Research Article

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Area-wide mass trapping by pheromone-based attractants for the control of sugar beet weevil (*Bothynoderes punctiventris* Germar, Coleoptera: Curculionidae)

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Abstract

BACKGROUND: Owing to the changing climate, narrow crop rotation, and changes in insecticide application practice, sugar beet weevil (SBW) (*Bothynoderes punctiventris* Germar) has become the most important economic pest in sugar beet. To develop alternative control methods, an area-wide (AW) control program using aggregation pheromones was implemented over 4 years on an area of 6 and 14.8 km^2 in east Croatia.

RESULTS: The mass trapping of SBW on the 'old' sugar beet fields reduced the population from 0.73% to 11.59%. Owing to the strong attack, mass trapping was not effective enough to avoid an insecticide application. However, it significantly reduced the number of insecticide applications, the amount of insecticide used, and the damage compared to the fields outside the mass trapping area.

CONCLUSION: This is the first study to implement an AW program for SBW. It may not be possible to state from this study that trapping alone can reduce the SBW population below the economic threshold level. However, the data do suggest that trapping can play an important role in the reduction of insecticide applications and in creating an integrated pest management plan for dealing with SBW under similar circumstances.

Keywords: aggregation pheromones; area-wide control program; integrated pest management; mass trapping; sugar beet weevil

1 INTRODUCTION

The sugar beet weevil (*Bothynoderes punctiventris* Germar) (Coleoptera: Curculionidae) is an important pest of sugar beet throughout the central, eastern and southeastern parts of Europe.[1,2] In areas with drier climate, the weevil is the most destructive insect pest of sugar beet, causing severe losses especially during outbreak periods that are frequently up to 50% of the fields. Due to the changing climate and narrow crop rotation, the population in eastern Croatia has reached its economic threshold, and sugar beet weevil has become the most important economic pest of sugar beet.[3]

Sugar beet weevils (SBW) spend the winter as adults in the soil at a depth of 15–35 cm, and 80–90% of individuals overwinter in old sugar beet fields.[3] In the early spring, the beetles emerge from the soil and begin to search for food. After a period of intensive feeding on sugar beet seedlings (when the weevils cause the most damage), the beetles mate, and eggs are deposited in the soil. The larvae live in the soil from May until autumn while feeding on the roots of sugar beet. The larvae rarely cause serious damage, and complete larval development occurs in late summer. Currently, for sugar beet weevil control in Croatia, three insecticides based on four active substances are approved: lambda-cyhalothrin (Karate Zeon, Syngenta), a combination of chlorpyrifos and cypermethrin (Chromorel D, Agriphar) and acetamiprid (Mospilan, Nippon).[4] Owing to the specific morphological structure of weevils (the body is covered with a rough, hard integument), their large feeding capacity and the small leaf area of the plants (seedlings) at the time of insecticide application, insecticides often give very poor results. If the treatment is not applied in warm sunny weather, a large proportion of the weevils are unaffected because they remain hidden in the upper layer of soil during unfavorable weather. Therefore, repeated insecticide treatments are often required.[5] This is not in accordance with the principles of integrated pest management (IPM) nor with the rational use of pesticides as proposed by the

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Directive on the sustainable use of pesticides.[6] Therefore, it is necessary to consider non-pesticide plant protection measures and to develop a strategy to control sugar beet weevil. The strategy should include all available methods that are compatible with all agricultural practices, such as crop rotation, the proper choice of fields, early sowing, sowing of the edges of a field in high-density areas and measures that contribute to rapid plant development.[7] During vegetative growth, it is advisable to plow between rows, thus reducing the number of eggs and hatched larvae.[8] The construction of ditches around the previous year's sugar beet fields has been used to suppress weevils in Vojvodina (north Serbia).[8] Microbial insecticides based on entomopathogenic fungi have been shown to reduce the number of larvae and pupae by 85%[9] and contribute to the reduction of the population by 74%.[10] The use of the nematodes *Steinernema* and *Heterorhabditis* (together with symbiotic bacteria *Xenorhabdus* and *Photorhabdus*) for the suppression of weevils is currently under investigation.[11,12] To date, the commercial use of products based on the aforementioned organisms has not been reported.

Although there are numerous examples of area-wide (AW) pest management,[13–15] the scientific basis of this approach has been ascribed to Knipling.[16,17] AW is the systematic organized control of all pest populations over a wide area.[15] 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. Some of the methods used in AW include the planting of resistant varieties and hybrids,[15] the release of natural enemies,[18] the release of sterile males (SIT),[19,20] and the so-called 'attract and kill' method, in which an attractant is used with a low dose of insecticide[21] or a sex attractant is used for male confusion.[22] Described methods are not considered as relevant for SBW control. The only relevant method for the control of SBW could be mass trapping. Mass trapping is based on the use of an attractant that draws insects to the traps, where they are caught in large numbers. Attractants include food baits,[23] sex pheromones[23,24] or aggregation pheromones.[25,26] Mass trapping offers promising perspectives with beetle pests, where aggregation pheromones are frequently found.[27]

