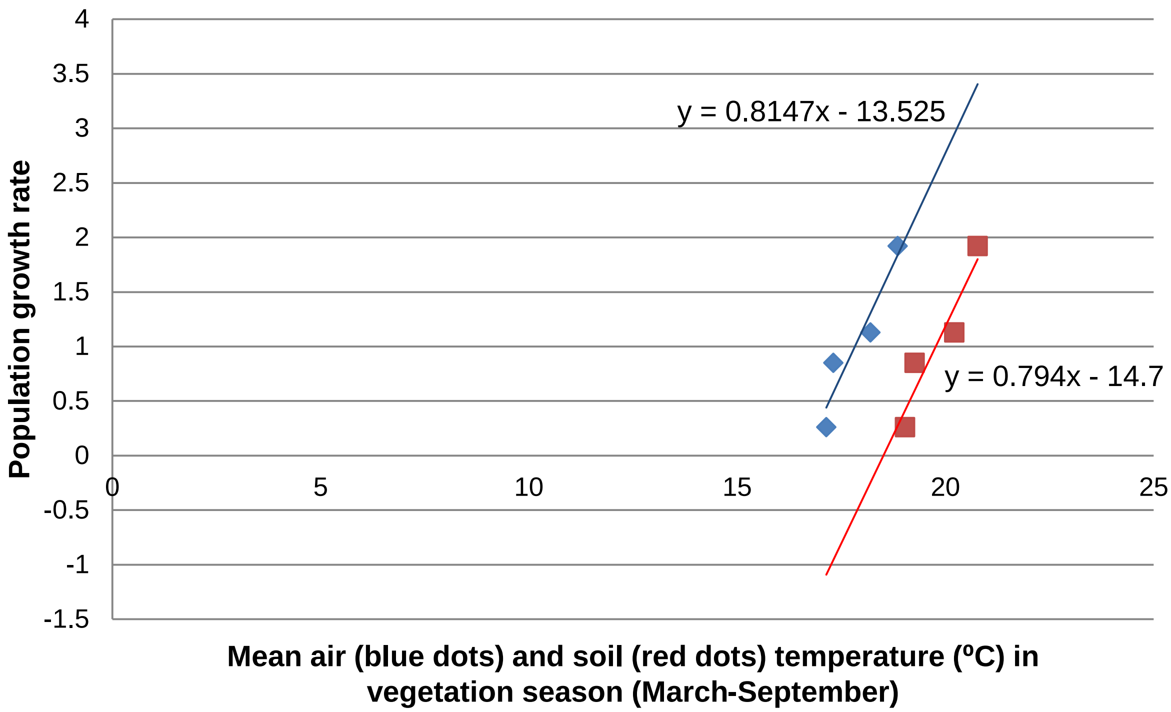


Figure 39. Regression analysis of the population growth index (y) versus average air (x_1) (blue line) and soil (x_2) (red line) temperatures in the vegetation period, Tovarnik, 2012-2015.

Regression analysis of the population growth index (y) versus total amount of rainfall (x) in vegetation season (Figure 40) and versus total amount of rainfall in May (Figure 41, Table 13) showed that the regression curves are linear and correlations (measured by Pearson's coefficient of correlations) between those variables are negative and very strong, according to Roemer and Orphal (Vasilj, 2000).

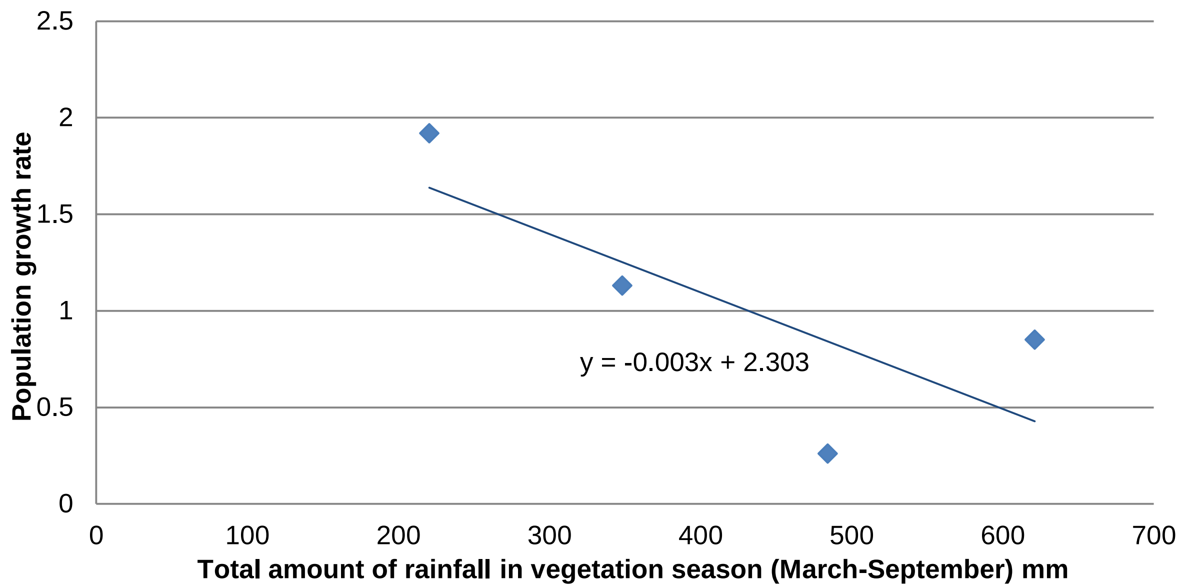


Figure 40. Regression analysis of the population growth index versus total amount of rainfall in the vegetation period (March-September), Tovarnik, 2012.-2015.

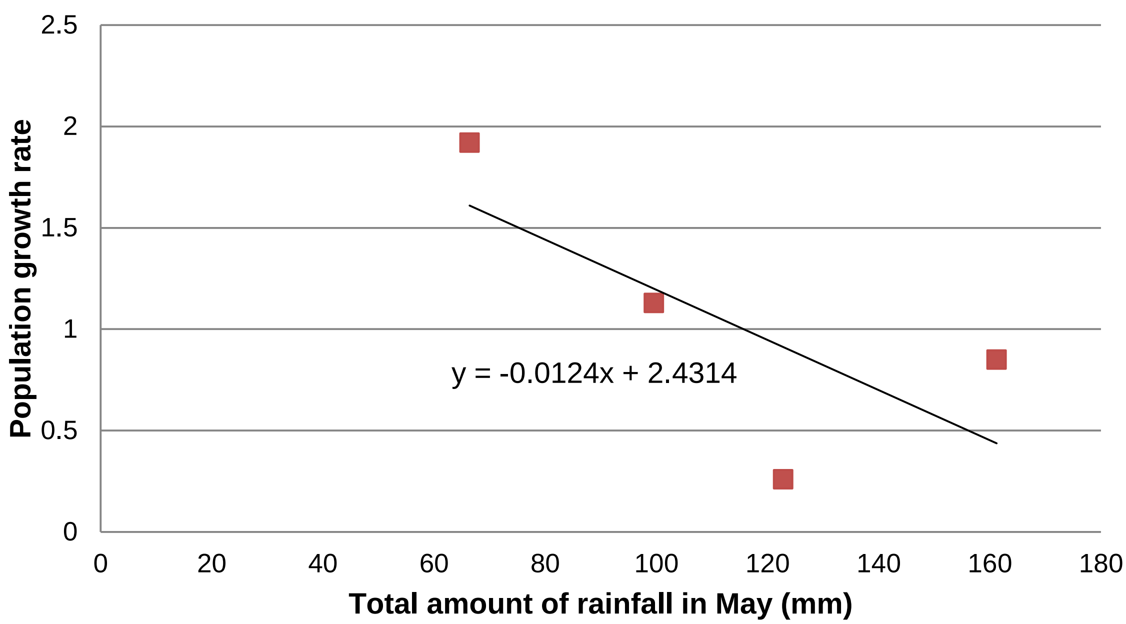


Figure 41. Regression analysis of the population growth index versus total amount of rainfall in May, Tovarnik, 2012-2015

Regression analysis of the population growth index (y) versus the ratio between the new and old sugar beet fields in the marked area (Figure 42, Table 13) showed that the regression curve is linear and correlation (measured by Pearson's coefficient of correlations) between those variables is positive and very strong according to Roemer and Orphal (Vasilj, 2000).

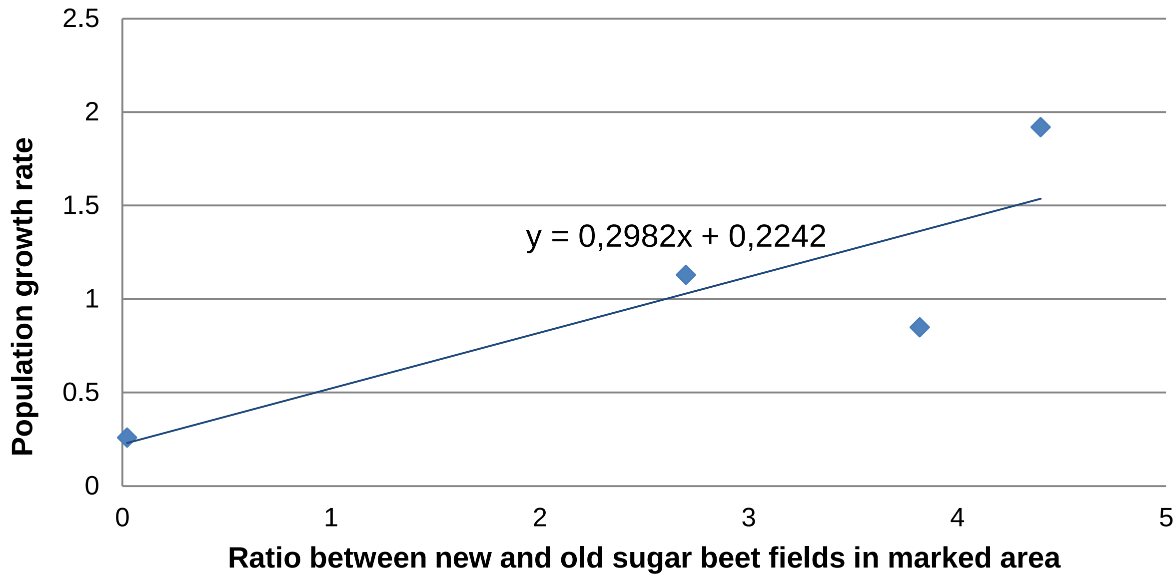


Figure 42. Regression analysis of the population growth index versus the ratio between new and old sugar beet fields, Tovarnik, 2012-2015

The summarised results of the analysis of correlations between different climate data (independent variables) and overwintering success as a dependent variable are shown in Table 14.

Table 14. Correlation coefficients, coefficients of determination and regression equations for overwintering success of sugar beet weevil (y) on mean average air (x_1) and soil temperature (x_2) and total amount of rainfall in the overwintering period (October-February) (x_3)

Dependent variable (y)	Independent variable x	n	Correlation coefficient r	Coefficient of determination r^2	Probability P^1
Overwintering success	Average air temperature in overwintering period (October-February)	27	0.5431	0.2950	0.0019**
	Average soil temperature in overwintering period (October-February)	27	0.3890	0.1513	0.036*
	Total amount of precipitation in overwintering period (October-February)	27	-0.2772	0.0768	0.1381

[1]*significant at the level of 95%
[1]**significant at the level of 99%

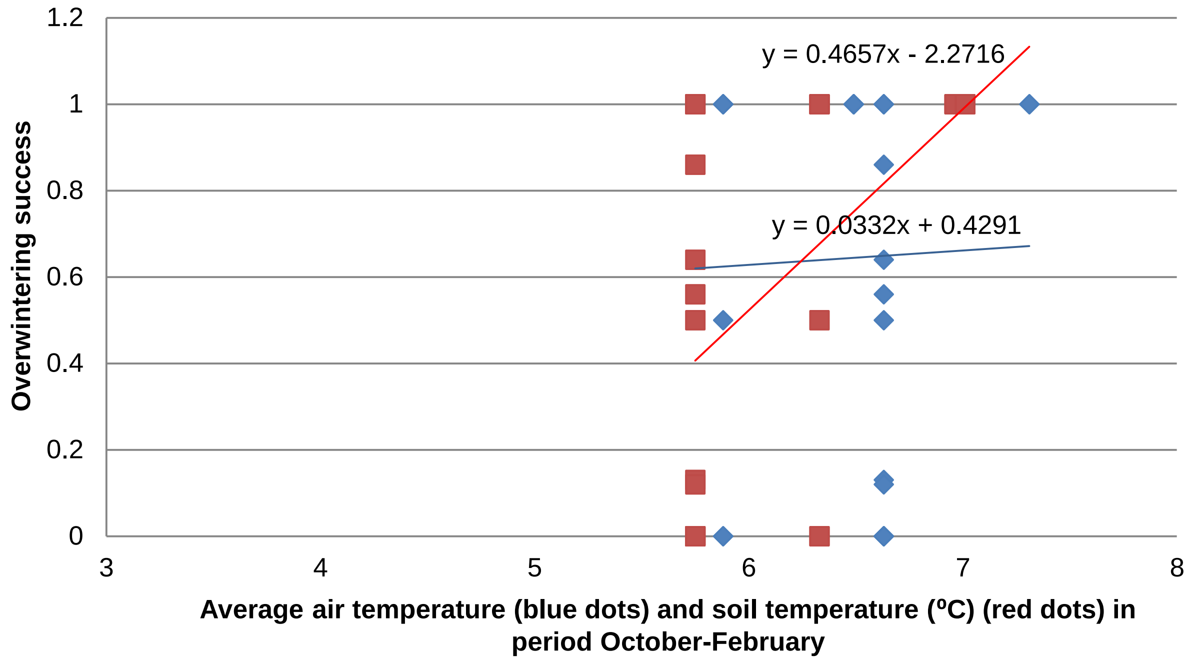


Figure 43. Regression analysis of the overwintering success rate versus average air temperature (blue dots) and average soil temperature (red dots) during the overwintering period (October-February), Tovarnik, 2012-2016

Regression analysis of the overwintering success (y) versus average air temperature (x) in overwintering period (Figure 43) showed that the regression curves are linear; correlations (measured by Pearson's coefficient of correlation) between those variables are positive and weak (average soil temperature) or medium (average air temperature), according to Roemer and Orphal (Vasilj, 2000).

5.3. AREA WIDE CONTROL OF SUGAR BEET WEEVILS BY MASS TRAPPING

As described in the methodology part, the success of mass trapping was measured by several different parameters.

a) Results of sugar beet weevil mass trapping in each year of infestation expressed as an average infestation of old sugar beet fields in the autumn and spring and average number of weevils per trap and total capture/field are shown in Tables 15-18.

Table 15. Results of mass trapping of sugar beet weevil on old sugar beet fields in 2012

Field number	Field size (ha)	Average infestation (weevils/m²) established in soil survey in spring	Average total capture/trap	Total population of sugar beet weevil before trapping on the entire field in spring*	Total number of caught weevils on the entire field
1	1.21	0 d**	119.53 cde	0	2,151
2	3.69	8.89 ab	139.76 cde	345,937.5	7,533
3	3.96	6.5 abc	119.78 cde	495,000	7,187
4	1.21	0.92 bcd	129.6 cde	37,812.5	2,282
5	1.35	2.15 a-d	149.15 cde	63,281.25	2,443
6	0.98	0.64 cd	57.58 e	15,312.5	845
7	2.03	0 d	79.54 de	0	2,364
8	1.98	0.64 cd	234.99 bcd	30,937.5	1,935
9	2.35	3.04 a-d	265.48 abc	183,593.8	9,260
10	1,76	2.15 a-d	367.1 ab	82,500	9,429
11	2.03	15.47 a	420.4 a	348,906.3	12,418
12	4.6	0 d	90.78 cde	0	6,300
13	1.83	2.15 a-d	163.28 cde	85,781.25	4,422
14	36	0.92 bcd	181.91 cde	1,125,000	90,072
total	64.98			2,814,063	158,641
LSD p=5%		0.465t***	149.304		

* based on the average infestation per m² and the total size of the field

** Means followed by same letter are not significantly different according to Duncans' multiple range test (P=0.05)

*** Mean descriptions are reported in transformed data units (*log x+1* transformation has been applied) and are not de-transformed

Table 16. Results of mass trapping of sugar beet weevil on old sugar beet fields in 2013

Field number	Field size (ha)	Average infestation (weevils/m²) established in soil survey in		Average capture/ trap	Total estimated population of sugar beet weevil before trapping on the entire field in		Total number of caught weevils on the entire field
		autumn	spring		autumn*	spring*	
1	5.90	3.57	6.1 ab	24.52 c	645,312.5	645,312.5	2,107
2	4.85	16.72	0.0 c	16.85 c	1,060,937.5	0	1,231
3	4.98	2.15	1.69 abc	19.93 c	233,437.5	155,625	1,345
4	4.82	2.46	3.09 abc	26.08 c	301,250	527,187.5	1,878
5	3.86	0.64	0.0 c	19.86 c	60,312.5	0	1,151
6	1.84	3.04	0.0 c	37.36 c	143,750	0	1,007
7	3.35	10.56	0.64 c	23.01 c	261,718.75	52,343.75	1,150
8	4.88	7.3	0.0 c	28.86 c	560,000	0	1,960
9	3.00	1.69	4.16 abc	19.77 c	93,750	187,500	885
10	5.94	11.71	0.0 c	17.30 c	117,500	0	1,537
11	0.94	5.63	5.63 ab	29.46 c	556,875	88,125	406
12	2.83	3.76	14.35 a	38.08 c	309,531.25	795,937.5	1,631
13	4.05	3.04	0.64 c	23.49 c	316,406.25	63,281.25	1,431
14	60.00	10.96	4.08 abc	664.57 b	7,837,500	4,087,500	598,113
15	130.18	14.24	6.83 ab	798.24 a	18,713,375	11,472,113	1,479175
total	241.42				31,211,656.25	18,074,925	2,095,007
LSD p=5%		ns	0.462t***	33.61			