As reported by Park and Goh,[28] Yongmo *et al.*,[29] Zhu *et al.*,[30] Borden *et al.*,[31] Wawrzynski and Ascerno,[32], James *et al.*,[33] Beevor *et al.*,[34] Oehlschlager *et al.*[35] and Podleckis,[36] AW mass trapping programs have been shown to be successful against many pests. Insects from all orders can be controlled by mass trapping. In some cases, several tools are combined in order to achieve success. If sex pheromone traps are used, only one sex (usually males are attracted) is trapped, as was the case with beet armyworm *Spodoptera exigua* (Hübner) control in welsh onion,[28] tea tussock moth (*Euproctis pseudoconspersa* (Strand)) control in peach orchards[29] and the control of stem borers in rice.[30] Aggregation pheromones or other lures that attract both sexes are more suitable for mass trapping programs. The use of aggregation pheromones (or a combination of sexual pheromones with other attractants) has been reported to control mountain pine beetle (*Dendroctonus ponderosae* Hopkins) in lodgepole pine,[31] Japanese beetle (*Popillia japonica* Newman) in an isolated area,[32] *Carpophilus mutilatus* Erichson and *Carpophilus davidsoni* Dobson in stone fruit orchards,[33] cocoa pod borer (*Conopomorpha cramerella* Snellen) in coconut palms,[34] American palm weevil (*Rhynchophorus palmarum* L.) in oil palms,[35] Mediterranean fruit fly (*Ceratitis capitata* Wiedemann) in paw-paw (*Asimina triloba* L.)[36] and stinkbug (*Plautia stali* Scott) in persimmon.[26]

Toth *et al.*[37,38] discovered that the mixture of Grandlure III–IV [(*Z*)- and (*E*)-2-ochtodenal; (*Z*)- and (*E*)-(3,3-dimethyl)cyclohexylidene acetaldehyde] can be used as a sensitive and powerful trapping tool in the control of the SBW. Tomašev *et al.*[25] proposed mass trapping of SBW using baits with aggregation pheromones.

Basically, there are two key points where mass trapping could be used to reduce adult SBW populations. The first is to trap a significant percentage of the overwintering weevils by mass trapping the emerging beetles at the overwintering sites (the 'old' sugar beet fields), and the second is to try to intercept beetles that emerged elsewhere when they arrive at the sugar beet fields with beet seedlings ('new' fields). Tomašev *et al.*[25] proposed that a density of 30 pheromone traps ha^{-1} should be used in donor fields in the case of mass trapping on a single field. Some studies in Croatia have indicated that there is a risk of extensive damage on 'new' fields due to large numbers of weevils because they continue to feed and cause damage before they arrive at the bait. Čamprag,[8] Sekulić *et al.*[39] and Maceljski[40] agree that SBW should be confined to the location where they overwinter and not be allowed to enter new areas.

Based on the described facts, we hypothesized that the mass trapping of SBW using aggregation pheromones in the 'old' sugar beet fields within a particular larger area will provide the possibility of reducing the pest population within that area. Therefore, the goal of the research was to implement mass trappings at all of the 'old' sugar beet fields within the larger area (i.e., AW) and to evaluate the success of mass trapping and discuss the possibility of implementing this method into agricultural practices.

2 MATERIALS AND METHODS

2.1 Research area

The mass trapping of SBW was implemented over 4 years (2012–2015) and within the vicinity of the village Tovarnik (east Croatia). In the spring of 2012, the borders of the AW or mass trapping area (MTA), which included 111 crop fields, were determined. Every year, mass trapping was implemented within a total area of 6 km^2 (i.e., approximately 600 ha), and in 2014 mass trapping was implemented within a total area of 14.8 km^2 (see Fig. 1). The enlargement in 2014 occurred because of the small area of sugar beet cultivation in the study area in 2013. The MTA in 2012, 2013 and 2015 expanded between the coordinates of 45°12′ 34.22″ N, 19° 06′ 59.86 ″ S (northwest point) and 45° 11′ 05.02″ N, 19° 08′ 40.41″ S (southeast point). The MTA in 2014 enlarged between the coordinates of 45° 12′ 32.50″ N, 19° 06′ 26.59″ S (northwest point) and 45° 11′ 05.02″ N, 19° 08′ 40.41″ S (southeast point). The 'old' (or donor) and 'new' sugar beet fields were identified in the MTA. The donor ('old' sugar beet) fields were either planted with wheat or barley in autumn, or fields were prepared for planting with soybean, corn or sunflower in spring. Common agronomic practices on newly sown sugar beet fields applied in the whole area. All seeds were treated with insecticides (neonicotinoids were used), and thus during the early developmental stage of the plants no other pests were recorded. Pre-emergence herbicide application has been conducted with reduced doses of herbicides and, after plant emergence, several treatments with reduced doses of post-emergence herbicides were applied. The fields within the MTA varied in size from less than 1 ha to 130 ha. Therefore, when analyzing the data, the fields

Figure 1. The location of area-wide program in the Vukovar-Sirmium region and map of the mass trapping area from 2012 to 2015.

were grouped according to field size into three groups: less than 6 ha in size, between 6 and 59 ha, and over 60 ha in size.

Every year, two newly sown sugar beet fields outside the mass trapping area were chosen for damage inspection; additionally, three fields were chosen to monitor insecticide applications.

2.2 Initial population

To determine the population of SBW on donor fields, the soil was surveyed in the fall (except in 2011) and in the spring (early March) before placing the pheromone traps. Surveying was performed by digging 0.5 × 0.5 m pits to a depth of 30 cm. The excavated soil was examined in detail and all arthropods were collected. The number of pits depended on the size of the inspected fields. In fields up to 10 ha four pits were dug, whereas in larger fields 12–16 pits were dug. The weevil population was calculated and was represented as the average number of weevils per square meter.

2.3 Mass trapping

CSALOMON® TAL traps (modified pitfall traps) baited with aggregation pheromones for SBW (Plant Protection Institute, CAR HAS, Budapest, Hungary) were placed at a density of 15 pheromone traps ha^{-1} in all marked donor fields within the MTA, according to a predefined scheme that matched the size and shape of the individual fields. The traps were set up in the field at SBW emergence (as soon as it was possible; i.e., when snow melted) and trapping lasted until the adults started to fly (when the average daily temperatures increased above 20 °C). The traps were installed in all donor fields in the mass trapping area between 11 and 20 March in 2012, 2014 and 2015 (Julian days 70–80) and between 21 and 30 March in 2013 (Julian days 80–90). The traps were removed after 5–7 weeks (corresponding to Julian days between 105 and 130). Traps were inspected and emptied once a week. The mass trapping was conducted on all sugar beet fields within the mass trapping area. The number of donor fields where mass trapping was conducted in 2012, 2013, 2014 and 2015 was 14, 15, 19 and 7, the surfaces of the fields were 64.98, 241.42, 40.97 and 12.93 ha, and the number of traps employed was 929, 3518, 614 and 191, respectively.

2.4 Establishing infestation and damage caused by SBW

All fields sown with sugar beet in the mass trapping area were marked. The inspection started immediately after the first plant emergence (BBCH 09) and continued to the end of May or to when the plants reached BBCH 3:31–3:33 (i.e., from the beginning of crop cover when the leaves covered 10% of the ground to when the leaves covered 30% of the ground).[41] The fields were regularly inspected once a week for seven surveys. The infestation was expressed as the average number of weevils per square meter, and the damage caused by weevils was established. At each inspection date, a wooden square (area of 1 m^2) was randomly cast on the surface and all weevils inside the square were counted. Within the same square, all emerged plants were counted. Of the counted plants, the damaged ones were classified into five categories (0–4) based on the percentage of damaged leaf area.[42] The classes in which the plants were classified are as follows:[42] 0, no damage; 1, 1–25% of plant parts damaged; 2, 26–50% of plant parts damaged; 3, 51–75% of plant parts damaged; and 4, over 76% of plant parts damaged. Based on the frequency of

Table 1. Details of the visual inspection of newly sown sugar beet fields for establishing infestation with SBW adults and the damage caused

	Inside the mass trapping area		Outside mass trapping area		The period of survey (Julian days)		
Year	Number of fields	Total size (ha)	Number of fields	Total size (ha)	From	To	Number of inspections
2012	17	237.19	2	51.05	87	130	7
2013	2	5.54	2	89.0	104	137	6
2014	23	157.66	2	129.0	84	126	7
2015	4	170.19	2	82.0	92	134	7

plants within each of the five categories, the percent of damage using the Townsend–Heuberger[43] equation was calculated as follows:

$$\%\ \text{damage} = \frac{\Sigma\left(f \times n\right)}{a \times N} \times 100 \qquad (1)$$

where f is the number of plants in the group, n = score of the group (0–4), a = the number of groups (in this case, 5), and N = total number of plants in the sample examined.

Each observation was replicated four times at each inspection date. The same was done in each year on two control fields outside the mass trapping area where no mass trapping had been conducted in order to compare the infestation and damage. Details of the inspected fields where the infestation and damage caused by SBW occurred are given in Table 1.

2.5 Insecticide application

The use of insecticides on all newly sown sugar beet fields inside the mass trapping area was advised to farmers based on the visual inspection results. The type and average amount of insecticides used for SBW control inside the mass trapping area was recorded.

The decisions regarding insecticide applications on the fields outside the mass trapping area were made by farmers themselves without clear justification on the established infestation level. For comparison, the five fields outside the mass trapping area were monitored for the use of insecticides for SBW control. In addition to two fields where the damage was established, three additional fields were recorded for the use of insecticides for SBW control.

2.6 Data analysis

For each field, the average infestation per square meter was based on the average number of weevils found in the soil samples, and the average surface of the soil examined (0.25 m^2) was calculated. Based on the average infestation per square meter and the field size, the total population for each field was estimated. For each field, the number of weevils caught in pheromone traps at each inspection was recorded, and the total caught population was estimated. The population reduction was expressed as the percentage of caught beetles in pheromone traps compared to the estimated spring population.

The collected data (the field infestation with SBW established in autumn and in spring soil surveys, the data on the weevil capture in pheromone traps, infestation of newly sown sugar beet fields, damage established on newly sown sugar beet fields and average insecticide consumption) were compared among the fields (or between the inside and outside of the mass trapping area) and among years by analysis of variance (ARM 2016 GDM® software, Revision 2016.2, 6 May 2016),[44] and means separation was estimated using Tukey's HSD test. Where appropriate, data were log $x + 1$ or arc.sin $\sqrt{x}$ transformed.