* based on the average infestation per m² and the total size of the field

** Means followed by same letter are not significantly different according to Duncans' multiple range test (P=0.05)

*** Mean descriptions are reported in transformed data units (*log x+1* transformation has been applied) and are not de-transformed

Table 17. Results of mass trapping of sugar beet weevil on old sugar beet fields in 2014

Field number	Field size (ha)	Average infestation (weevils/m^2) established in soil survey in		Average capture/trap	Total population of sugar beet weevil before trapping on the entire field in		Total number of caught weevils on the entire field
		autumn	spring		autumn*	spring*	
1	4.45	2.0	1.59 b-e**	35.72 cde	89,000	133,500	2,429
2	0.58	1.0	16.67 a	48.29 ab	5,800	92,800	510
3	1.1		5.59 abc	38.96 bcd		121,000	576
4	3.48		0.0 e	21.67 f		0	1,127
5	3.16		1.59 b-e	23.37 f		94,800	1,097
6	1.07		0.0 e	46.31 abc		0	741
7	1.61		1.24 cde	44.25 abc		32,200	1,062
8	1.29		0.0 e	32.1 def		0	611
9	1.22		0.73 de	22.69 f		24,400	402
10	2.12		0.0 e	27.09 ef		0	867
11	0.59		0.5 de	42.67 abc		5,900	366
12	0.72		5.59 abc	43.71 abc		79,200	466
13	4.95		0.0 e	10.67 g		0	790
14	3.81		0.0 e	27.94 ef		0	1,588
15	2.1		3.92 bcd	45.13 abc		126,000	1,444
16	0.42		0.0 e	44.5 abc		0	252
17	5.74		7.52 ab	26.03 ef		459,200	2,239
18	1.17		0.5 de	35.91 cde		11,700	641
19	1.29		0.0 e	50.58 a		0	959
total	40.87				94,800	1,180,700	18,167
LSD p=5%		ns	0.339t***	9.446			

* based on the average infestation per m^2 and the total size of the field

** Means followed by same letter are not significantly different according to Duncans' multiple range test (P=0.05)

*** Mean descriptions are reported in transformed data units (*log x+1* transformation has been applied) and are not de-transformed

Table 18. Results of mass trapping of sugar beet weevil on old sugar beet fields in 2015

Field number	Field size (ha)	Average infestation (weevils/m^2) established in soil survey in		Average capture/trap	Total population of sugar beet weevil before trapping on the entire field in		Total number of caught weevils on the entire field
		autumn	spring		autumn*	spring*	
1	1.1	0.0 b**	1.5 b	38.96 b	0	22,000	576
2	3.48	17.73 a	17.72 a	21.67 d	626,400	626,400	1,127
3	3.16	0.0 b	0.63 b	23.37 d	0	31,600	1,097
4	1.07	0.25 b	0.36 b	46.31 a	10,700	5,350	741
5	1.61	0.25 b	1.5 b	44.25 ab	16,100	32,200	1,062
6	1.29	0.0 b	1.5 b	32.1 c	0	25,800	611
7	1.22	0.0 b	2.17 b	22.54 d	0	30,500	402
total	12.93				653,200	773,850	5,616
LSD p=5%		3.936 t***	0.708t****	6.080			

* based on the average infestation per m^2 and the total size of the field
** Means followed by same letter are not significantly different according to Duncans' multiple range test (P=0.05)
*** Mean descriptions are reported in transformed data units (*arc.sin* $\sqrt{x}$ transformation has been applied) and are not de-transformed
**** Mean descriptions are reported in transformed data units (*log x+1* transformation has been applied) and are not de-transformed

The summarised results of the overall success of mass trapping over five years of the program are shown in Table 19.

Table 19. Results of the mass trapping of sugar beet weevils carried out in Tovarnik, Croatia from 2012 to 2015

<table>
<tr><th colspan="2" rowspan="2">Year</th><th rowspan="2">Established infestation of weevils/m² on fields involved in mass trapping (from-to)</th><th rowspan="2">Total estimated population of the previous year sugar beet fields in the area where mass trapping is carried out</th><th rowspan="2">Number of trapped weevils in spring</th><th rowspan="2">Percent of the mass trapping success in relation to autumn or spring population</th><th colspan="2">Area (ha) that the weevil population had the ability to destroy</th></tr>
<tr><th>Estimated</th><th>Trapped</th></tr>
<tr><td>2012</td><td>Spring</td><td>0-15.47</td><td>2,814,063</td><td>158.641</td><td>5.64</td><td>938</td><td>52.88</td></tr>
<tr><td rowspan="2">2013</td><td>Autumn</td><td>0.64-16.72</td><td>31,211,656</td><td rowspan="2">2,095,007</td><td>6.71</td><td>10,403</td><td rowspan="2">698.33</td></tr>
<tr><td>Spring</td><td>0-14.35</td><td>18,074,925</td><td>11.59</td><td>6,024</td></tr>
<tr><td rowspan="2">2014</td><td>Autumn</td><td>1-2</td><td>94,880*</td><td>2,939*</td><td>3.1</td><td>31.60</td><td>0.98</td></tr>
<tr><td>Spring</td><td>0-16.67</td><td>1,180,700**</td><td>18,167**</td><td>1.53</td><td>393.60</td><td>6.05</td></tr>
<tr><td rowspan="2">2015</td><td>Autumn</td><td>0-17.73</td><td>653,200</td><td rowspan="2">5,616</td><td>0.86</td><td>217.73</td><td rowspan="2">1.87</td></tr>
<tr><td>Spring</td><td>0.63-17.72</td><td>773,850</td><td>0.73</td><td>258</td></tr>
</table>

* Population established on old sugar beet fields in area in which mass trapping has been carried out in 2013 by soil survey and pheromone traps
** Population established by soil survey and mass trapping on the whole area in 2014 (the area has been enlarged)

The level of infestation with sugar beet weevils and forecast of sugar beet weevil attack in the following season has been estimated; the results, expressed as number of fields belonging to different categories regarding infestation level (according to Petruha, 1971), are shown in Table 20.

Table 20. Forecast of sugar beet weevil attack based on soil survey conducted in the autumn and spring

Season and year of the soil survey	The vegetation for which attack is forecasted	Number of fields on which weevil population is forecasted as			
		poor ≤ 0.5 weevils/m^2	mean 0.6-3.0 weevils/m^2	strong 3.1-10 weevils/m^2	very strong ≥ 10.1 weevils/m^2
spring 2012	2012	3	2	8	1
autumn 2012	2013	0	1	7	7
spring 2013	2013	5	2	5	3
autumn 2013	2014	0	2	0	0
spring 2014	2014	8	6	2	3
autumn 2014	2015	4	2	0	1
spring 2015	2015	1	5	0	1
		21	20	22	16

b) and c) The success of mass trapping is estimated using two additional parameters, average infestation of sugar beet fields with sugar beet weevil adults (number of adults/m^2) in the marked area and sugar beet fields outside the marked area expressed as number of adults/m^2 and average damage caused by sugar beet weevils established on the same fields expressed as % of damage calculated according to Townsend-Heuberger. The results of those two parameters are shown in Figures 44-51.

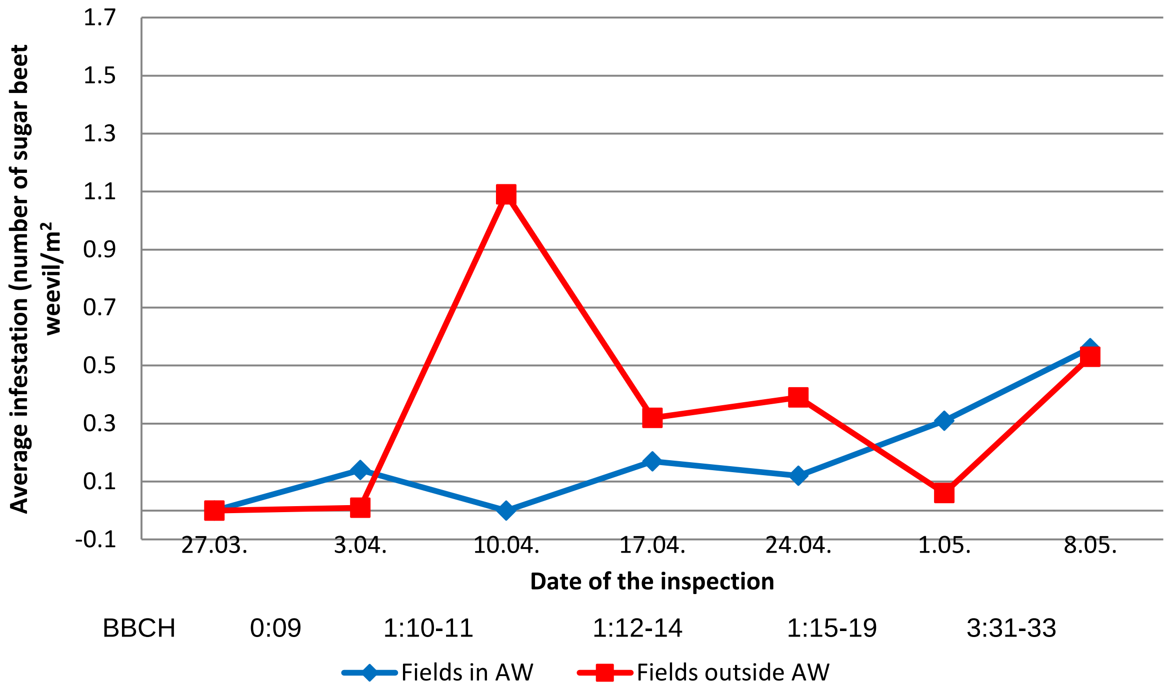


Figure 44. Average infestation of sugar beet fields with sugar beet weevil adults (number of adults/m^2) inside and outside the marked area established on different dates of survey, Tovarnik, 2012

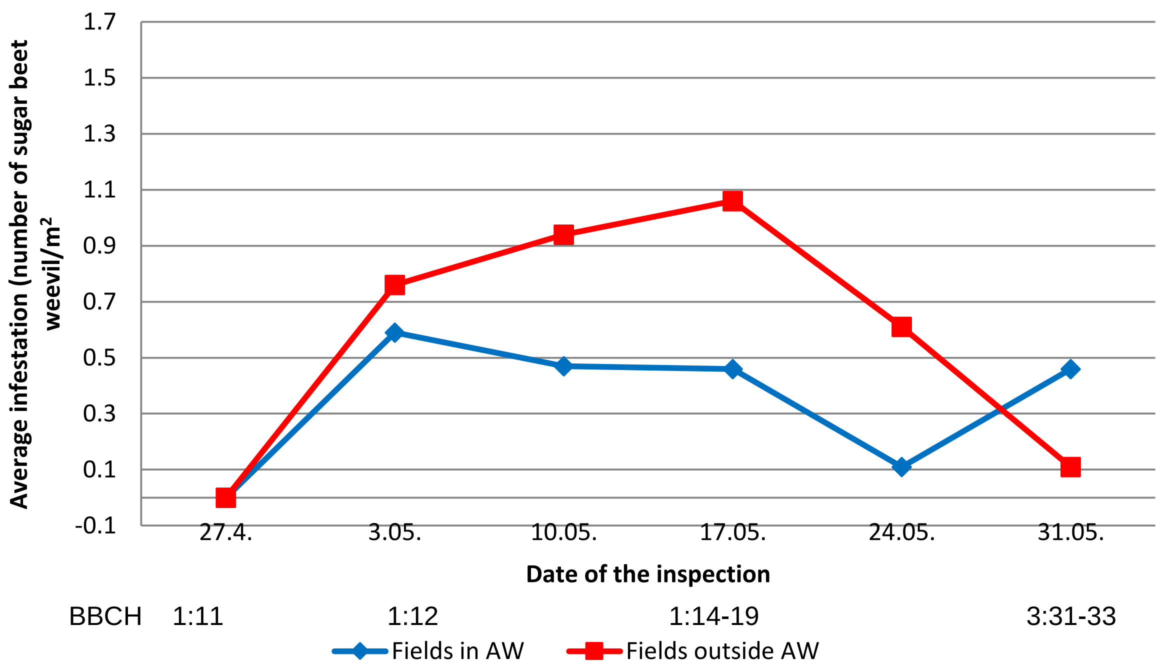


Figure 45. Average infestation of sugar beet fields with sugar beet weevil adults (number of adults/m^2) inside and outside the marked area established on different dates of survey, Tovarnik, 2013

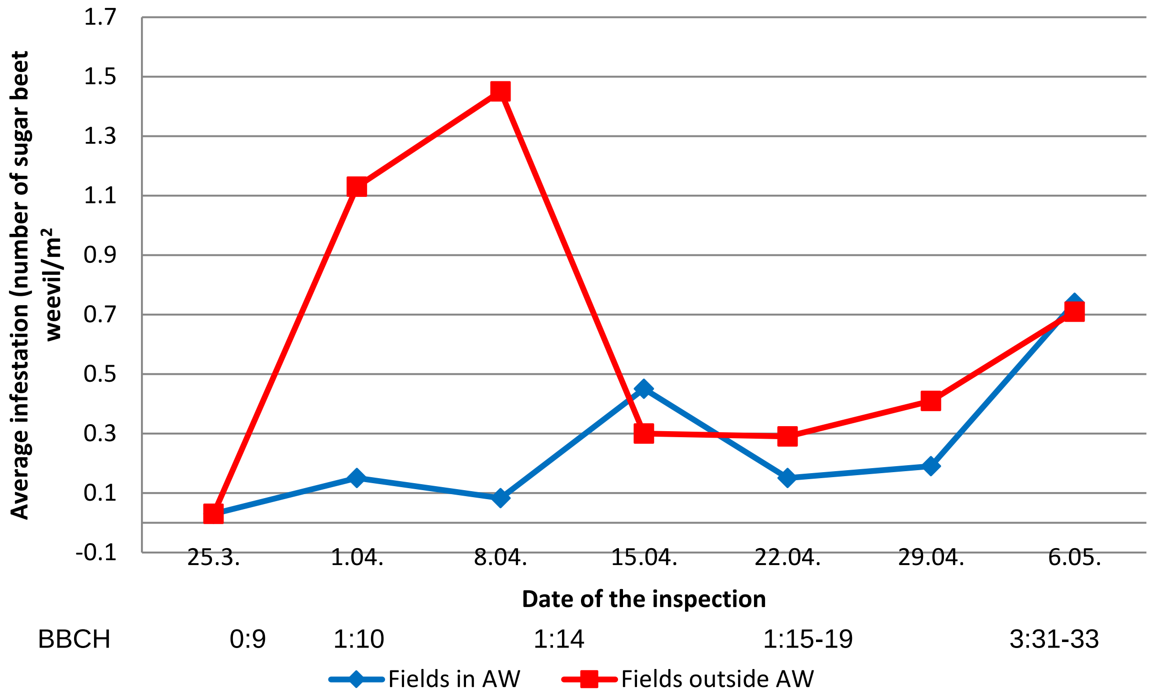


Figure 46. Average infestation of sugar beet fields with sugar beet weevil adults (number of adults/m^2) inside and outside the marked area established on different dates of survey, Tovarnik, 2014

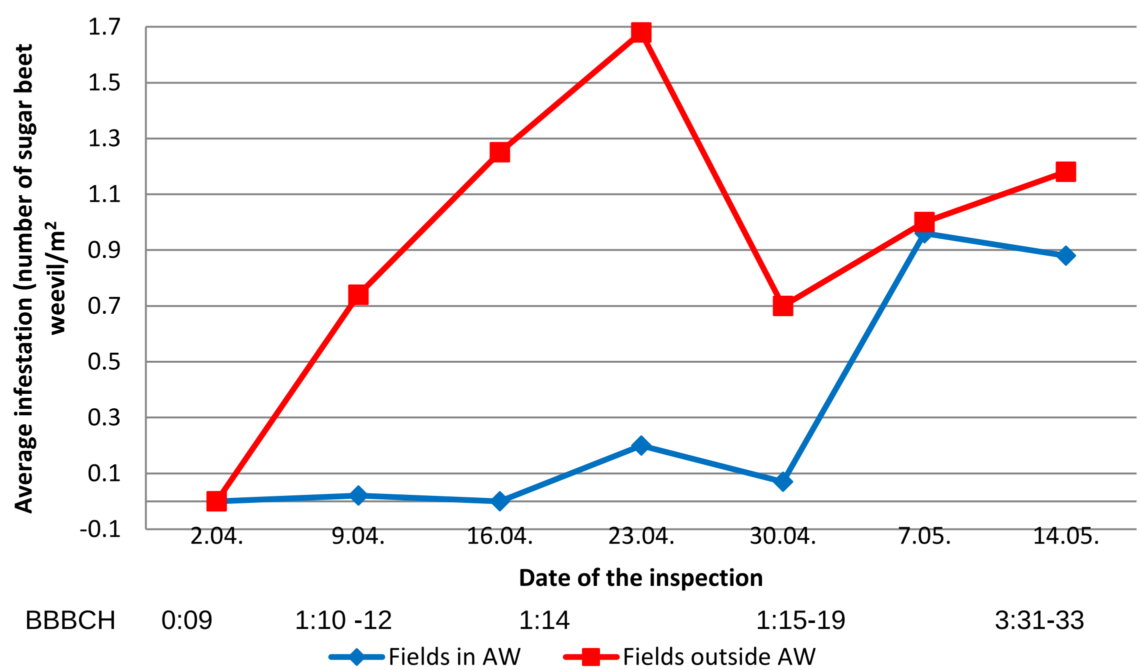


Figure 47. Average infestation of sugar beet fields with sugar beet weevil adults (number of adults/m^2) inside and outside the marked area established on different dates of survey, Tovarnik, 2015