To determine the success of AW mass trapping, three basic parameters were used:

(a) comparison of the number of weevils in the MTA estimated from the soil samples taken in donor fields in the spring to the number of weevils caught in pheromone traps;
(b) comparison of the average infestation and average damage of sugar beet fields in the mass trapping area to the average infestation of sugar beet fields outside the mass trapping area, expressed as number of adults per square meter and average damage established on sugar beet plants;
(c) comparison of average number of insecticide treatments and amount of insecticide applied per hectare on the fields inside the MTA to the fields outside the MTA.

3 RESULTS

Within the mass trapping area in each of the investigated years, we found very high variability in terms of population level estimates based on the soil samples taken in donor fields in the spring, as well as in the number of caught weevils in the pheromone traps. In all years of investigations, we found that all fields were infested with SBW, but the infestation differed among the fields. On some of the donor fields in each year, an infestation of 10–17.73 weevils m^{-2} was established. According to Čamprag,[42] this is considered a very strong infestation. In 2012, the average trap capture varied from 57.58 and 420.4 weevils per trap per season. The mean trap capture significantly differed among the fields, and the average was 162.71 weevils per trap. In 2013, the mean number of caught weevils per trap significantly differed among the fields and varied between 16.85 and 798.28, with an average of 578.57 weevils per trap. The average total capture of weevils per trap established in the fields that were smaller (less than 6 ha) varied from 16.85 to 38.08 weevils per trap and was significantly lower compared to the average total capture of weevils per trap caught in fields sized 60 and 130.18 ha (LSD = 33.61). On fields sized 60 and 130.18 ha, the average total capture was 664.57 and 798.24 weevils per trap, respectively. In 2014, a very low average total capture at 29.54 weevils per trap was observed. No fields larger than 6 ha were included in the investigation in 2014. A similar situation was recorded in 2015, and the mean number of captured weevils per trap significantly differed among the fields, with an average of 28.95 weevils per trap per season.

The summarized results of the overall success of mass trapping over the 4 years of conducting the program are shown in the Table 2.

Table 2. Results of the area-wide mass trapping of SBW carried out in Tovarnik, Croatia from 2012 to 2015

Year		Established infestation of weevils m^{-2} on fields involved in mass trapping (from–to)	Total estimated population of the previous year sugar beet fields in the area where mass trapping is carried out	Number of trapped weevils	Percent of reduction in population in relation to autumn or spring population
2012	Spring	0–15.47	2 814 063	158641	5.64
2013	Autumn	0.64–16.72	31 211 656	2 095 007	6.71
	Spring	0–14.35	18 074 925		11.59
2014	Autumn	1–2	94 880[a]	2 939[a]	3.1
	Spring	0–16.67	1 180 700[b]	18 167[b]	1.53
2015	Autumn	0–17.73	653 200	5 616	0.86
	Spring	0.64–17.72	773850		0.73

[a] Population established on donor fields in area in which mass trapping has been carried out in 2013 by soil survey and by pheromone traps.
[b] Population established by soil survey and by mass trapping in the whole area in 2014 (the area has been enlarged).

The fields inside and outside the mass trapping area were treated with insecticides in order to prevent serious damage and the need for resowing the fields. The results presented in Table 3 on established infestations with SBW adults from the seven surveys conducted from the emergence of sugar beet plants (BBCH stage 09) until the plants reached the developmental stages 3:31–3:33[41] revealed that sugar beet fields outside the mass trapping area were infested by SBW at a higher intensity than the fields inside the mass trapping area, although the differences were often not significant. This higher infestation resulted in greater damage (Table 4). It should be taken into account that the fields outside and inside the mass trapping area were not treated equally.

For SBW control in Croatia, three insecticide products based on four active substances are approved.[4] Inside and outside the mass trapping area, farmers used two products: one is based on lambda-cyhalothrin (Karate Zeon, Syngenta, Basel, Switzerland) and the second is a combination of chlorpyrifos and cypermethrin (Nurelle D, Agriphar SA, Ougrée, Belgium). In each treatment, farmers applied insecticides at the doses registered for SBW control in Croatia.[4] For the control of SBW, it is recommended to apply the highest approved dose of the product, 2.0 L ha^{-1} (i.e., 1000 g a.i. ha^{-1} chlorpyrifos + 100 g a.i. ha^{-1} cypermethrin), which is much higher compared to the doses approved for other pests (0.8 1.0 L ha^{-1}). When applying lambda-cyhalothrin, farmers applied a dose at 0.15 L ha^{-1} (i.e., 7.5 g a.i. ha^{-1}). Instead of treating the whole field area at the beginning of the infestation, farmers were advised to treat only field edges in order to control weevils the moment they walked into the field. In that case, the applied amount of lambda-cyhalothrin was 1.5 g a.i. ha^{-1}. No field edges were treated with Nurelle D.

The results of the statistical analysis on the average amount of active substance per hectare used (Table 5) showed that fields outside the mass trapping area were treated with more insecticides than the fields inside the MTA. Inside the mass trapping area, insecticides were applied twice: the first time was on the field edges, and the second application was on the whole surface, making 1.2 insecticide treatments per field. Contrary to this, outside the mass trapping area farmers applied insecticides 3.5–4 times (see Fig. 2). There was a significant difference between the fields inside and outside the mass trapping area in the number of insecticide treatments (LSD = 0.366).

4 DISCUSSION

Mass trapping with pheromone-baited traps has been successfully attempted in the family of weevils.[25,35,45] Of all examples of successful cases of mass trapping, it is important to note the successful control of American palm weevil (*R. palmarum)* in oil palms,[35] and sugar cane weevil *Metamasius hemipterus* L. and American palm weevil (*R. palmarum)* in palmito palm.[45] The use of the aggregation pheromone of *R. palmarum*[35] proved to be effective in maintaining red ring disease (RRD) (transmitted by R. *palmarum*) at very low levels over several years. Both species of the insect belong to the same family (Curculionidae) as SBW, and thus probably have similar patterns in the reaction toward aggregation pheromones. All cited attempts were applied on a single plantation. No attempts to organize an AW program for the control of any weevil species have been made. Only one AW program was organized to control a field crop pest; it was an AW program to control western corn rootworm (*Diabrotica virgifera virgifera* LeConte), which was organized by USDA.[21]

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

4.1 Population reduction

We compared the estimated number of weevils in the MTA based on the soil samples taken on donor fields in the spring and number of caught weevils in the pheromone traps.

The baited traps were useful in terms of lowering the SBW population. By mass trapping, we reduced the weevil population up to 11.59% of the total estimated population established by the soil surveys in the donor fields in spring in the mass trapping area. This is much lower compared to the results reported by Tomašev *et al.*[25] The observed differences in the success could be influenced by the field size, SBW population level and the trap density per hectare. The results of Tomašev *et al.*[25] were obtained on a few small fields under conditions of lower pest population. Additionally, they used 30 traps per hectare, whereas in our investigation 15 traps per hectare were used. The decision on trap density per hectare in our investigation was made based on the fact that traps were employed over a large area and on the fact that the traps were grouped on the field borders.

Table 3. Average infestation with SBW (number of insects m^{-2}) established on the newly sown sugar beet fields inside and outside mass trapping area (MTA) on different rating dates

Plant stage at survey (BBCH scale)		0:09	→					3:31–3:33
		Rating number						
Year	Location of fields	1	2	3	4	5	6	7
2012	Inside MTA	0.0	0.14	0.96	0.17	0.12	0.31	0.56
	Outside MTA	0.0	0.01	1.09	0.32	0.39	0.06	0.53
	$LSD_{P=5\%}$	ns	ns	ns	ns	ns	ns	ns
2013	Inside MTA	0.0	0.0	0.59	0.47	0.46	0.11b	0.46
	Outside MTA	0.0	0.0	0.76	0.94	1.06	0.61a	0.11
	$LSD_{P=5\%}$	ns	ns	ns	ns	ns	0.35	ns
2014	Inside MTA	0.03	0.15	0.83	0.45	0.15	0.19	0.74
	Outside MTA	0.03	1.13	1.45	0.3	0.29	0.41	0.71
	$LSD_{P=5\%}$	ns	ns	ns	ns	ns	ns	ns
2015	Inside MTA	0.0	0.02	0.0	0.2	0.07	0.96	0.88
	Outside MTA	0.0	0.74	1.25	1.68	0.7	1.0	1.18
	$LSD_{P=5\%}$	ns	ns	ns	ns	ns	ns	ns

Means followed by the same letter are not significantly different according to Tukey's HSD test ($P = 0.05$); ns, not significant.

Table 4. Damage (according to Townsend and Heuberger[43]) caused by SBW (%) established on newly sown sugar beet fields inside and outside mass trapping area (MTA) on different rating dates

Plant stage at survey (BBCH scale)		0:09	→					3:31-3:33
		Rating number						
Year	Location of fields	1	2	3	4	5	6	7
2012	Inside MTA	0.0	0.8	1.48	1.45	0.81	0.49	1.55
	Outside MTA	0.14	1.3	1.54	1.98	3.01	1.33	1.92
	$LSD_{P=5\%}$	ns	ns	ns	ns	ns	ns	ns
2013	Inside MTA	0.0	0.5	1.28	0.96	0.68	0.95b	0.37
	Outside MTA	0.07	0.81	1.1	2.18	2.62	1.82a	1.44
	$LSD_{P=5\%}$	ns	ns	ns	ns	ns	0.139	ns
2014	Inside MTA	0.0	0.13	0.65	0.99	1.0	0.86	0.99
	Outside MTA	0.0	1.62	2.23	0.25	1.16	3.29	1.15
	$LSD_{P=5\%}$	ns	ns	ns	ns	ns	ns	ns
2015	Inside MTA	0.0	0.0	0.98	1.52	1.57	1.65	0.78
	Outside MTA	0.0	0.86	1.11	2.30	3.18	4.17	1.35
	$LSD_{P=5\%}$	ns	ns	ns	ns	ns	ns	ns

Means followed by the same letter are not significantly different according to Tukey's HSD test ($P = 0.05$); ns, not significant.