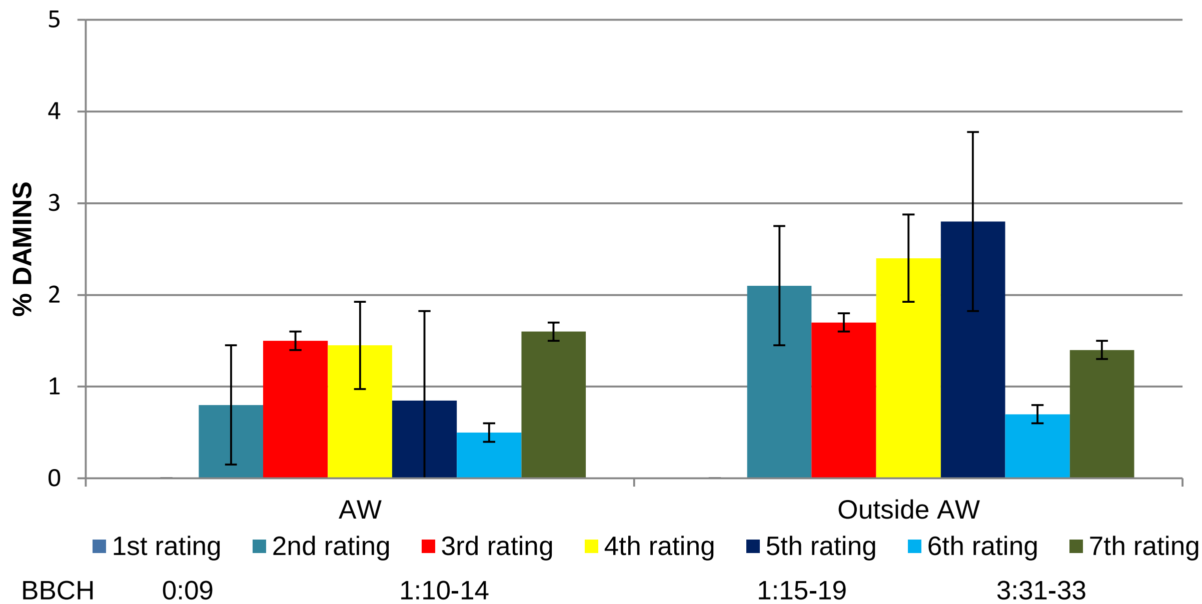


Figure 48. Average damage caused by sugar beet weevils adults (in %) established on sugar beet fields inside and outside the marked area established on different dates of survey, Tovarnik,2012

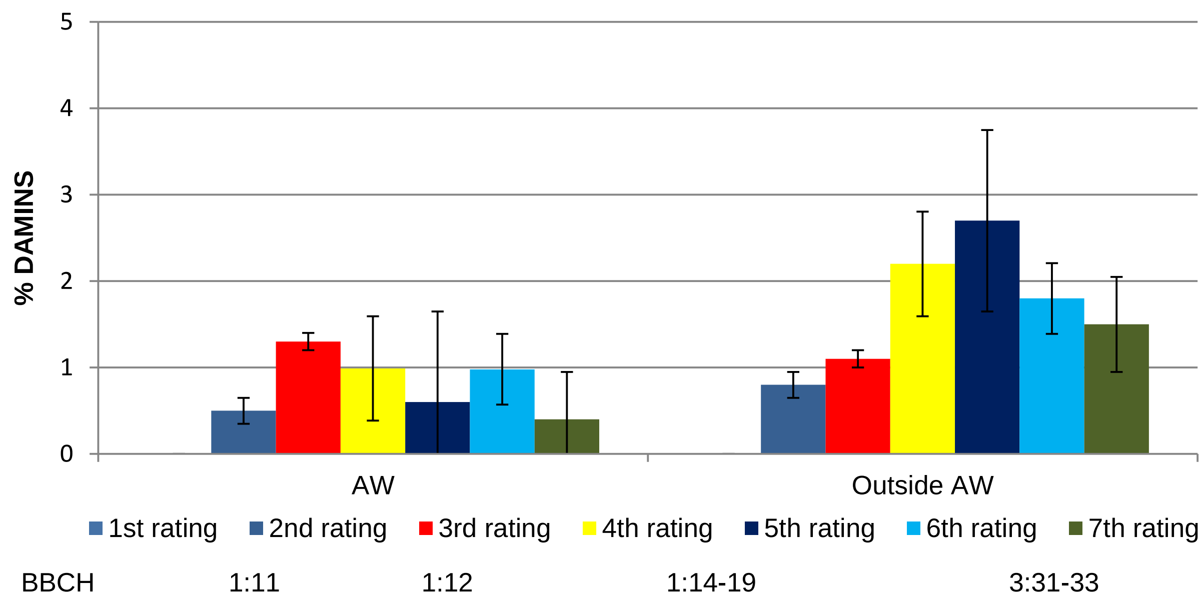
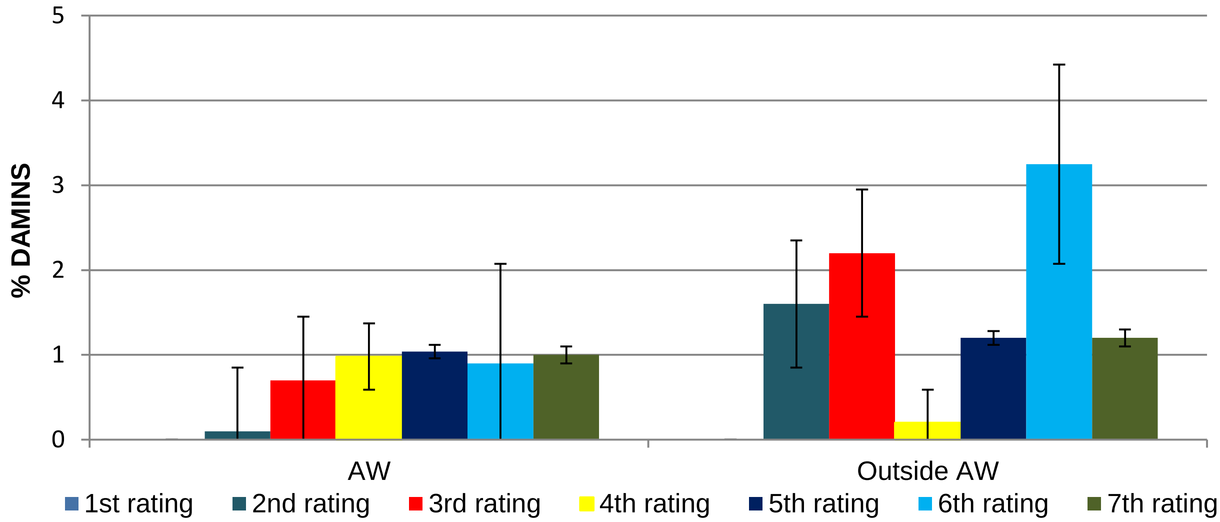
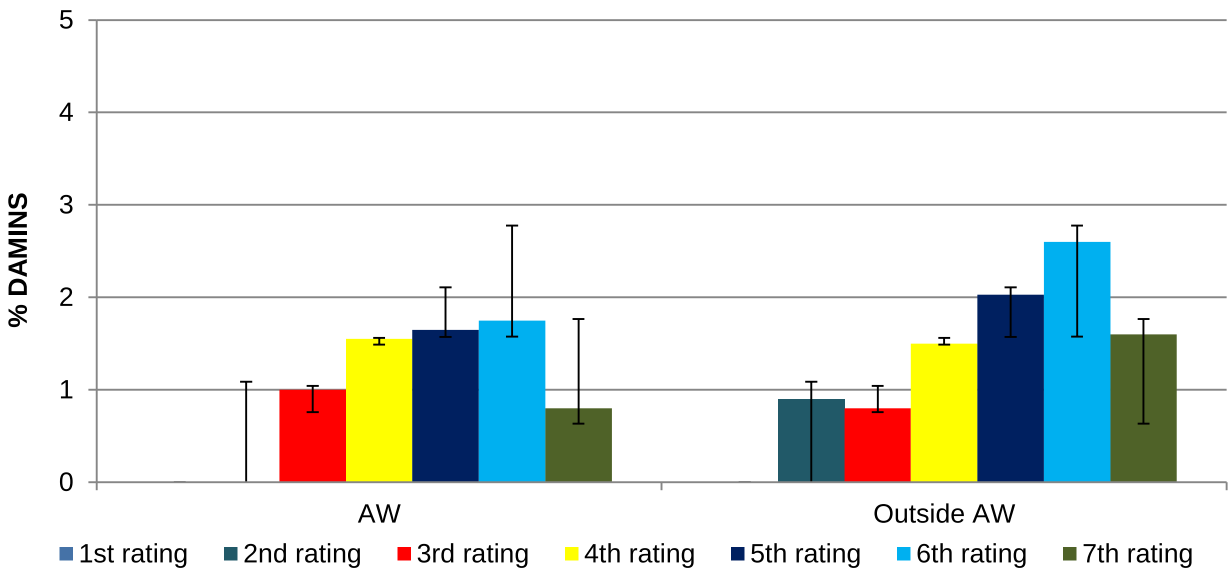


Figure 49. Average damage caused by sugar beet weevils adults (in %) established on sugar beet fields inside and outside the marked area established on different dates of survey, Tovarnik, 2013



BBCH 0:09 1:10 1:14 1:15-19 3:31-33

Figure 50. Average damage caused by sugar beet weevils adults (in %) established on sugar beet fields inside and outside the marked area established on different dates of survey, Tovarnik, 2014



BBCH 0:09 1:10-12 1:14 1:15-19 3:31-33

Figure 51. Average damage caused by sugar beet weevils adults (in %) established on sugar beet fields inside and outside the marked area established on different dates of survey, Tovarnik, 2015

d) The fourth parameter used for establishing the success of mass trapping is the amount of insecticide used for sugar beet weevil control on the fields inside and outside the marked area. In Table 21, the basic characteristics of the products used for sugar beet weevil control inside the marked area (AW) and outside the marked (AW) area.

Table 21. Basic characteristics of insecticides used for sugar beet weevil in the investigated area in the period from 2012 to 2015

Product name	Producer	Active ingradient	Content of active ingradient in the product (g/l)	Applied dose of the product l/ha		Applied amount of the active ingradient g/ha	
				Broad application	Field edges	Broad application	Field edges
Karate Zeon 5 CS	Syngenta	Lambda-cychalothrin	5%	0.15	0.03	7.5	1.5
Nurelle D	Chromos	Chlorpyriphos + cypermetrin	500 g/l + 50 g/l	2.0	-	1,000+ 100	-

The first treatment of sugar beet weevil was usually conducted on field edges with Karate Zeon 5CS. This treatment was applied on approximately 20% of the total surface of the field. Later on, Karate Zeon 5 CS and Nurelle D were applied either alone or in combination over the entire surface. If the combination was applied, both products were used in full doses.

In the Figure 52, the summary of insecticide treatments inside and outside the marked area is shown.

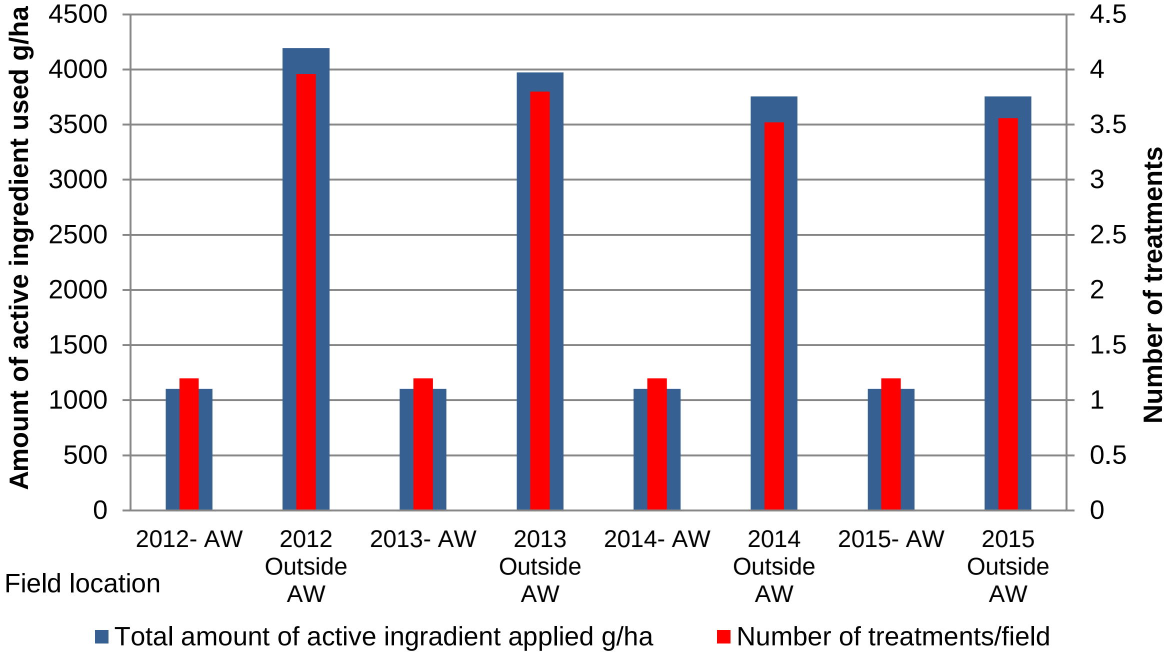


Figure 52. Number of treatments and average consumption of insecticides (g active ingredient/ha) applied on fields inside and outside marked area in which AW control of sugar beet weevil was carried out

5.4. EFFICIACY OF ENTOMOPATHOGENIC NEMATODES (EPN) ON SUGAR BEET WEEVIL

Efficiacy of EPN on sugar beet weevil in 2014

The number of sugar beet weevil adults determined prior to the application of Nematop in 2014 was 0.75 weevil/m^2 (7,500 adults/ha).

The total number of sugar beet weevil larvae found in the field experiment in 2014 was very low (18 larvae) (Figure 53). Nevertheless, as expected, the highest total number of larvae (10) was determined in the untreated plot. During all field inspections of sugar beet roots in 2014, no larvae were found in the treatment with 7 million/10 m^2. The average monthly air and soil temperatures were highest in July (air: 22.3°C; soil: 24.9°C), while the total monthly precipitation was highest in May (157.5 mm).

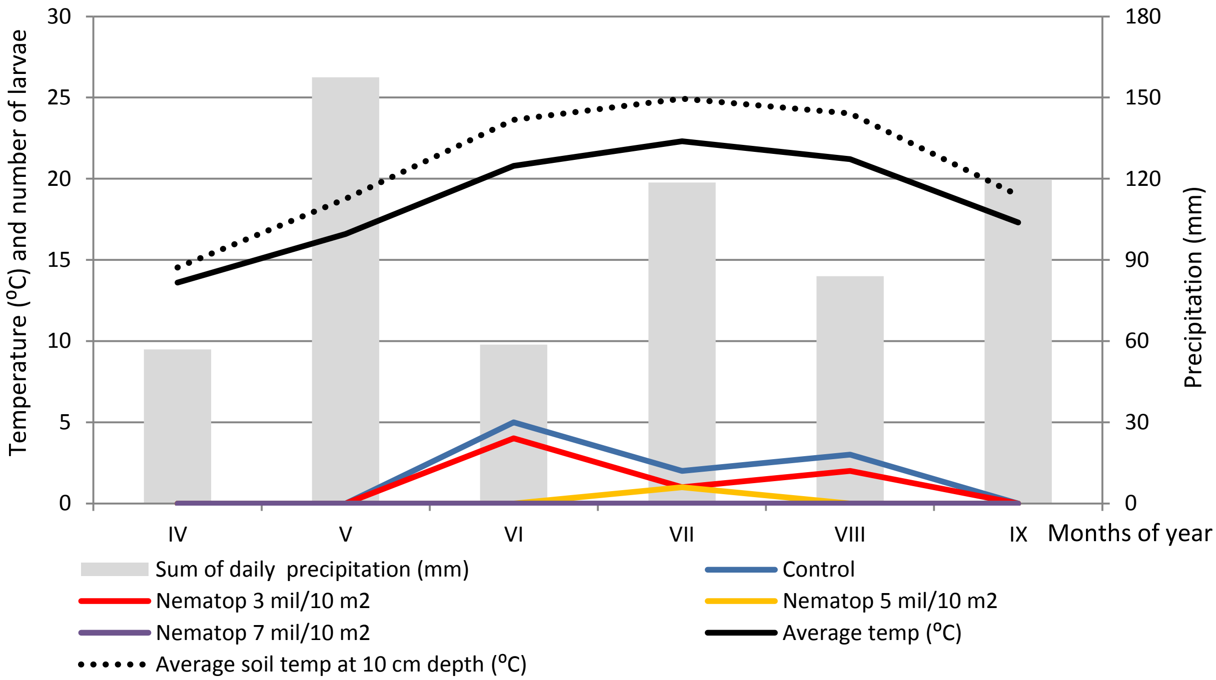


Figure 53. The number of sugar beet weevil (*Bothynoderes punctiventris*) larvae after the application of EPN *Heterorhabditis bacteriophora* and prevailing climatic conditions, Tovarnik, 2014

Efficiacy of EPN on sugar beet weevil in 2015

The average infestation of the study field before the application of Nematop in 2015 was 0.5 weevils/m^2 (5,000 adults/ha). The infestation determined during the last visual inspection was 1.75 weevils/m^2. The total number of sugar beet weevil larvae counted in field experiments during 2015 was 73 larvae (Figure 54). The average monthly air temperature was highest (22.3°C) in August, while the soil temperature was highest (28.3°C) in July 2015. The highest (100.4 mm) total monthly precipitation was observed in May 2015.