The average trap capture in our investigation varied among years and among fields, ranging from 10.67 to 798.24 weevils per trap per trapping season. The trap design was suitable for capturing a higher number of weevils, and we did not observe that the saturation of traps with weevils influenced the mass trapping success as mentioned by Jones.[46] Compared to the capture of 1000–2000 weevils per trap per 3 weeks reported by Tomašev *et al.*,[25] we recorded much lower captures. Moreover, very low captures were recorded in 2014 and 2015. The temperature and amounts of precipitation in March and April could be excluded as a possible reason for the lower number of trapped weevils in 2014 and 2015 because the differences in climatic conditions in March and April among the years do not support this statement. Lower capture in 2014 and 2015 could be influenced by weevil population level, the average size of the fields in the mass trapping area or lower share of sugar beet fields in the mass trapping area. Tomašev *et al.*[25] reported that their results were obtained under conditions where the average infestation was up to 30 000 weevils ha^{-1}. Under our conditions, the average population of weevils varied from 28 818 weevils ha^{-1} in 2014 up to 74 869 weevils ha^{-1} in 2013, and the traps were exposed for 7 weeks. Longer trap exposure in our investigation should result in higher captures, but it did not occur. In 2014 and 2015, there were no large fields involved in the investigation, as was the case in 2012 and 2013, and the trap capture on larger fields (over 60 ha in size) was significantly higher than smaller fields. The number and size of the fields sown by sugar beet in the mass trapping area over the years of investigation decreased due to the farmers' decisions. This fact could influence the capture of weevils in pheromone traps, since Čamprag[47] suggested that the best prevention for protecting crops from SBW attack was a 1–3 km spatial isolation from the previous year's sugar beet fields.

By mass trapping, a significant number of weevils were caught. The captured weevils had the ability to destroy a large area of

Table 5. Average consumption of active ingredient of insecticides (g ha^{-1}) used for SBW control on fields inside and outside the mass trapping area (MTA) over the period 2012–2015

Field location	Year	Insecticide (g ha^{-1}) Chlopyriphos	Cypermethrin	Lambdacychalothrin	Total amount of active ingredient (g ha^{-1})
Inside MTA	2012	1000b	100b	1.5b	1101.5b
	2013	1040b	104b	0.81b	1144.81b
	2014	1040b	104b	0.81b	1144,81b
	2015	1040b	104b	0.81b	1144.81b
Outside MTA	2012	3800a	380a	14.7a	4194.7a
	2013	3600a	360a	15.0a	3975.0a
	2014	3400a	340a	15.9a	3755.9a
	2015	3400a	340a	16.2a	3756.0a
HSD, $P = 5\%$		574.96	57.57	3.58	635.32

Means followed by the same letter are not significantly different according to Tukey's HSD test ($P = 0.05$).

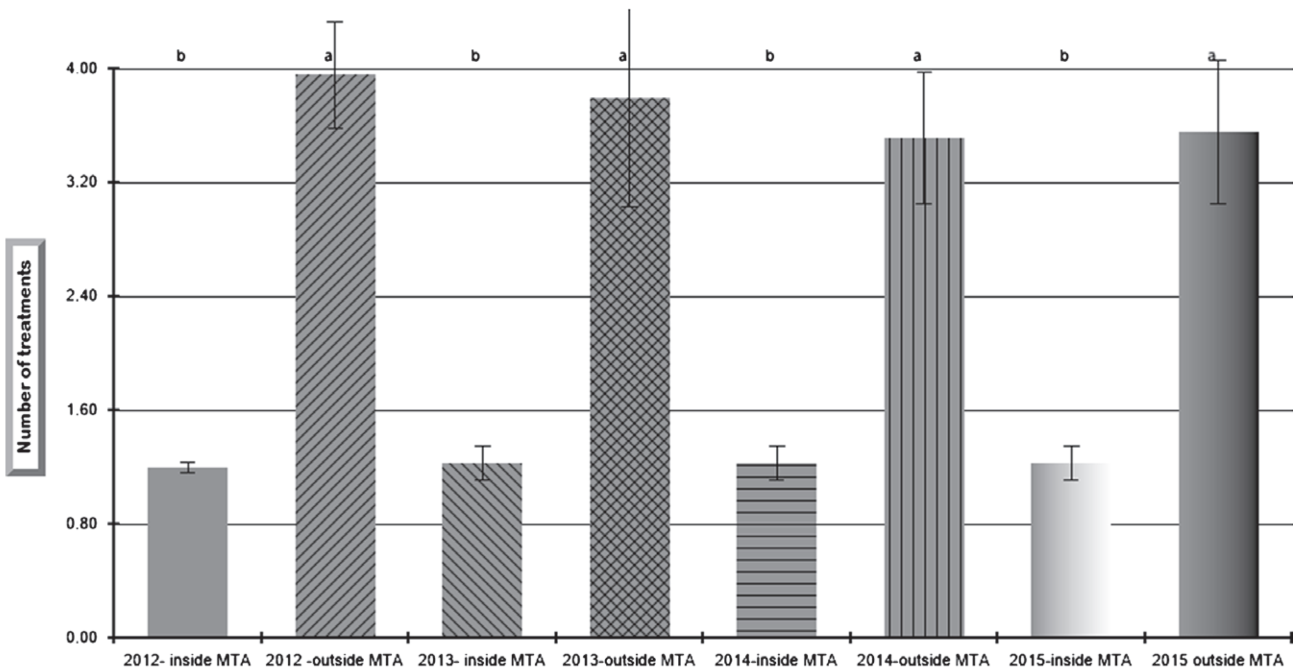

Figure 2. Number of insecticide treatments applied on fields inside and outside the mass trapping area (MTA) and results of statistical analysis.