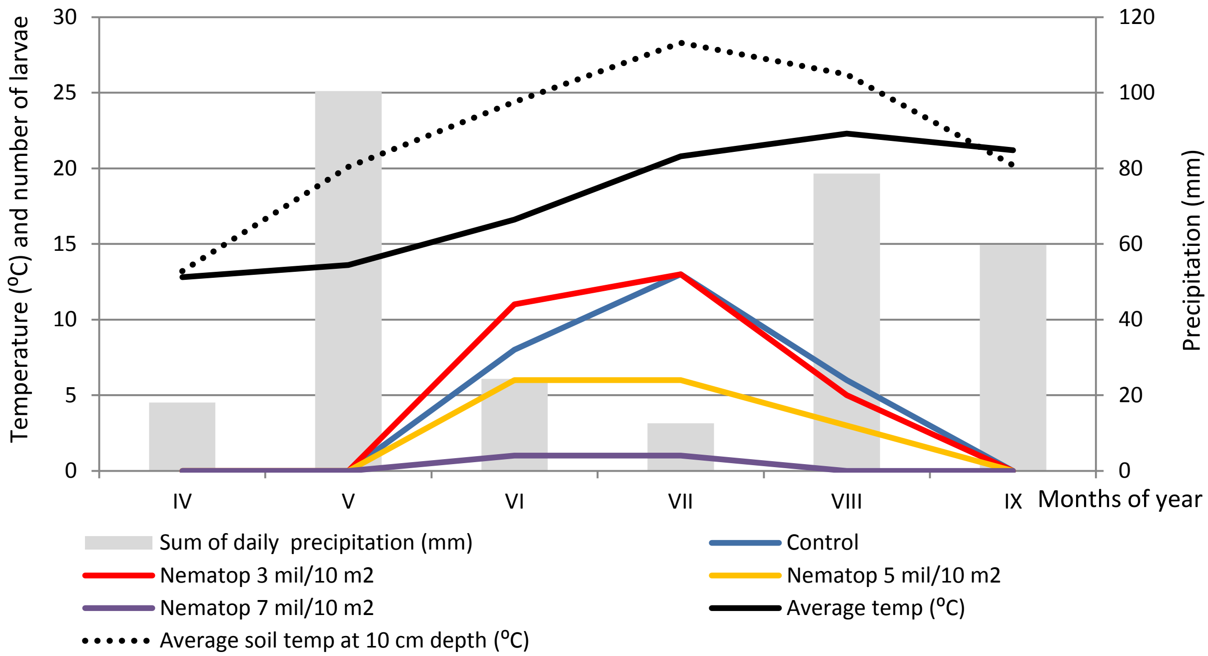


Figure 54. The number of sugar beet weevil (*Bothynoderes punctiventris*) larvae after the application of EPN *Heterorhabditis bacteriophora* and prevailing climatic conditions, Tovarnik, 2015

5.4.1. Efficacy assessment

Significant reduction of sugar beet weevil larvae in 2014 has been observed after the application of EPN at doses of 5 and 7 million IJs/10 m^2, while the dose of 3 million IJs/10 m^2 did not result in a significant reduction in the number of larvae compared to the control (Figure 55). No significant reduction in the number of larvae was established after the application of EPN in a dose of 3 million IJs/10m^2 in 2015. The doses of 5 and 7 million IJs/10 m^2 proved to be efficient and ensured a significant reduction in the number of larvae compared to the control.

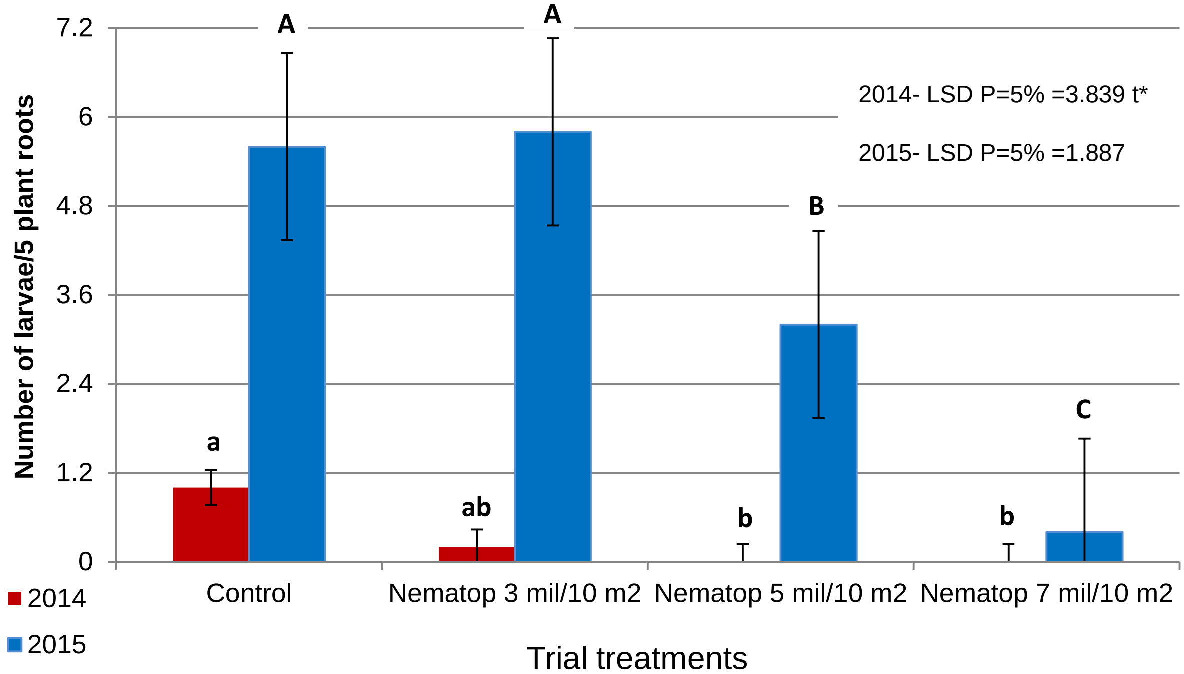


Figure 55. Number of sugar beet weevil (*Bothynoderes punctiventris*) larvae after the application of EPN *Heterorhabditis bacteriophora* in a two-year field experiment, Tovarnik, Croatia, 2014, 2015

In 2014, the efficacy of the lowest applied dose of EPN was 80% when both of the higher doses applied (i.e. 5 and 7 mill. IJs/10m^2) in 2014 resulted in 100% of the control. However the larval density was very low.

In the condition of much higher, but still moderate larval density, in 2015, the lowest dose of EPN did not result in a significant reduction of larvae. The efficacy of EPN applied at a dose of 5 mill. IJs/10 m^2 was 42.86% while the efficacy of higher dose was 92.86%.

6. DISCUSSION

6.1. CLIMATIC CONDITIONS

Climate types prevailing in Croatia are described as temperate/mesothermal climates (Cf) with dry winters (w) and overall higher amounts of precipitation. Penzar and Penzar (2000) reported that eastern Croatia where Tovarnik is situated, belongs to the Cfwbx climate type (according to Köppen's classification).The letter b indicates warmest month averaging below 22°C, but with at least 4 months averaging above 10°C. The letter x indicates that there is only one maximum rainfall event that mainly occurs in early summer (June).

The average yearly air temperature in 40 years long period in East Croatia is 11.4°C (Čačija, 2015) and the total amount of rainfall was 673.74 mm (Čačija, 2015).

In attachments climate diagrams according to Walter (Vukovar and Gradište) for every research year are present, with data obtained according to the Croatian Meteorological and Hydrological Service.

Since the conditions prevailed in 2011 could significantly influence the overwintering success of the weevil, we analysed climate data from the period between 2011 and 2016. Figures 24-26 show the differences in monthly air and soil temperatures and precipitation in each month between years. Although many significant differences of all three climate data set among years were established, some of them were quite obvious and may influence sugar beet weevil development. Thus, those deifference shall be discussed.

A very significant difference is recorded in mean temperatures in February (Figure 24). A very low average temperature of -3.4°C was recorded in 2012, while a very high temperature of 6.2°C was recorded in 2014. The differences between years were established for average temperatures in November. The lowest average temperature of 2.8°C was recorded in 2011, and the highest temperature of 9.55°C was recorded in 2012. Similar differences were recorded in average soil temperatures but to a somewhat lesser extent (Figure 25).

From the climate diagrams according to Walter (Appendix 1), it is clear that in three out of four years (2012, 2013 and 2015) there was a precipitation deficit in the summer months. Very dry conditions in March 2012, when only 3.3 mm of precipitation were reported. In the same month in 2013, the total amount of precipitation was 83.65 mm. In 2014 and 2015 total amouts of rainfall were 37.10 mm and 46.40 mm, respectively. March is a very important month in sugar beet development because the weevil emergence mainly occurs in March.

The total amount of precipitation in May (when sugar beet weevil is ovipositing the eggs) varied between years. The lowest amount of 66.5 mm was recorded in 2012 and an amount that was almost three times higher (161.25 mm) was recorded in 2014. The amount of precipitation in May 2013 and 2015 was 122.85 mm and 99.55 mm, respectively (Table 10). If the total amount of precipitation in two months (April and May) is less than 90 mm and the average temperature exceeds 24.5°C, conditions are favourable for mass reproduction of pests (Manninger, 1967). These conditions happened in 2011, when the total precipitation in April and May was 64.4 mm and the average temperature was 30.8°C. The described conditions actually resulted in a very high weevil population in spring 2012. The amount of precipitation in July, August and September 2014 was significantly higher compared to the other three years of investigations, leading us to conclude that the climatic conditions in 2014 were extremely wet.

Weather conditions, expressed as average air and soil temperature and total amount of precipitation, varied between the investigated years (2012-2015) (Table 7).

The conditions of 2012 were characterised by a moderate average yearly air temperature of 12.82°C, but a total amount of rainfall in the same period which was significantly lower (487.10 mm) than in other years of investigation. According to the Croatian Meteorological and Hydrological Service in 2012, Croatia was characterised as having an extremely hot and dry year, and this was the only year of research in which the conditions were not favourable for mass reproduction. Compared with the rest of the 40-year period, 2012 was significantly drier and warmer than the 40-year average. In contrast, 2013 was characterised as a moderate year with average yearly air temperatures of 12.49°C and average soil temperatures of 13.8°C, with a total rainfall amount of 721.35 mm. 2013 had a somewhat higher amount of precipitation than the 40-year average and a higher average air temperature, while 2014 was characterised as cold and moist. The investigation period in 2014 was characterised by lower average yearly air temperatures (13.19°C), average soil temperatures (14.56°C) and a significantly higher amount of rainfall (823.10 mm) compared to 2012 and 2013. 2014 was extremely wet, with the amount of rainfall being 25% higher than the 40-year average. 2015 was characterised as a moderate year with average yearly air temperatures of 13.05°C and average soil temperatures of 14.44°C, with a total amount of rainfall of 629.65 mm.

The comparison of the average climatic data during the overwintering period (October-February) resulted in significant differences in the average air temperatures, average soil temperatures and total amount of precipitation (Table 8). The highest temperature in the overwintering period was recorded in 2012/2013 (6.96°C), while the overwintering population in 2011-2012 went through the coldest period with an average annual air temperature of only 3.38°C. Also, the average annual temperature of the soil in the colder part of the year differed between years. The highest soil temperatures were recorded during the overwintering period of 2012/2013, while the soil temperatures were not significantly different in the overwintering period of 2014/2015 and 2015/2016. The amount of precipitation in the overwintering period was lowest in 2011/2012 (176.70). A statistically non-significant difference was noted between 2012/2013 (312.75) and 2014/2015 (286.70) in terms of the amount of precipitation.

The climate data (average air and soil temperature and total amount of precipitations) which prevailed during the vegetation period, i.e. the period of seven months (sugar beet weevil development from adult to adult lasts seven months) in four vegetation seasons (2012, 2013, 2014, and 2015) differed. Average air temperatures in 2012 were highest (18.86°C), with 18.20°C in 2015, while in 2013 and 2014 they were slightly lower (17.14°C and 17.31°C); there was no significant difference between them. Mass reproduction of the insect is favoured by higher temperatures and drought during the growing season, especially when there are two to three such years in succession (Čamprag et al., 2006). Average soil temperatures correlate with air temperatures during the same period (Table 9). According to the amount of precipitation during the vegetation period, the highest amount of precipitation was recorded in 2014.

Many authors reported that the climatic conditions in May are very important for pest population growth. According to the comparison of climate data in May in the study area over five years (2011 was taken into consideration since it could have a significant influence on weevil population in 2012, the first year of investigation), we found significant differences (Table 10) between years for average air temperatures, average soil temperatures and the total amount of rainfall. Although there was no significant difference between 2012 and 2013 in terms of average air temperature and soil temperature, they differed in the total amount of precipitation, with only 66.4 mm in May in 2012 and 122.85 mm in the same period in 2013.

6.2. LIFE TABLE PARAMETARS AND POPULATIONS CHARACTERISTICS

6.2.1. Dynamic of adults' emergence

Baited pheromone (TAL) pitfall traps (Figure 11) were used to catch adult insects which were emerging on a previous sugar beet field (Table 2). In research conducted from 2012 to 2015, we explored the emergence dynamics through all years in the period from the 12th till the 19th week of the year (Figure 28). During 2012, the highest rate of weevil emergence was recorded in the 14th week, while in 2014 and 2015 the same moment was for one week shifted, in the 15th week of the year. In 2013, the dynamic of adult's emergence was shifted in accordance with the prevailing weather conditions (Table 7). The first catches were recorded in the 15th week and the maximum in the 17th week of the year. According to data analysis, the largest share of the catch was recorded in the 15th and the 16th weeks of year (22.39% and 21.68%, respectively) and the lowest in the beginning (12th week of year) of the observation (1.83%) and at the end (19th week of year) with 1.93% (Figure 28).

Since many authors reported that first adults appear in spring, and the first specimens can be observed when the soil temperature at 5-10 cm raises between 6-10°C, we decided to use the temperature of 5°C as the thermal threshold for calculating DDA. The DDAs in three out of four analyzed year's (2012, 2013 and 2015) show the similar patterns (Figure 29). In 2014, DDA has been increasing much faster. This was the result of very warm January, February and March in 2014.

The correlation coefficients between DDA and the average share of adult emergence from the soil ranged from 0.8779 and 0.9774 what may be described as very strong to full. The correlation coefficients were significant at the level of 99% in all four years (Table 11). This finding confirms the statements of Rozsypal (1930) and Čamprag (1984) who reported that the time of appearance of the adults in the field depends on the temperature in spring and the depth at which hibernation occurs (and that weevils do not emerge all at the same time. First emerge those individuals that have spent diapause in the top layer of soil and then individuals who overwinter at greater depths. Regression analyse done based on the four year data (Figure 30) shows that the regression line is linear and described by the equation **$y= 0.243x - 4.0294$**, where x is DDA and y is the percent of the total weevil emergence. By the use of this equation it is possible to calculate DDA when 1% of all weevils emerge, as well as the DDA when 50 % and 100 % of all weevils emerge.

The calculated DDA for 1 % of emergence is 20.7°C. According to the data on DDA, the 1% of emergence occurred in three out of four years in March, on 18th, 8th and 10th of March in 2012, 2013 and 2015, respectively. Although in 2013 the emergence of 1% of weevils could occur on March 8th, we did not observe the weevil emergence until the beginning of April since there was a snow layer covering the soil by the end of March. In 2014 due to the extremely warm January, the occurrence of 1% of weevils could happen on January 20th. Since there was no available food on the fields we did not follow emergence so early in the season. In terms of the eastern part of Croatia soil temperature reaches 6-10°C usually at the end of the first and beginning of the second week in March. The second decade of March is the most appropriate period for weevil emergence but other factors as are snow layer and availability of the food shall be taken into account when predicting weevil emergence.

According to established regression line, the emergence of 50 % of weevils may occur when DDA reaches 222°C. It happened in our conditions in April, on April 14th 2012, April 23rd 2013, April 1st 2014 and April 21st 2015, respectively.

Weevils complete the emergence when DDA reaches 428°C. It happened on May 4th in 2012, May, 6th in 2013 and 2015 and on April 24th in 2014, respectively.

Our findings corresponds with those reported by other authors, The peak of weevil's emergence takes place in terms of sunny weather, when average air temperature reaches 15-25°C and soil surface temperature reaches 25-35°C (Petruha, 1959). In Hungary and Romania the highest percentage of pest's emergence was recorded in late March and early April, as well as in our conditions, according to Kovačević (1929) and according our findings. During 1923 in Czechoslovakia weevils continued to emerge from the soil until mid-August and in 1924 until mid-June, while most of them, however, appear in the middle of May, when they migrated to new beet plantations and paired (Rozsypal, 1930). In Vojvodina (area similar in terms of climate and edaphic conditions of eastern Slavonia) during 1981, was observed the dynamics of pest emergence. Both studies have resulted with a maximum catch (32.65% and 41.4%) of adult pests at the end of the first decade of April, in the 14th week of year (Radin, 1982) what is only one weak earlier than we established in our study. Deep autumn ploughing reduces the population and somewhat accelerates emergence from the soil in spring (Pyatnitzkiï, 1940). In our investigation we noted somewhat earlier emergence of adults from the fields that were not covered by crops in spring but we did not analyse the differences.

6.2.2. Appearance of eggs, larvae and pupal stages

As seen from Figures 29-32, adult weevils are present in fields from March (61 Julian day) through to the entire vegetation season (until Julian day 275), with two peaks of appearance: one in the spring between Julian day 110 and 150 (corresponds to April and May) and the second in autumn, between Julian day 220 and 250 (corresponds to August and September). It is important to note that until the 19^{th} week, we followed adults on previous year sugar beet fields using pheromone traps; later on, to monitor the dynamics of pest development, sugar beet plant surveys were conducted periodically. The lowest share of adults in the total population of weevils was established in July (between Julian day 184 and Julian day 204). In August, the share of adults in the total population started to increase (Figure 33). The results do not correspond completely with the results of Čamprag (1963), who reported that a few individuals can be found in the first half of August. The first eggs were observed on Julian day 102 and 122 in 2012, 2013 and 2015 and 132 in 2014, respectively. Eggs were observed in the field until Julian day 154, 173, 183 and 203, in 2012, 2013, 2014 and 2015, respectively. Although the development stage of eggs takes 10-15 days, due to the expanded time of weevil emergence, in prevailing conditions, eggs were found during days 52 to 71. The decreased presence of eggs in 2012 compared to the other three years of investigations could be explained by the fact that the average air temperature in June was highest in 2012 compared to the other three years of infestation. High temperatures in June (22.85°C average) was accompanied by very low amounts of precipitation (36.85 mm). The conditions described probably stopped egg laying. Additionally, the oviposition in 2012 started 20-30 days earlier than in the other three years, which also could influence the termination of oviposition. The optimum temperature for laying eggs is between 25 and 29°C (Bogdanov, 1961). First larvae were found on Julian day 122 or 132, depending on the year. In 2012 and 2015 first larvae were found 10 days after the first eggs, while in 2013 and 2014 we found the first eggs and the first larvae in the same survey. Since the time span between the surveys was 10 days, we may conclude that embryonic development lasts for less than 10 days, or it is possible that when conducting the survey due to the large sampling area we did not pick up any plants infested with eggs. The last larvae were found on Julian day 245 in three years of the investigation. The only exception was 2013, when the last larvae were found on Julian day 265. The first pupae were found on Julian day 143, 153 or 163 in years 2012, 2013 and 2015 and in 2014, respectively. This means that the shortest larval development time lasted between 20 and 30 days, which is much shorter than the data reported by Petruha (1959), who stated a shorter larval development of 45 days. The longest larval development time in our conditions (if calculated as the time span between the first and last date when larvae were observed in the field), lasted for approximately 120 days (four months), which is much longer than the data presented by Petruha (1959), who stated that larval development may last for up to 91 days. Since the first pupae were found between Julian days 143 and 163, we may conclude that a part of the adult population recorded in July consisted of freshly developed adults. It could be seen in the field from their body shape and other properties. It is difficult to state exactly what the shortest period of development from egg to the adult was, but we may conclude that approximately 60 days was the shortest period. This corresponds with the data presented by Petruha (1959), who reported that the overall development of sugar beet weevil, from egg to adult insect, takes 67-148 days. Steiner (1936) reported a period of 133 days. In Romania, the development lasts from 70 to 82 days, in Bulgaria it is about 75 days, in Hungary it is about three months, and in the area of Vojvodina it ranges from 2.5-3.5 months, but is usually about three months (Čamprag, 1984). The adult population started to increase in the beginning of August (Julian day 210) so we may conclude that the increase in adult population is a result of completing adult development from pupae. If analysing the average share of different developmental stages of sugar beet weevils established in surveys (2012-2015) (Figure 33) we can see that adults dominated until Julian day 122 and after Julian day 255. The share of the other developmental stages started to increase from Julan day 122 until Julian day 255. During the 50 day period (between Julian day 164 and Julian day 215), the share of larvae was significantly higher than the share of other developmental stages. During the one month period (in July) the share of larvae in the total population was over 60%. The share of larval population in September was 20% or less. Similar results were reported by Auersch (1954) in eastern Germany, during the second decade of September, where 6% of the larvae, 22% of pupae and 72% of adults were found. In Turkeyin mid-September, Steiner (1936) has found 57% of the larvae, 33% of the pupae and 10% of the adults.

Our data correspond the most with data reported by Čamprag (1984). In mid-September 1959 in the area of Vojvodina, the shares of developmental stages were as follows: 14.8% larvae, 27.2% pupae and 58% adults. The largest proportion of larvae in 2012 (47.22%) was found in the soil survey on the 154th Julian day (Figure 29), as well as the largest share of pupae (11.11%). 2013 deviated from the usual weather conditions and the attitude of certain developmental stages was shifted (Figure 30). On the 214th Julian day, the largest proportion of larvae (94.23%) was found, and on the 234th Julian day, the largest proportion of pupae (22.22%). Furthermore, in 2014 (Figure 31), 64.1% of larvae were recorded on the 193rd

Julian day and 30.43% of pupae on the 203rd Julian day. In the final study year (Figure 32), on the 183rd Julian day, 68.42% of larvae and 21.43% of pupae were found on the 173rd Julian day.

Based on the research results, we composed a phenogram of the sugar beet weevil development in Croatia (Figure 34). The life cycle of sugar beet weevil in Croatia is very similar to that reported in neighbouring countries (Serbia, Hungary or Romania) (Čamprag, 1984, Manninger, 1967, Petruha, 1971). We could expect this because the climate conditions in the area of investigation are very similar to those in other countries. Although many authors in Croatia (Kovačević, 1959, Maceljski, 2002) have reported on the sugar beet weevil life cycle, they just used the data available from other countries and assumed without any investigation that the life cycle in Croatia fully corresponds with life cycle in neighbouring countries. Here, we confirmed that fact.

6.2.3. Sex ratio

The number of males and females should be at least equal in a biotope (Kovačević, 1959). If males prevail in the population, the further spread of the population is endangered, whereas if females prevail, population increase could be expected. From a biological point of view, insect species in which females prevail have better biotic potential. Males can pair with more than one female which is why they do not have to be present in the same numbers as females.

In our investigation, we followed a sex ratio during the entire period of beetle emergence from the soil (Figure 35). The sex ratio of the emerged sugar beet weevil, after an overwintering period, is changing. In the early stages of pest emergence, males dominate. This relationship changes in equality, and in the end, after migration to the newly sown sugar beet field, the population is dominated by females. At the beginning of emergence, almost 90% of weevils are males. After 3-4 weeks, the share of males and females is equal and in the following three weeks the share of females is increasing (Figure 35). The same scenario has been established in all four years of investigation. The only difference was established in 2013 when the emergence of weevils was delayed. In 2013, the emergence lasted only 4-5 weeks and the described dynamic followed the same tendency but in a shorter time. Summarising the results obtained in all four years of investigation, we may say that in the week 15, the share of males and females did not significantly differ. Before the 15th week, the share of males was significantly higher and starting from the 16th week, the share of females is significantly higher than the share of males (Figure 36).

The appearance of different sexes in an insect population depends on the biological characteristic of the species. Protandry is the tendency for males to emerge before females (Bulmer, 1983), and it is common in insects with discrete, non-overlapping generations in which females mate only once, soon after emergence. In these circumstances, males which emerge early will have more opportunities to mate than those which emerge late, meaning that protandry would be expected to evolve through sexual selection. Very common cases of protandry in European Corn Borer (*Ostrinia nubilalis* Hubn.) (Bažok et al., 2009) and in Western Corn Rotworm (*Diabrotica virgifera virgifera* LeConte) are listed in the literature and proved in Croatian conditions (Igrc Barčić et al., 2003). A weekly review of samples (4*100 individuals) gave the same results as the research by Bogdanova (1965) in Bulgaria. In 2012 and 2015, we explored the equalisation in the ratio of sexes in the 15th week of the year (Figure 35). Our results partially correspond with the data reported by Manolache (1961) for the conditions in Romania. From pest emergence until mid-April, the ratio was 63:37 in favour of males, while the same ratio from the second half of April to the end of May changes in favour of females (47:53). In our investigation, we did not establish the sex ratio of the weevils in autumn.

6.2.4. Population fluctuation

To determine the biotic capacity of the species with its capacity for overwintering and its capacity to build up the population in one season, we analysed the population growth rate (in vegetation period) and overwintering success during the following winter (2012-2016) (Table 12). According to Chapman (1928), biotic potential indicates the value of any animal species as an environmental factor in one community (which is the ability of an organism or species for breeding and spreading). Biotic potential depends on the type of fertility, the number of offspring, diet, reproduction and the life span (Kovačević, 1961). Biotic factors that influence the biotic potential are the presence of predators, parasites and pathogens, while humidity, wind, light and, the most important, temperature are the abiotic factors.

Population growth rate was calculated as the ratio of the average infestation of the sugar beet fields in the autumn (expressed as the average number of weevil/m^2) vs. average infestation of the old sugar beet fields in the same area established in the spring, highly varied from year to year (Table 12). The highest population growth rate has been established in 2012 and the lowest in 2013. In order to determine how temperature and moisture influence the population growth rate, we correlated population growth rate with average air and soil temperature in the vegetation season and with the total amount of precipitation in the vegetation period. As vegetation period, we considered the period from March until September. It is obvious (Table 13) that correlation coefficients with all three parameters were significant (p=0.0001). Regression analysis of the population growth rate versus average air and soil temperature (Figure 39, Table 13) showed that the regression curves are linear, and correlations (measured by Pearson's coefficient of correlations) between those variables are positive and full according to Roemer and Orphal (Vasilj, 2000). Also, regression analysis of the population growth rate versus the total amount of rainfall in the vegetation season (Figure 40) and versus the total amount of rainfall in May (Figure 41, Table 13) showed that the regression curves are linear, and correlations (measured by Pearson's coefficient of correlations) between those variables are negative and very strong according to Roemer and Orphal (Vasilj, 2000).

Population growth rate is directly influenced by the oviposition success. Most oviposition occurs in May. Some literature data (Manninger, 1967; Maceljski, 2002) have reported that the temperatures and amount of precipitation in May have an impact on the level of infestation in the next year. Therefore, we correlated population growth rate with average air and soil temperature in May and with total amount of precipitation in May. Out of the three parameters analysed, only a correlation between population growth rate *vs.* the amount of precipitation in May (Table 13) was established as significant (p=0.0002). Regression analysis of the population growth rate versus total amount of rainfall in May (Figure 41, Table 13) showed that the regression curve is linear, and correlations (measured by Pearson's coefficient of correlations) are negative and very strong (r=-0.7794) according to Roemer and Orphal (Vasilj, 2000).

Research results confirm the previous reports (Manninger, 1967, Maceljski, 2002) that the sugar beet weevil prefers dry and warm climatic conditions and that the pest population growth is increasing with the increase in mean temperature. Additionally, the amount of precipitation negatively influences the pest population growth. Out of two basic conditions in May, only the amount of precipitation significantly influences population growth. According to Petruha (1959), the area of mass reproduction is characterised by climate factors, where the amount of total annual rainfall is between 400-450 mm during the whole year and 300-400 mm of rainfall occurs in the growing season. Out of the four years of investigations, only in 2012 (Table 7) did the total amount of precipitation correspond with the conditions proposed by Petruha (1959). In the other three years, the total amount of precipitation was between 600 and 800 mm, which probably negatively influenced the population growth. Comparing the amount of precipitation during the vegetation season, only in 2015 did the amount of precipitation correspond (348.15 mm) with the favourable conditions proposed by Petruha (1959). In 2012, the amount of precipitation during the vegetation season was lower, and in 2013 and 2014 the amounts of precipitation were higher (Table 9).

Favourable conditions for weevil propagation will occur if in the previous year (in May), the number of sunny and warm days (with temperatures above 25°C) was over 12, rainfall does not exceed 50 mm and the number of hours of sunshine is close to 300 (Maceljski, 2002). In our research, the average air and soil temperatures in May do not have any significant impact on population growth in the same year. In the literature, the authors do not report on the significant influence of temperature in May; they instead reported on the significant influence on the number of sunny days in May on pest population growth. We did not analyse this parameter.

Some other factors might influence the pest population growth. One very important parameter is the availability of food for insects (the presence of last year's area of sugar beet in relation to areas newly sown in the current year). Therefore, we calculated for each year the ratio between new and old sugar beet fields in the marked area and this value was correlated with population growth rate. The correlation is very strong, by increasing the average of new sugar beet fields in marked area the population growth rate increases. This finding could explain the fact that the weevil population has increased in Croatia in the past period where the acreage sown with sugar beet has increased significantly.

To influence the pest population growth, one cannot influence climatic conditions. However, we can manage and plan the distribution of newly sown sugar beet fields in one area. The acreage sown with sugar beet in particular areas shall remain constant. This shall be the responsibility of the organisers of sugar beet production.

The overwintering period is essential to maintain the population, and the overwintered weevils begin to make their way up to the surface of the soil as soon as the soil temperature increases above 2°C provided that the upper layers of the soil are warmer than those below; this movement of the population continues more or less until the end of the summer when the temperature near the surface falls below that of the lower layers (Pyatnitzkiï, 1940). However, if larvae and pupae do not complete development before the colder period, they die in late autumn or early winter (Čamprag, 1984).

According to Mansigh (1971), overwintering (hibernation) is defined as a physiological condition of growth retardation or arrest, primarily designed to overcome lower then optimum temperatures during the summer or winter. The size of insect population entering the overwintering stages and the subsequent survival of these stages play a major role in determining the population levels encountered in the following spring and summer. For overwintering success, three cues are known, photoperiod, temperature and moisture, as well as two biotic factors, nutrition and crowding (Leather et al., 1995). According to the cited authors, the first two of the three abiotic cues predominate in most insects.

In our research, we compared the overwintering success among four overwintering periods. Significant differences have been established among the seasons in the overwintering success. During the winter period of 2012/2013, only 42% of weevils were successfully surveyed, while in the next two winters, 100% of beetles were surveyed in the winter (Table 12). When the effect of air and soil temperature and the amount of rainfall in overwintering period (from October to February) on overwintering success were analysed (Table 14), we established significant correlations between average air ($p=0.0019$) and soil ($p=0.036$) temperature on overwintering success. Regression analysis of the overwintering success versus average air temperature in the overwintering period (Figure 43) showed that the regression curves are linear, and correlation (measured by Pearson's coefficient of correlation) between those variables are positive and medium according to Roemer and Orphal (Vasilj, 2000).

The amount of rainfall during the overwintering season does not influence the overwintering success.

6.3. AREA WIDE CONTROL OF SUGAR BEET WEEVIL BY MASS TRAPPING

Mass trapping with pheromone-baited traps has been successfully attempted in the family of weevils before (Tomašev et al., 2007).

In our investigation, in order to establish the success of mass trapping, we analysed several different parameters.

Comparison of the number of estimated weevils in the AW and number of caught weevils in pheromone traps

In 2012, 14 fields of previous year sugar beet were included in mass trapping, with a total area of 64.98 ha (Table 15). The average infestation of weevils/m^2 was established in the spring soil survey between 0 and 15.47, which showed the presence of a total population of

2,814,304 weevils in an area of 64.98 ha. Average infestation established by soil sampling varied between the fields. The number of captured weevils was 158.641. The mean trap capture significantly differed among the fields. It varied from 57.88 to 420.40 weevils/trap with an average of 162.71 weevils/trap. Overall, 5.64% of the estimated weevil population was trapped in 2012 (Table 19). In 2013, 15 fields of the previous year's sugar beet fields were included with a total area of 241.42 ha (Table 16). The established average infestation of weevils/m^2 in the autumn varied between 0.64 and 16.72 and in spring between 0 and 14.35. Over the entire area, sugar beet weevil population in the spring soil survey was established with 18,074,925 specimens. With over 3,600 traps, 2,095,007 weevils were captured. The mean number of weevils/trap significantly differed between fields and varied between 16.85 and 798.28 with an average of 578.57 weevils/ trap. The average total capture of weevils per trap established on the fields which are smaller in size (less than 6 ha) was significantly lower compared to the average total capture of weevils/trap caught in fields sized 60 and 130.18 ha, with the average total capture of 664.57 and 798.24 weevils/trap, respectively. Overall, 11.59% of the estimated population of weevils was trapped, which was the best result between the search area of wide mass trapping (Table 19). In the third year of research, in 2014, 19 fields of previous year sugar beet fields were included in the extended area of mass trapping with a total size of 40.97 ha. In spring, the soil survey established the population of 1,180,700 weevils. Total captured was maintained on 18.167 weevils. The third year of research resulted in only 1.53% of the captured population, with a very low average total capture of 29.54 weevils/trap. Although significant differences in average total capture were established (Table 17), it was not possible to find any correlation between field size and the average total capture of weevils. A similar situation was recorded in 2015. Only seven previous year sugar beet fields were involved, accounting for only 12.93 ha. The average infestation of weevil/m^2 established in spring varied from 0.36 to 17.72 weevils/m^2 (Table 18). Of the estimated population in the spring soil survey (773,850 weevils), 5,616 weevils were trapped, which is 0.73% of the estimated population. The mean number of captured weevils/trap significantly differed between the fields with an average of 28.95 weevils/trap/season. Again, it was not possible to find any correlation between field size and the average number of caught weevils.