sugar beet. According to Čamprag *et al.*,[7] the threshold decision for SBW is 0.1–0.3 weevils m^{-2}. This means that 1000–3000 weevils ha^{-1} are able to cause economic damage on sugar beet field in the early developmental stages. In 2012, 2013, 2014 and 2015, we caught 158 641, 2 095 007, 18 167 and 5616 weevils, respectively. The caught weevils had the ability to cause economic damage on 53–158, 698–2095, 6–18 and 2–5.6 ha in 2012, 2013, 2014 and 2015, respectively.

4.2 Infestation and damage caused by SBW

To establish the success of the mass trapping, we surveyed all new sugar beet fields in the MTA, and the established average infestation of sugar beet fields was expressed by the number of adults per square meter and the damage to sugar beet plants. At the same time, we surveyed fields outside the MTA and established the damage and average infestation. On the fields inside the MTA, the number of adults per square meter, as well as the established damage, was lower compared to the fields outside the MTA, although the differences were not significant at each observation date. The maximal infestation of SBW on the fields inside the MTA was 0.96, 0.59, 0.83 and 0.96 weevils m^{-2} in 2012, 2013, 2014 and 2015, respectively. Since the established infestation was over the economic threshold for the current plant developmental stage, we advised an application of insecticide. At the same time, the maximal SBW infestation on the fields outside the MTA in spite of insecticide treatments applied was 1.1, 1.1, 1.45 and 1.68 weevils m^{-2} in 2012, 2013, 2014 and 2015, respectively. Inside the MTA, farmers followed the advice and applied insecticides based on the established thresholds. Outside the MTA, farmers applied insecticides according to their own decisions, which were mainly based on experience.

The damage caused by SBW adults (in %) in all 4 years on both fields, inside and outside the MTA, was between 1.0% and 4.17%. The differences between the fields inside and outside the MTA were significant at only one observation date due to the fact that fields outside the mass trapping area received insecticide treatments regularly. However, it was visible that the damage was lower (but not significantly different) on the fields inside the mass trapping area. It should be noted that the infestation and damage on the fields outside the mass trapping area were similar to those inside the MTA, although the fields outside the MTA received more insecticide treatments than fields inside the MTA.

4.3 Insecticide treatments

The comparison of insecticide treatments and amount of insecticide applied per hectare on the fields inside the MTA and the fields outside MTA can serve as the most accurate measure of the success of mass trapping.

Currently in Croatia, the active substances approved for SBW control are acetamiprid, chlorpyrifos, chlorpyrifos + cypermethrin, lambda-cyhalothrin and thiamethoxam (as a seed treatment).[4] Seed treatments with insecticides are regularly conducted at seed producers, and all sugar beet seeds in Croatia are treated with neonicotinoid insecticides. It has been proven that a seed treatment with neonicotinoids does not provide effective protection against SBW under the conditions of a medium or high population level.[5,48] Therefore, a foliar application of insecticides against SBW should be conducted if a pest attack is established. The approved active substances for foliar treatments belong to organophosphate insecticides (chlorpyrifos), pyrethroids (lambda-cyhalothrin and cypermethrin) and neonicotinoids (acetamiprid). Two insecticides based on three active substances were used for SBW control inside and outside the mass trapping area during the 2012–2015 research period. The first treatment for SBW control is usually conducted on the field edges with lambda-cyhalothrin (Karate Zeon 5 CS). This treatment was applied on approximately 20% of the total surface of the field. Since the pests approach the new crop by walking from last year's sugar beet fields after overwintering, the treatment of the edges is recommended by many authors.[39,49] It is a common practice and lowers the amount of insecticides used on the fields, but increases the number of treatments. Later, lambda-cyhalothrin (Karate Zeon 5 CS) and the combination of chlorpyrifos and cypermethrin (product Nurelle D) were applied either alone or in combination on the whole surface. If the combination was applied, both products were used in full doses.