The average trap capture in our investigation varied between years and fields. It varied from 10.67 to 798.24 weevils/trap/trapping seasons. The trap design is suitable to even capture much higher numbers of weevils; we did not observe problems of high density of the insect populations; saturation of the traps with weevils may influence the mass trapping success, as mentioned by Howse et al. (1998). Compared to the capture reported by Tomašev et al. (2007) of 1,000 to 2,000 weevils/trap/3 weeks, we recorded much lower captures. Very low captures were recorded in 2014 and 2015. It is difficult to conclude the reason for the lower number of trapped weevils in 2014 and 2015. It could be the weather conditions, due to the amounts of precipitation and average monthly temperatures in March and April. However, the differences related to the conditions in March and April between years do not support this statement. Tomašev et al. (2007) reported that their results were obtained in the conditions of the average infestation up to 30,000 weevils/ha. In our experiment, the average population of weevils varied from 28,818 weevils/ha in 2014 up to 74,869 weevils/ha in 2013, and the traps were exposed for seven weeks. Longer trap exposure did not resulted with higher number of captured weevils in our conditions. Also, the area of sugar beet sowed fields was reduced over years by farmer's decisions. The best prevention to protect crops represents spatial isolation of 1-3 km from the previous year's sugar beet fields (Čamprag, 2000).

Baited traps were useful in terms of low sugar beet weevil population. By mass trapping, we reduced the weevil population by up to 11.59% (Table 19). This is much lower than the results reported by Tomašev et al. (2007); however, their results were obtained on few small fields in conditions of lower infestation. Additionally, they used 30 traps/ha, while our investigation used only 15 traps/ha. However, by mass trapping, the infestation was postponed and the population reduction was accomplished in 2014 and 2015 compared to 2012 and 2013 in the marked area. According to Petruha (1971), the forecast of sugar beet weevil attack in the following vegetation season can be established on soil survey. Fields were categorised regarding the level of infestation as follows: (a) poor, with ≤0.5 weevils/m^2; b) mean, with 0.6-3 weevils/m^2; c) strong, 3.1-10 weevils/m^2; and d) very strong ≥10.1 weevils/m^2. The infestation according to soil survey level is given in Table 20. Regardless of the high number of pests in the area surveyed, there is still a risk of the stronger attack of pests, especially in already known pests (Sekulić et al., 2005). For vegetation season 2012, the forecast showed a strong attack on 8 fields and very strong attack on 1 field. The weevils captured in pheromone traps in 2012 had the ability to destroy 938 ha of sugar beet plants. In 2013, 2 fields showed mean, 5 fields showed strong and 3 fields showed very strong infestation levels. Total capture in the second year of research resulted in the preservation of 6,024 ha of sugar beet. In 2014 (6 fields mean infestation, 2 fields strong, 3 fields very strong infection) and 2015 (5 fields mean, 1 field with very strong infection), the forecasts of pest were not negligible, but because of the prevailing weather conditions in the period of mass trapping, the number of captured weevils and the result of the damage caused was not proportionate. In 2014 and 2015, there were fewer fields on which the weevil infestation was classified as poor or medium (Table 20). As established on soil survey over the years of research, a total number of 61,207,074 weevils had the ability to destroy 14,279,43 hectares (according to Čamprag et al. (2003) where threshold decision for sugar beet weevil is presented as 0.1-0.3 weevils/m^2 (representing 1,000-3,000 weevils per hectare). Overall, 2,280,370 weevils were trapped, which had the ability to destroy 777.72 hectares of newly sown sugar beet.

Average infestation of sugar beet fields in and outside AW

In order to establish the success of mass trapping, we surveyed all new sugar beet fields in the marked area and the average infestation of sugar beet fields in the marked area was established and expressed as the number of adults/m^2; also, damage of sugar beet plants was established. At the same time, we surveyed fields outside the marked area and established the damage and average infestation. It is obvious that the average infestation and average damage on the fields inside and outside the marked area were very similar (Figures 41-51). It shall be taken into account that commercial fields were observed on which farmers applied insecticides. In the marked area, farmers followed our forecast and applied insecticides when infestation close to the threshold was established by visual inspection. Each insecticide application was registered. Outside of the marked area, farmers applied insecticides according to their own decision based mainly on experience. Insecticide applications outside the marked area were also recorded.

In 2012 the highest infestation level was reached in time of leaf development; BBCH 1:10-11 (first pair of leaves visible, not yet unfolded (pea-size) outside the AW, on 10th April (1.09 weevil/m^2) and inside the AW (0.96 weevil/m^2). A second moment of infestation of pests is recorded inside (0.56 weevils/m^2) and outside AW (0.53 weevil/m^2) on the 8th May (BBCH 3:33, rosette growth (crop cover) - leaves cover 30% of ground). The next year, 2013, was recorded as more infestated, with an average infestation of 0.59 weevil/m^2 on 3rd May inside and 0.76 weevils/m^2 outside the AW. The highest infestation level outside AW was reached on 17th May (1.06 weevils/m^2) (BBCH 1:15; youth stage; 5 leaves unfolded. The highest infestation (0.74 weevil/m^2) in 2014 was recorded on 6th May (BBCH 3:31-33; rosette growth (crop cover) from beginning of crop cover, leaves cover 10% - 30% of ground) inside AW. Outside the AW was a different view in terms of infestation, where the level of damage reaches earlier the maximum with 1.45 weevil/m^2 on 8th April, in time of sugar beet youth stage development (4 leaves - 2nd pair of leaves unfolded). Different in terms of infestation definitely was 2015, where the most differences in level of infestation inside and outside the AW were recorded. The highest average number of weevil/m^2 inside AW was recorded just in development stages of sugar beet (BBCH 3:31-33; crop cover - leaves cover 30% of ground) on 7th and 14th May with a 0.96 and 0.88 weevil/m^2. On the contrary, outside the AW, an infestation with 0.74 weevil/m^2 was recorded already on 9th April (BBCH 1:14, youth stage - 4 leaves (2nd pair of leaves) unfolded) and rised to 1.68 weevil/m^2 on 23rd April (BBCH 1:15, youth stage - 5 leaves unfolded).

Established average damage caused by sugar beet weevil adults on sugar beet fields inside and outside the AW

According to the average damage caused by sugar beet weevil adults (in %) established on sugar beet fields inside and outside the marked area established on different dates of the survey, we can say that in four years there was a clearly visible difference. Within the study area, damage was significantly less compared to fields that were outside the area of research. In 2012 (Figure 46), damage from the 2nd to the 5th inspection was doubled (BBCH 1:10-19). The difference was already visible on the 2nd sugar beet review (BBCH 1:10), where the damage recorded within AW was below 1%, while in the fields outside the AW in the same period, the damage was recorded as over 1%. Next, 2013 (Figure 47) showed damage on the fields outside the AW, with an even greater difference compared to the fields inside the AW. The largest of the established damage was found in the 4th and 5th survey of sugar beet (BBCH 1: 14-19), where the percentage of damage was over 2%. In 2014 (Figure 48) damage during the fourth survey was observed to be close to 1% in the AW, while in the same reviews on the fields outside the AW, the percentage of damage was 0%. However, the previous (2nd and 3rd survey, BBCH 1:10-14) measurements showed the opposite results, and the 5th damage observation had the most significant offset between the fields, showing that the damage within the AW was close to 1%, and outside the AW was over 3%. Over the last year of the survey (Figure 49), the damage identified in the fields outside the AW was higher, especially in the 5th (BBCH 1:19) and 6th (BBCH 3:31) surveys of sugar beet plants.

Average number of insecticides treatments and amount of applied insecticides on the fields inside and outside the marked area

Currently in Croatia, active ingredients allowed for sugar beet weevil control are acetamiprid, chlorpyriphos, chlorpyriphos + cypermethrin, lambdacychalothrin and tiametoksam (as seed treatment) (Bažok, 2016a). Seed treatment with insecticides is regularly conducted at seed producers and all sugar beet seeds in Croatia are treated with neonicotinoid insecticides. However, it has been proven that seed treatment with neonicotinoids does not provide effective protection against sugar beet weevil in the conditions of medium and strong attack (Igrc Barčić et al., 2000; Bažok et al., 2012). Therefore, the foliar application of insecticides against sugar beet weevil is often conducted if the pest attack is established. Permitted active ingredients for foliar treatment belong to the group of OP insecticides (chlorpyriphos), pyrethroids (lambdacyhalothrin and cypermethrin) and neonicotinoids (acetamyprid). There is an intention in the European Union to limit the use of all of these insecticides in the future (Bažok et al., 2016b). According to Inđić (1998), chemical control of this pest is the main method of control and will probably be the same in the near future, although the economically rational measures of pest control include agrotechnical, mechanical and biological measures.

Two insecticides based on three active ingredients were used for sugar beet weevil control during the research period from 2012-2015 (Table 21). The first treatment of sugar beet weevil was usually conducted on field edges with lambdacychalothrin (product Karate Zeon 5 CS). This treatment has been applied on approximately 20% of the total surface of the field. The percentage of the treated field was calculated according to the range of the sprayer which farmers were using for sugar beet weevil treatment. Since the pest overruns by walking after overwintering from last year's sugar beet fields in the new crop, treatment of the edges is a common practice, which lowers the consumption of insecticides on fields, but increases the number of treatments. Later on, lambdacychalothrin (Karate Zeon 5 CS) and combination of chlorpyriphos and cypermethrin (product Nurelle D) were applied either alone or in combination on the whole surface. If the combination has been applied, both products were used in full doses.

The number of treatments and average consumption of insecticides (amount of active ingredient/ha) applied on fields inside and outside marked area in which area wide control of sugar beet weevil has been carried out, varied between years. For the purpose of this research, every year we followed five fields outside the AW. The collected information on insecticide application was systematically analysed. Additionally, we collected information from farmers about pesticide application and damage caused by sugar beet weevil on other fields in the non-marked area. That information was not collected systematically so we did not analyse them statistically.

Through all of the years of investigation, the numbers of insecticide applications and the amounts of active substance/ha were 3.5-4 times greater on fields outside the AW compared to the fields inside the AW (Figure 52). Selected fields outside the AW were randomly chosen to be observed. We recorded differences between those fields in the consumption of active ingredients per hectare. Also, we observed differences between years as the average consumption of insecticides on the fields' outside the AW. However, generally, the fields outside the AW received between 3.5 and 4.2 treatments per season. The amount of insecticides applied depended on the insecticides used in the treatments. If lambdacychalothrin was used, due to the lower recommended dose per ha, the amount of insecticide used was lower. All insecticides allowed for sugar beet weevil control in Croatia could be applied on up to two occasions on one field (Bažok, 2016a). It shall be pointed out that fields outside the AW were very often treated with two insecticides simultaneously. Therefore, on the fields which were treated four or more times, the number of applications and the amount of insecticides exceeded the permitted rate (Bažok, 2016b).

Due to the specific morphological structure of weevils, their large feeding capacity and the small leaf area of plants at the time of insecticide application, even the permitted insecticides often give very poor results and require repeated treatment (Bažok et al. 2012). This is not in accordance with the principles of integrated pest management (IPM) nor with the rational use of pesticides in modern agriculture. Among the many factors that influence the toxicity and efficacy of insecticides, temperature has a significant role in time before and after the application (Vuković et al., 2004). 2012 was recorded as a warmer year in terms of average monthly air and soil temperatures. For comparison, during the research, we had fields that were not in extreme conditions, or attacks, and the number of treatments was an average of 4, or 4 times greater than the application of insecticides inside the AW. In our investigation, we also observed that some fields in the region of Tovarnik which were outside the AW were treated up to 12 times. In spite of such a large number of treatments and spending significant amounts of active ingredients/ha, it was not rare for farmers to have their fields lean because of the thinning set resulting from harmful eating by pests.

Eastern part of Croatia is covered in chernozem. Area of mass reproduction of sugar beet weevil is limited to the area of chernozem and meadow black soil (Čamprag, 2000). Years 2012 and 2013 were very suitable for pest's development and estimated was replanting of fields about 35-40% of the total area sown with sugar beet that surround Tovarnik.

Mass trapping of sugar beet weevil on the "old" sugar beet fields in marked area significantly reduced the number of insecticide applications and the amout of used insecticides (Figure 52) with keeping the damage and weevil infestation (Figures 4-51) on the same or even lower level comparing to the fields outside AW. The eastern part of Croatia is covered in chernozem. The area of mass reproduction of sugar beet weevils is limited to the area of chernozem and meadow black soil (Čamprag, 2000). 2012 and 2013 were very suitable for pest development and it was estimated that about 35-40% of the total area sown with sugar beet that surround Tovarnik was replanted.

Mass trapping of sugar beet weevil on the "old" sugar beet fields in the marked area significantly reduced the number of insecticide applications and the amount of insecticides used (Figure 52), keeping the damage and weevil infestation (Figures 44-51) the same or even lower compared to with fields outside the AW .

There are two ways in which mass trapping could be used to reduce adult weevil populations. The first is to try to trap out the overwintering weevils by mass trapping the emerging pest at the overwintering sites (”old” sugar-beet fields), while the second is to try to intercept weevils which emerged elsewhere when they arrive at the sugar-beet fields with beet seedlings ('newly sown sugar beet fields'). The method of mass trapping by the aggregation of pheromones on an AW basis according to the basic principles of AW programs as proposed by Knipling (1979) is not only a short-term goal, tp control pests in a field or a season, but the long-term goal of these methods, is to reduce the population of pests in a particular area.

In years of strong attack, as seen in 2012, 2013, 2014 and 2015 in the researched area, mass trapping was not efficient enough to avoid the insecticide application. AW mass-trapping with pheromones is capable of reducing the population (population density estimated by soil sampling of overwintering weevils). An additional advantage of the present attractant-baited traps for mass trapping was that they showed considerable specificity in catching the sugar-beet weevil and caught non-target, in part beneficial insects in very low percentages compared to the masses of weevils caught. The area of mass trapping contained different numbers and sizes of fields among years. On those fields, different crop rotations have been utilised. According to most farmers, sugar beet was sown every two, three or four years in the same field. This is not always (in the case of two or three years' crop rotation) in accordance with the principles of integrated crop production.

As it was reported (Table 1) in the chapter 3.3., area wide mass trapping programs showed to be successful against many pests. Insects from all orders could be controlled by mass trapping. In some cases several tools are combined in order to achive success. If sexual pheromone traps are used, only one sex (usually males are attracted) as it was the case with beet armyworm (*Spodoptera exiqua*) control in welsh onion (Park and Goh, 1992), tea tussock moth (*Euproctis pseudoconspers*) control in peach orchards (Yongomo et al., 2005) and the control of stem borers in rice (Zhu et al., 2007). Aggregation pheromones or other lures that are attracting both sexes are more suitable for mass trapping programs. The use of aggregation pheromones (or combination of sexual pheromones with other attractants) is reported for the control of mountain pine beetle (*Dendroctonus ponderosae*) in ladgepole pine (Borden et al., 1993), Japanes beetle (*Popilia japonica*) in isolated area (Wawrzynski and Ascerno, 1998), *Carpophilus spp*. in stone fruit orchards (James et al., 1998), cocoa pod borer (*Conomorpha cramerella*) in coconut palms (Beevor et al., 1993), American palm weevil (*Rhyhoporus* palmarum) in oil palms (Oehlschlager et al., 2002), Mediterranean fruit fly (*Ceratitis capitata*) in paw-paw (*Asimina triloba)* (Podleckis, 2007), stinkbug (*Plautia stali*) in persimmon (Yamanaka et al., 2011) etc. Out of all examples, it is important to point out the successful control of American palm weevil (*R. palmarum)* on oil palms (Oehlschlager et al., 2002). The use of a slow release formulation (~3 mg/day under field conditions) of 6-methylhept-2-en-4-ol, the aggregation pheromone of *R. palmarum* (Oehlschlager et al. 2002) proved to bee effective to maintained red ring disease (RRD) (transmitted by R. *palmarum*) at very low levels over several years. American palm weevil is the insect belonging to the same family (Curculionidae) as sugar beet weevil, thus probably having some similar patterns.in reaction toward aggregation pheromones.

During the process of joining the EU, Croatian agriculture was exposed to the strong demands of producers to follow IPM principles in the whole agricultural production. IPM is a decision-making process for managing pests using monitoring to determine pest-caused injury levels and combining biological control, cultural practices, mechanical and physical tools, and chemicals to minimise pesticide usage. The common codex for integrated farming in which IPM is very important part was developed in January 2001 by the members of the European Initiative for Sustainable Development in Agriculture (EISA). Studies have shown that IPM systems yield greater biodiversity and reduce pesticide use by at least 20% compared to conventional farming, as assessed using the treatment index (Barzman et al., 2015). Many EU countries including Croatia have developed national pesticide reduction programs. EU Regulation 1107/2009/EC on the placing of plant protection products on the market requires that pesticides be "used properly", where proper use "shall also comply with (...) general principles of integrated pest management (...)"(European Union 2009). More simply put, the new set of legislation -- the so-called "EU pesticides package" -- which includes two Directives and two Regulations, aims at risk reduction during the use phase of pesticides and demands that all pesticide users adopt IPM.

The AW-IPM approach is proactive, i.e. action is taken before a pest population reaches damaging levels, and aims at protecting agriculture and/or human health in an entire area

(Vreysen, et al., 2007b). Each AW-IPM programme requires a regulatory framework according to its specific needs. Consequently, after defining the strategic approach, e.g. suppression, containment/ prevention or eradication (Hendrichs et al. 2007), each campaign requires the development of an appropriate strategy and corresponding thorough and detailed operational planning well before it is initiated. It is very often that the AW strategy is not based only on one tool used for pest suppression; very often, several tools are combined. The use of insecticides is the last tool. We used insecticides in our investigation because the pest population was still high enough to cause serious damage; however, due to the situation described with the pesticide use in the EU, we tried to find an additional solution to be used in AW control. It appears that the mass trapping of sugar beet weevil played a large role in reducing the damage. We realize the lack of strict statistical controls limits the scope of this study, but we certainly feel that trapping was a significant factor in the population control effort at our study site. It may not be possible to state from this study that trapping alone will reduce sugar beet weevil population. The data do suggest however, that trapping can play an important role in an integrated pest management plan for dealing with sugar beet weevil under similar circumstances.

Since entomopathogenic nematodes were listed as potentially available non-pesticide tools for sugar beet control, we decided to explore their potential to be an additional tool when AW sugar beet weevil control by mass trapping is conducted.

6.4. EFFICIACY OF ENTOMOPATHOGENIC NEMATODES (EPN) ON SUGAR BEET WEEVIL

In spite of the fact that the initial population of adult weevils in 2014 was higher (0.75 adults/m^2) than in 2015 (0.5 adults/m^2), the infestation of plants in the trial was much higher in 2015 than in 2014. The reason for this could be weather conditions. According to Maceljski (2002), increased rainfall in April and May negatively affects egg laying, probably due to the disruption of weevil activities, and may also lead to egg deterioration or development difficulties of the egg and larval stages. The amount of rainfall in April and May 2014 was 214 mm. In the same period in 2015, the amount of rainfall was about two times lower (119 mm). Therefore, less rainfall in 2015 could have enabled oviposition and led to higher infection with larvae than in 2014. The ratio of autumn comparing to spring population of adults in 2015 was

3.5 and, in 2014 it was 1.5. The observed difference also indicates that weather conditions in 2015 were more favourable for sugar beet weevil. However, the population growth in both years was below the growth which could be expected if weather conditions were optimal. Maceljski (2002) stated that the higher population of sugar beet weevil could be expected if less than 50 mm of rainfall was observed in May of the previous year. Based on the available data on pest biology (Drmić and Bažok, 2015) and the average number of adults determined on study fields (7,500 adults/ha in 2014, and 5,000 adults/ha in 2015) the infestation between 2.5 and 3.75 larvae/plant could be expected. However, in 2015, the maximal number of larvae found in untreated plots was 7 larvae on 25 plants, which makes an average infestation of 0.28 larvae/plant. The maximum infestation on the untreated plot was reported on 6th July 2015. These data indicate that the amount of rainfall (as observed in May), although much lower than in 2014, still might have exceeded the optimal conditions for sugar beet weevil larval development.

Since in 2015, as well as in the previous year, the amount of rainfall has been much higher than mentioned, so it has been assumed that these climatic conditions might have led to lower egg disposal and increased egg and larval mortality. However, there are probably still some unknown factors which contributed to lower development of eggs or the larvae.

The observed results indicate the high efficacy of all three doses of EPNs in the condition of very low attack intensity. Also, EPN shows a dose response in the conditions of moderate attack intensity. In such conditions, the lowest dose was not effective while the highest dose resulted in 92.46% efficacy. The dose of 5 million IJs/10m^2 is recommended by producers for the control of other weevils and it is obvious that this did not result in satisfactory efficacy in the conditions of moderate (or even low) attack. Therefore, the question of the efficacy of the moderate dose could be raised.

According to Susurluk (2008), soil depth and temperature have an important influence on sugar beet weevil infection and mortality caused by the nematode *H. bacteriophora*. The efficiency of this nematode species increases as the soil temperature rises. *H. bacteriophora* shows the highest performance at soil temperatures between 20°C and 25°C, and at a depth of between 5 cm and 10 cm, where the larval stages are mobile (Čamprag, 1984). Temperatures <8°C and >40°C are lethal to most EPNs (Griffin, 1993; Grewal et al., 1994). The soil temperatures detected during this research were in the optimum range for tested nematode *H. bacteriophora*. Other species of nematodes, such as *Steinernema feltiae* Filipjev and *Steinernema weiseri* Mracek, Sturhan & Reed, 2003, perform better at lower temperatures (15 °C), but are not as effective on sugar beet weevil as *H. bacteriophora*.

Since EPN affects only the larval stages of weevils, the critical point to achieve success is application timing. According to the literature, oviposition chiefly occurs at the end of May and beginning of June, and the larvae hatch on the third day after the eggs are laid (Rozsypal, 1930). We applied EPN ten days after copulation was observed, to make sure that the eggs had hatched. In both years, the oviposition started earlier than is reported in literature. It is possible that we applied EPNs when the oviposition started and that the maximum oviposition occurred subsequently. Thus it might happen that at the time of EPN application, most of the eggs had not been laid jet and the nematodes did not have enough available eggs to attack. According to Čamprag (1963), sugar beet weevil larva passes through five developmental stages, which may raise the question of which of these stages is the most sensitive for the penetration and effectiveness of nematodes? New laboratory researches are needed in order to determine the most sensitive larval stage and the optimal period of treatment after oviposition.

The number of adults which entered overwintering stage, detected in the autumn on investigated fields, was 1 weevil/m^2 in 2014 and 1.75 weevils/m^2 in 2015. Both numbers are considered medium infestation in long-term forecasts.

The low larval population in 2014 enabled us to draw reliable conclusions about the efficacy of the EPNs against sugar beet larvae. However, the results achieved in 2015 show clear dose responses and indicate that EPNs could have a satisfactory effect on the larvae. To make more reliable conclusions, additional researches are needed.

The use of EPNs for sugar beet weevil control focuses on the prevention of larvae, which actually rarely damage the root (Maceljski, 2002). Therefore this is not a strategy for preventing damage on one field in a particular season. This is a measure that is aimed at the suppression of the adult population and should be implemented within a well-developed strategy, in which a number of different joint measures should be developed to suppress the population of pests in the wider area. This kind of suppression is very expensive and has a long-term impact. It is unlikely that the farmers themselves will find this measure. Therefore this measure will have to be organised and (semi)financed by the organisers of sugar beet production (sugar factories).

7. CONCLUSIONS

During the research, all goals were set up to prove that the hypotheses are accomplished. In accordance with the hypothesis and objectives, based on the results, the conclusions of the study are as follows:

- Weather conditions in East Croatia during growing season significantly varied among the investigated years (2012-2015). Out of the four years of investigation, significantly lower amounts of precipitation were recorded in 2012. The amount of precipitation on the level of the 40 years average was recorded in 2013 and 2015. In 2013 the amount of precipitation was 30% over the average. Vegetation period in 2012 was characterised by extremely high temperatures, thus not making them favourable for sugar beet weevil mass reproduction. In other three years, the temperatures were favourable for sugar beet weevil development.
- In the investigation the timing of the occurrence of different developmental stages of the sugar beet weevil under the ecological conditions of eastern Croatia was determined. The degree day accumulation (DDA) for weevil emergence can be calculated based on the soil temperature at 10 cm depth by the use the temperature of 5°C as the thermal threshold. The first emergence started when DDA reaches 20°C (first two decades of March). However, the emergence depends on the existing snow layer as well as on the availability of food. Weevils completed emergence when DDA reaches 428°C what usually happen in the first week of May.The results show how the largest proportions of specimens (which emerge from overwintering) were established in the 14th and 15th weeks of the year.
- Male sugar beet weevils emerge first and dominate in the weevil population up to the 15th week of the year. Sugar beet weevils have an equal sex ratio in the 15th week of the year. Afterwards, however, the sugar beet weevil population is dominated by females.
- Sugar beet weevil development in eastern Croatia is very similar to that in neighbouring countries (Serbia and Hungary). Overwintering adults are present in the fields up to the beginning of July. Newly developed adults emerge from the soil in July. The average time of pest development for all stages lasts about three to four months. Although the development stage of the egg takes 10-15 days, due to the expanded time of weevil emergence, in prevailing conditions, eggs were found on average in 102 days (between 112th and 214th Julian day), larvae development was

established up to 143 days (between 122th and 265th Julian day) and pupae development up to 102 days (between 143th and 245th Julian day).

- The population growth positively correlate with air (r=0.9409**) and soil temperature (r= 0.9307**) during the vegetation period and negatively correlate with the amount of precipitation in vegetation period (r= -7971**) as well as with the amount of precipitation in May (r= -0.7794**). Population growth rate depends on the ratio between new and old sugar beet fields in marked area (r= 0.7813**). With increasing the share of newly sown sugar beet field, the population growth increases. Therefore, the distribution of newly sown sugar beet fields in one area shall be carefully planned. The surface of fields sown with sugar beet in particular area shall keep constant in order to reduce the possibility of an increased population. This shall be the responsibility of the organisers of sugar beet production.
- Overwintering success depends on the air and soil temperature prevailed in the period of overwintering. No dependence of the overwintering success on the amount of precipitation during the overwintering period has been established.
- Sugar beet weevil population in the marked area was high during the entire period of investigation. Established spring populations on "old" sugar beet fields varied from approximately 28,000 to 78,000 weevils/ha, which is 10-20 times more than the economic threshold. In such conditions, baited traps were useful in terms of low sugar beet weevil population. By mass trapping, we reduced weevil populations by up to 11.59%. Although a less than 1% population reduction has been obtained in some years, by mass trapping, the infestation was postponed and the population reduction is accomplished in 2014 and 2015 comparing to 2012 and 2013 in the marked area.
- Mass trapping of sugar beet weevil on the "old" sugar beet fields in AW area significantly reduced the number of insecticide applications and the amount of insecticides used to keep the damage and weevil infestation on the same or even lower level comparing to the fields outside the AW.
- The number of treatments can be aligned with a rational use of insecticides. Only 1.2 treatments (one treatment applied on the field edges and one on the whole surface) of fields inside the AW program were sufficient to control the sugar beet weevil and keep damage below the economic threshold.
- The entomopathogenic nematode Heterorhabditis bacteriophora has a potential in suppressing the sugar beet weevil. Observed results indicate a high efficacy of all three doses of EPNs in the condition of very low attack intensity. EPNs show a dose

response in the conditions of moderate intensity attack. In such conditions, the lowest dose was not effective while the highest dose resulted in 92.46% of the efficacy. The dose of 5 million IJs/10m2 is recommended by producers for the control of other weevils and it is obvious that it did not result in satisfactory efficacy in the conditions of moderate (or even low) attack (42.86%). Therefore the question of the efficacy of the moderate dose could be raised.

- As a biological pest control measure, EPNs aimed at the suppression of the adult population should be implemented within a well-developed strategy in which a number of different joint measures including area wide mass trapping should be developed to suppress the population of sugar beet weevil in the wider area.
- Research results provided very detailed information on the life table parameters and sugar beet weevil life cycle in the conditions of eastern Croatia. Also, the main factors influencing population growth were determined. The knowledge obtained is necessary to develop different strategies for sugar beet weevil control according to the principles of IPM.
- Area-wide (AW) mass trapping of beet weevils using aggregation pheromones in the previous year's sugar beet fields provided the possibility of reducing the pest population and significantly reduced the need to apply insecticides. This enabled farmers to control sugar beet weevil according to the principles of IPM with no more than two insecticide treatments in the season.
- In order to achieve better success, AW mass trapping shall be combined with other non-pesticide measures for the sugar beet weevil control. The use of the entomopathogenic nematode (Heterorhabditis bacteriophora, Poinar 1976) has significant potential to reduce the sugar beet weevil population and might serve as a good tool to be implemented into AW programmes.
- The research results significantly contribute to the ability of sugar beet producers to introduce mandatory principles of integrated pest management in their production and enable environmentally acceptable control of sugar beet weevil which almost became a limiting factor in the production of sugar beet.

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9. BIOGRAPHY

Zrinka Drmić was born on 16th March 1984 in Vukovar. She graduated in 2010 at the Josip Juraj Strossmayer University of Osijek, Faculty of Agriculture in Osijek (Agronomy). First she was emploeed in Agro-Tovarnik d.o.o. as an agronomist. Since March of 2012 she has been employed as an expert associate in Department of Agricultural Zoology, Faculty of Agriculture, University of Zagreb.

She was a collaborator in a scientific project financed by the Ministry of Agriculture and the Croatian Science Foundation and associate in a scientific project financed from EU funds. Currently she is an associate in a scientific project financed from EU funds. Research interests are related to the pests of field crops. Postgraduate doctoral study "Agricultural Science", she enrolled in 2011/12. From the list of published papers she has three papers related to the topic of the thesis, and also have two published work of doctoral thesis indexed in a1 database. The total is co-author of the two papers a1 category, 3 papers a2 categories, 2 papers a3 categories, eight abstracts in conference proceedings with international review, 10 papers presented at national and international congresses and several research papers.

List of published works of authors

A1 papers:

1. Bažok, R., Šatvar, M., Radoš, I., Drmić, Z., Lemić, D., Čačija, M., Gašparić, H. V. (2016). Comparative Efficacy of Classical and Biorational Insecticides on Sugar Beet Weevil, *Bothynoderes punctiventris* Germar (Coleoptera: Curculionidae). Plant protection science, 52 (2), 134-141.
2. Lemic, D., Drmić, Z., Bažok, R. (2016). Population dynamics of noctuid moths and damage forecasting in sugar beet. Agricultural and Forest Entomology.