Through all years of the investigation, the number of insecticide applications and the amount of active substances per hectare were 3.5–4 times higher on the fields outside the mass trapping area compared to the fields inside the mass trapping area. We observed slight differences among the years in the average amount of insecticides used on the fields outside the mass trapping area. The fields outside the mass trapping area received between 3.5 and 4.2 treatments per season. The reason for the high number of treatments outside the mass trapping area lies in the fact that farmers are aware that SBW could cause the total damage. Also, farmers are aware that insecticides are not often very efficient against SBW. Therefore their decisions on insecticide treatment are not driven by the established economic threshold level (ETL). The decisions are rather driven by previous 'bad' experience and damage they experienced. The amount of applied insecticides depended on the insecticides used in the treatments. If lambda-cyhalothrin was used, due to the lower recommended dose per hectare, the amount of insecticides used was lower. All insecticides approved for SBW control in Croatia are approved for application up to two times on one field.[4] It should be noted that fields outside the mass trapping area were very often treated with two insecticides at once. Therefore, on the fields that were treated four and more times, the number of applications and the amount of insecticides exceeded the allowed rate.[4] Described practice is unlawful. AW mass trapping with pheromones is capable of reducing the population of SBW, but under the conditions of a high SBW density, as it was 2012, 2013, 2014 and 2015, mass trapping was not effective enough to avoid an insecticide application. However, mass trapping of the SBW on 'old' (donor) sugar beet fields contributed to the reduction of insecticide use as expressed as the amount of active substances and number of treatments on the fields inside MTA compared to the fields outside MTA. An additional advantage of the present attractant-baited traps for mass trapping was that they showed considerable specificity in catching SBW and caught non-target and occasional beneficial insects at very low percentages compared to the masses of weevils caught. The mass trapping of SBW on the 'old' sugar beet fields in the AW area significantly reduced the number of insecticide applications and the amount of insecticides used while lowering the damage and weevil infestation compared to the fields outside the mass trapping area.

Although the results of the conducted program are not spectacular, we can see many advantages of the conducted program. The method of mass trapping by aggregation pheromones on an AW basis according to the basic principles of AW programs as proposed by Knipling[17] is not a short-term goal for controlling pests in a field or a season. The long-term goal of these methods is to reduce the population of pests in a particular area, and our results significantly contributed to this goal.

Between the two possibilities on how mass trapping could be used to reduce adult weevil populations, we choose to trap the overwintering weevils by mass trapping the emerging pests at the overwintering sites ('old' sugar beet fields). This method has some advantages because Sekulić *et al.*, Maceljski, and Čamprag[39,40,42] agree that weevils should be kept at the overwintering sites in order to prevent them from creating damage on newly sown sugar beet fields. Keeping SBW at overwintering sites has to be organized as an AW program because it is not possible for one farmer to conduct this measure. As discussed by Lindquist,[50] AW insect control is applied over a relatively large area involving many producers of the same or similar crops. The owners of the donor fields are not always the same as the owners of newly sown sugar beet fields within a particular area. Therefore, they do not necessarily have the same short-term interests. Lindquist[50] mentioned that AW control should be conducted by a separate organization. In the case of Croatia, it was not possible, but the sugar-processing industry is contracting farmers for sugar beet production in a particular area. Therefore, we realize that they have strength and capacity to organize AW control. The question of financing AW programs is very important. Since the reduction of the weevil population in one area is a long-term goal for both farmers and the sugar-processing industry, it is necessary that the sugar-processing industry pay the expenses. However, farmers' participation in the costs should be expected as well, since economic analysis shows that the cost of pheromones and 1.2 applications of insecticides (as was needed inside the mass trapping area) is just slightly over the price of four applications of insecticides. Additionally, the amount of applied insecticides on the fields outside the mass trapping area exceeded the allowed level, which might result in residue levels over the approved amounts and limit the market of sugar beet.

The common codex for integrated farming,[51] where IPM is a very important part, was developed in January 2001 by 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.[52] Many EU countries, including Croatia, have developed national pesticide reduction programs in which they require that pesticides be 'used properly' and where proper use 'shall also comply with … general principles of integrated pest management'.[6]

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.[53] Each AW-IPM program requires a regulatory framework according to its specific needs. Consequently, after defining the strategic approach (e.g., suppression, containment/prevention or eradication),[15] each campaign requires the development of an appropriate strategy. It is very often that the AW strategy is not the only tool used for pest suppression. Usually, several tools are combined. The use of insecticide 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 with pesticide legislation in the EU, we tried to find additional solutions to be used in AW control. The EU intends to limit the use of all insecticides currently allowed for SBW control in the future.[54] Inđić *et al.*[55] state that chemical control of this pest is the main way to control SBW and probably will be the same in the near future, but they also note that the economically rational measures of pest control including agrotechnical, mechanical and biological measures should be further explored and developed.

This is the first study to implement an AW program for SBW by mass trapping. It appears that the mass trapping of SBW played a large role in reducing the damage and amount of insecticides used. Trapping was a significant factor in the population control effort at our study site. Owing to the lack of strict statistical control it is not possible to state that trapping alone will reduce the SBW population below the economic threshold level. However, the data do suggest that SBW trapping on donor fields can play an important role in the reduction of insect population and thus in the reduction of insecticide applications. It will be an important tool when an integrated pest management plan for dealing with SBW under similar circumstances is created. Mass trapping and AW management could be a relevant tool and approach in managing the pest, but the combination with other available pest control tools is needed. Of all available options for SBW, the most important are rotation and reduction of the share of sugar beet in the rotation system. Additionally, entomopathogenic nematodes (EPNs) were listed as a potentially available non-pesticide tool for sugar beet control[56] and obtained good preliminary results in small-scale trials[57] indicating that the EPN *Heterorhabditis bacteriophora* Poinar could have a satisfactory effect on SBW larvae. However, further investigations are needed in order to determine better the optimal dose and application timing and use them as an additional tool when AW SBW control by mass trapping is conducted.

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