A2 papers

3. Grubišić, D., Bažok, R., Drmić, Z., Kartelo, I., Mrganić, M. (2016). Distribution of the species *Heterodera schachtii* in the area of Tovarnik and current options for control. Poljoprivreda, 22 (1), 28-33.
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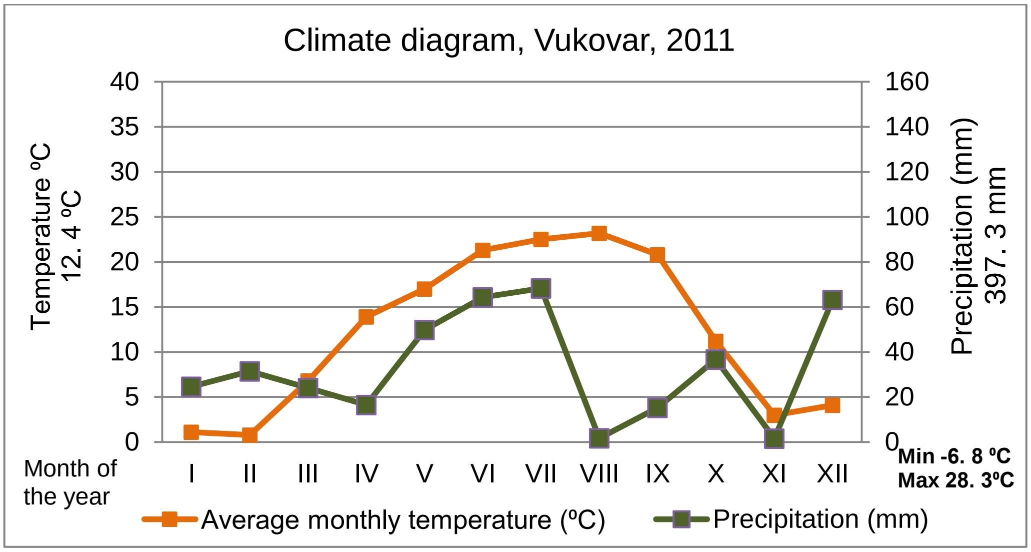
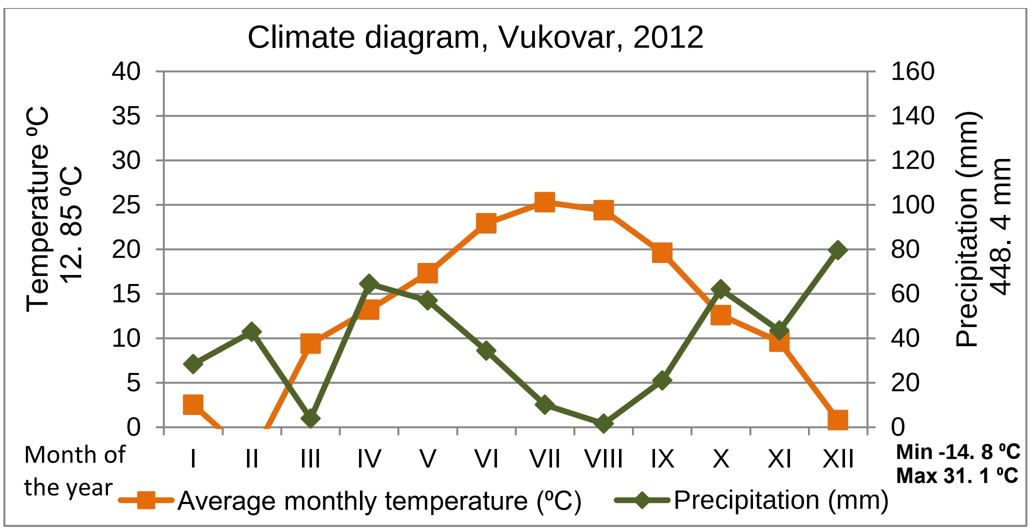
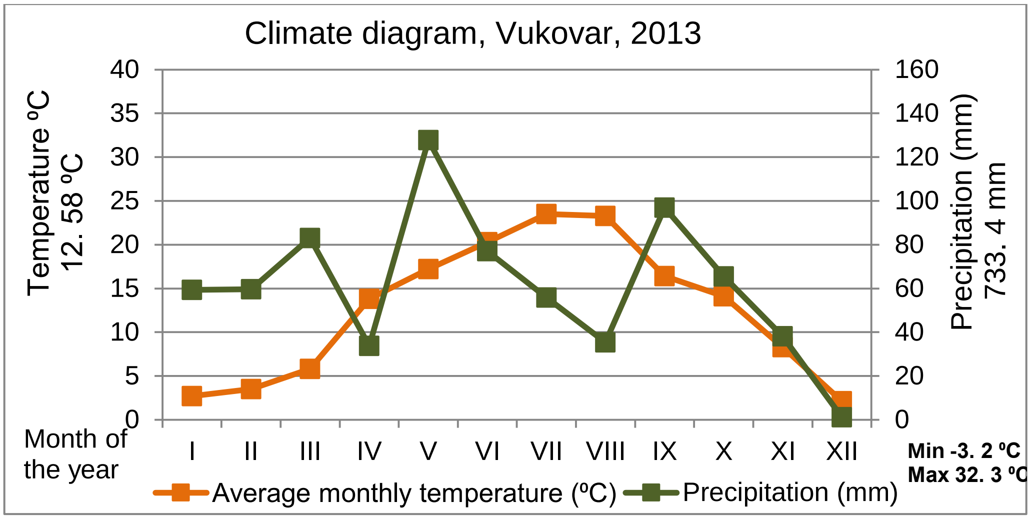
10. ATTACHMENTS

Attachment A.

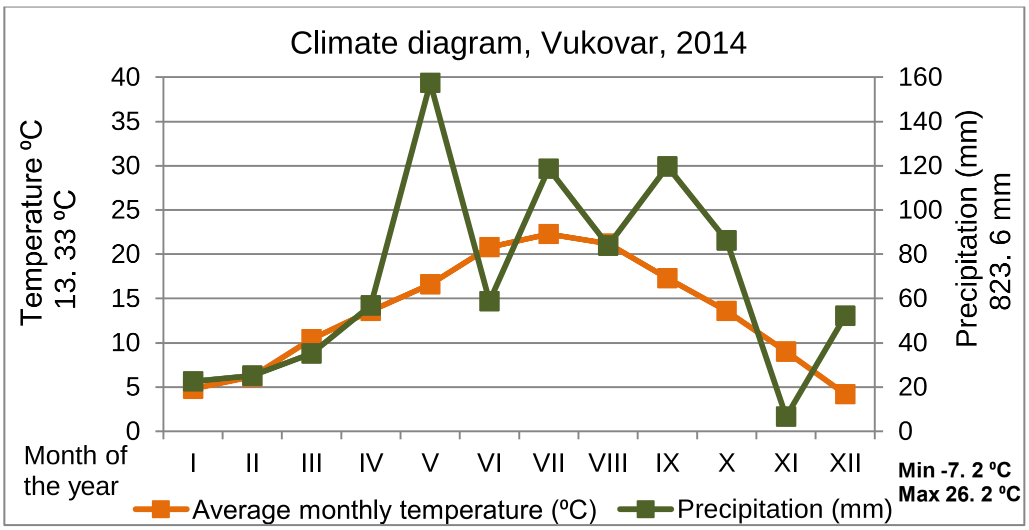
a) Display of climate diagram from the research area (Vukovar) for all exploration years (2011-2015)



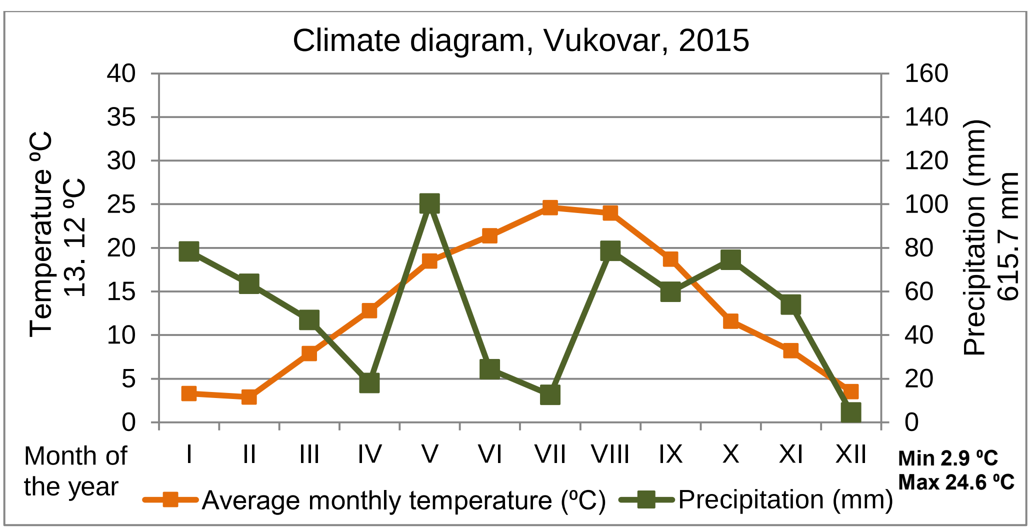




Climate diagram, Vukovar, 2013
Temperature ºC
12. 58 ºC
Precipitation (mm)
733. 4 mm
Month of the year
I II III IV V VI VII VIII IX X XI XII
Min -3. 2 ºC
Max 32. 3 ºC
Average monthly temperature (ºC)
Precipitation (mm)

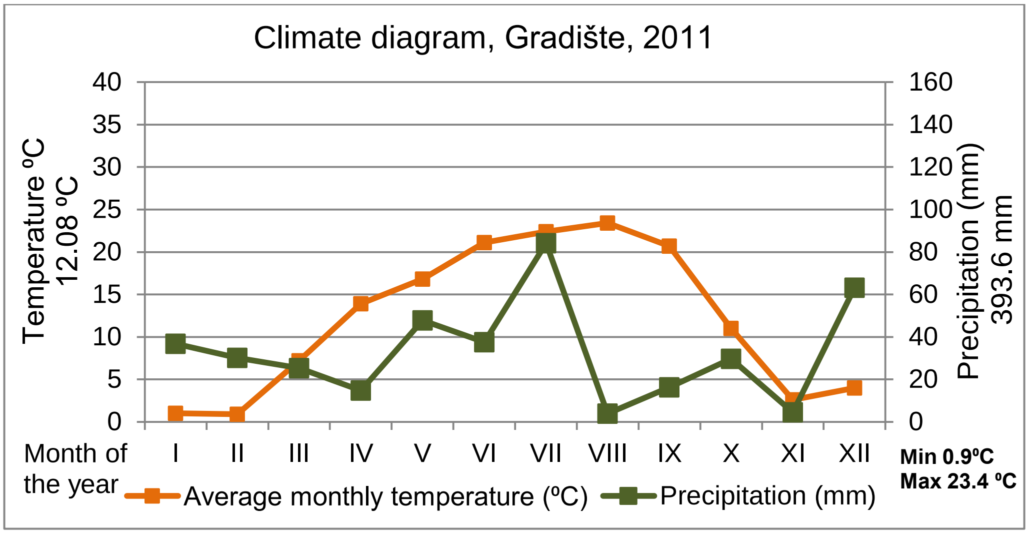


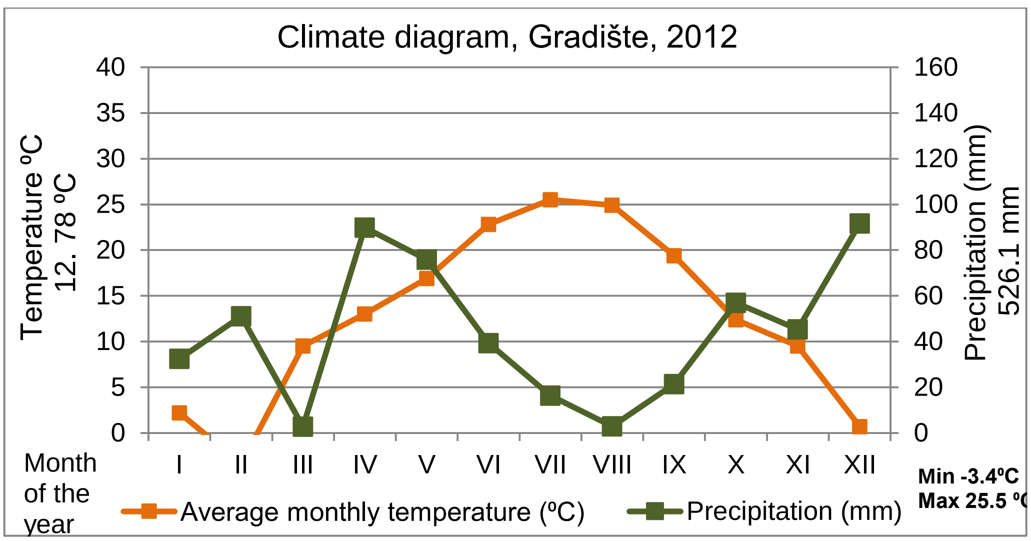
Climate diagram, Vukovar, 2014
Temperature ºC
13. 33 ºC
Precipitation (mm)
823. 6 mm
Month of the year
I II III IV V VI VII VIII IX X XI XII
Min -7. 2 ºC
Max 26. 2 ºC
Average monthly temperature (ºC)
Precipitation (mm)

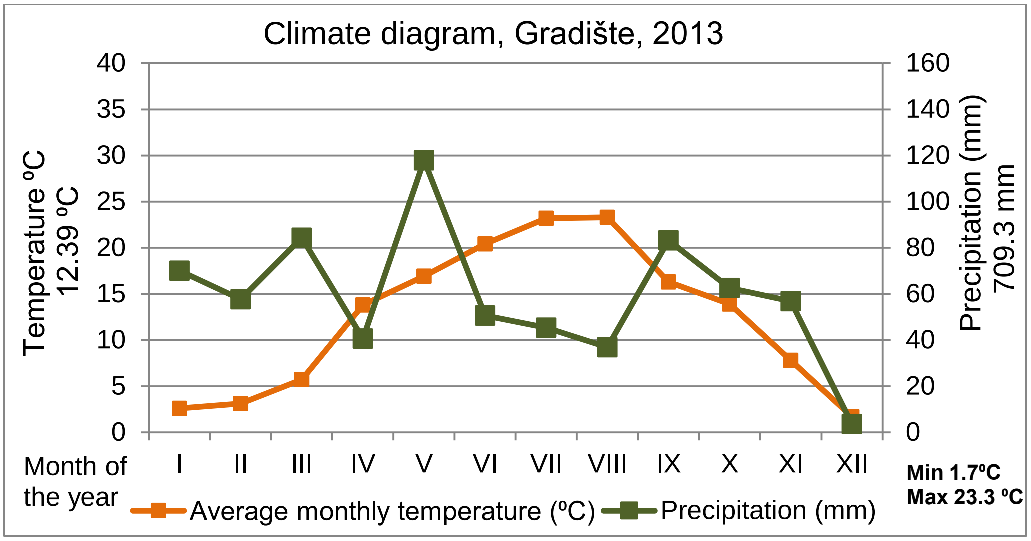


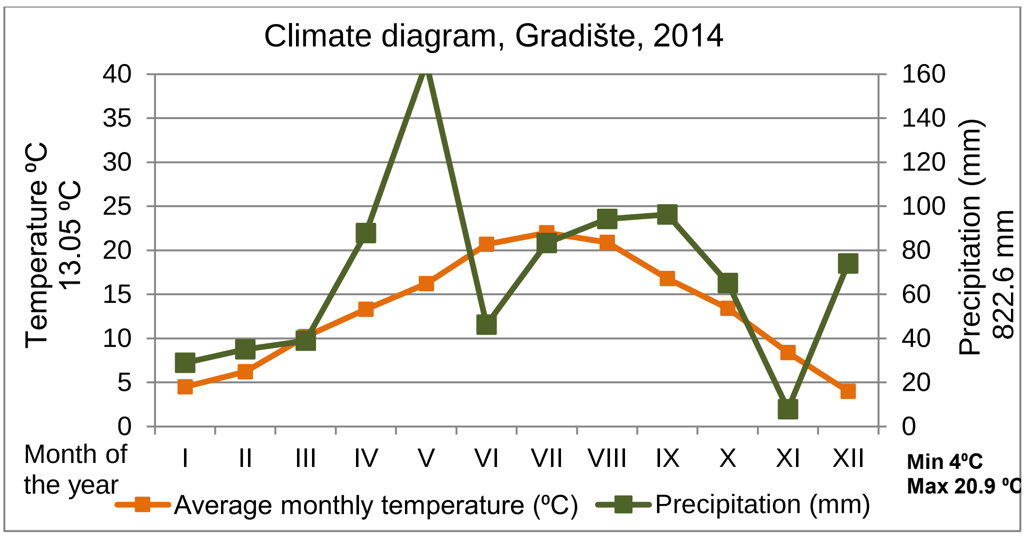
Climate diagram, Vukovar, 2015
Temperature ºC
13. 12 ºC
Precipitation (mm)
615.7 mm
Month of the year
I II III IV V VI VII VIII IX X XI XII
Min 2.9 ºC
Max 24.6 ºC
Average monthly temperature (ºC)
Precipitation (mm)

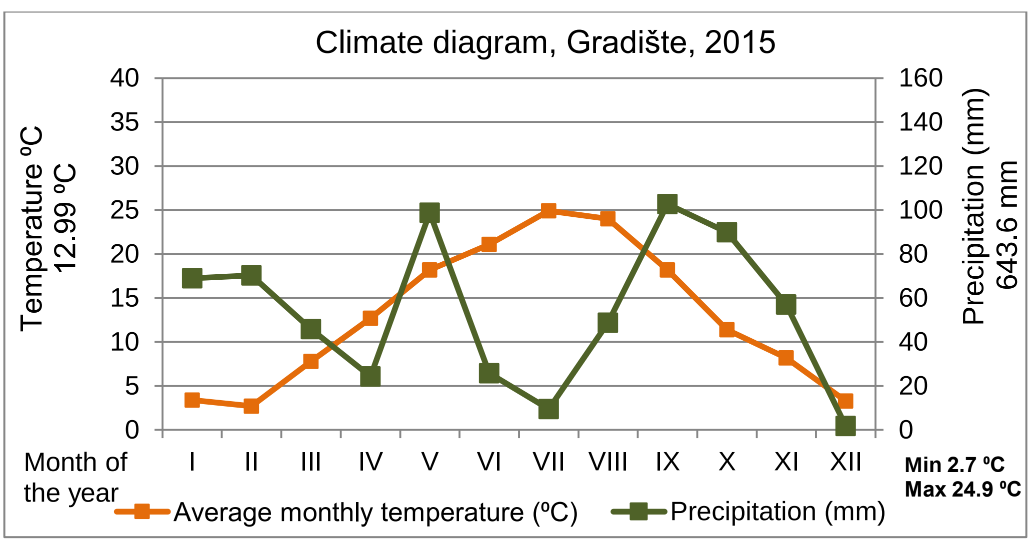
b) Display of climate diagram from the research area (Gradište) for all exploration years (2011-2015)






Climate diagram, Gradište, 2013
Temperature ºC
12.39 ºC
Precipitation (mm)
709.3 mm
Month of the year
I II III IV V VI VII VIII IX X XI XII
Average monthly temperature (ºC)
Precipitation (mm)
Min 1.7ºC
Max 23.3 ºC


Climate diagram, Gradište, 2014
Temperature ºC
13.05 ºC
Precipitation (mm)
822.6 mm
Month of the year
I II III IV V VI VII VIII IX X XI XII
Average monthly temperature (ºC)
Precipitation (mm)
Min 4ºC
Max 20.9 ºC


Climate diagram, Gradište, 2015
Temperature ºC
12.99 ºC
Precipitation (mm)
643.6 mm
Month of the year
I II III IV V VI VII VIII IX X XI XII
Average monthly temperature (ºC)
Precipitation (mm)
Min 2.7 ºC
Max 24.9 ºC