

Enhancing pest management in sugar beet cultivation: impact of variety selection and insecticide seed treatments on sugar beet flea beetles and weevils

DARIJA LEMIC^{1,2*}, MARIO SCHUMANN³, RALF TILCHER³, OLAF CZARNECKI³,
KATARINA M. MIKAC⁴, DOMAGOJ VUČEMILOVIĆ-JURIĆ¹, HELENA VIRIC GASPARIC^{1,2}

¹Faculty of Agriculture, University of Zagreb, Zagreb, Croatia

²Green Environmental Research Ltd., Zagreb, Croatia

³KWS SAAT SE & Co., Einbeck, Germany

⁴School of Earth, Atmospheric and Life Sciences, Faculty of Science, Medicine & Health,
University of Wollongong, Wollongong, Australia

*Corresponding author: dlemic@agr.hr

Citation: Lemic D., Schumann M., Tilcher R., Czarnecki O., Mikac K.M., Vučemolović-Jurić D., Viric Gasparic H. (2024): Enhancing pest management in sugar beet cultivation: impact of variety selection and insecticide seed treatments on sugar beet flea beetles and weevils. *Plant Protect. Sci.*, 60: 278–287.

Abstract: This study focused on evaluating the effectiveness of seed treatments and different sugar beet varieties in controlling flea beetles (*Chaetocnema tibialis*) and sugar beet weevils (*Asproparthenis punctiventris*) in Croatia. The field trials were conducted in Vukovar-Sirmia County and targeted the developmental stages of sugar beet from BBCH 12 to BBCH 31. Although the sowing was done within the optimal period, no clear pattern between germination of the seeds and susceptibility was identified as the results showed different responses at different development stages and among the three variants. The experimental design comprised no insecticide, thiamethoxam + tefluthrin, cyantraniliprole, flupyradifurone and *Beauveria bassiana* + *Metarhizium anisopliae*. The results show that the treatments with thiamethoxam + tefluthrin effectively reduced pest damage only at the critical stages of development. The current findings suggest that While some of these alternative methods offer good control, they may prove insufficient when applied individually. Hence, integrating them into a comprehensive pest management approach could be necessary for effectively safeguarding sugar beet yields. Further studies should explore potential additive or synergistic benefits to enhance these strategies.

Keywords: sugar beet varieties; IPM; *Chaetocnema tibialis*; *Asproparthenis punctiventris*; pesticides; seed coating

In 2021, sugar beet cultivation in Croatia spanned 10 000 ha, on which 717 000 t of raw material was grown, corresponding to an average of 70 t per hectare (CBS 2022). Due to the complicated production technology and the long vegetation period (~ 180 days), sugar beet is widely recognised as one of the most demanding agricultural crops (Pospišil 2013; Kristek

2015). Throughout the growing cycle, sugar beet faces a variety of pests that significantly affect yield, sugar content and root quality. The main pests that cause damage in the early stages of leaf development or in the juvenile phase include flea beetles (*Chaetocnema tibialis* Ill.) and sugar beet weevils (*Asproparthenis punctiventris* Germ.) (Čamprag 1983; Bažok et al. 2014).

Supported by the University of Zagreb, Faculty of Agriculture, Croatia and by KWS SAAT SE & Co., Einbeck, Germany.

© The authors. This work is licensed under a Creative Commons Attribution-NonCommercial 4.0 International (CC BY-NC 4.0).

Over the past two decades, the protection of sugar beet crops has relied on applying neonicotinoid insecticides for seed treatment. Neonicotinoid seed treatments effectively suppress flea beetles on sugar beet (Dobrinčić 2002; Bažok 2010), limiting the need for foliar insecticide applications during growth, which mainly target sugar beet weevils (Bažok et al. 2012). Effective protection against insect pests is achieved with concentrations 0.005–0.01 mg/kg in plant tissue (Castle et al. 2005; Byrne & Toscano 2006). The plants take up about 16–20% of the neonicotinoid active ingredients during germination from hulled seeds (Sur & Stork 2003). Alternatively, seed treatment uses lower doses of active ingredients per unit area, minimising the environmental impact and conferring an ecotoxicological and economic advantage (Vojvodić & Bažok 2021).

Insect pest control in the European Union recently changed significantly with the neonicotinoid ban because of their harmful effects on European honeybee (*Apis mellifera carnica*) colonies (Vojvodić & Bažok 2021). The European Commission Regulation (EU, 485/2013 of May 24, 2013), which provides for a temporary ban on thiamethoxam, imidacloprid and clothianidin for most agricultural crops (EC 2013), changed course. Sugar beet was originally exempted from this ban due to its limited attractiveness to bees and delayed flowering, which minimises the potential for beet pollen insecticide residues (Vojvodić & Bažok 2021). Finally, the European Commission decided to ban the use of imidacloprid, thiamethoxam and clothianidin completely, except in permanent greenhouses, on the recommendation of The European Food Safety Authority (EFSA) (EFSA 2018a, 2018b, 2018c). The decision has been applied in most EU Member States since 2019. However, with special authorisations, neonicotinoids can still be used for plant protection in the EU (Harrison-Dunn 2021). Nevertheless, there is an urgent need to test for alternative plant protection agents.

A promising candidate for replacing neonicotinoids should ideally be a systemic insecticide that can protect young plants from insect pest infestation. However, tefluthrin, the only insecticide currently available on the market, has no systemic properties (Vojvodić & Bažok 2021), which makes it unsuitable as a direct replacement for neonicotinoids.

Given the ongoing reduction in the use of pesticides and the frequent withdrawal of pesticide products from the market, coupled with the emer-

gence of insect pest resistance, the area of biological control is gaining importance in an integrated production approach (Barić & Pajač Živković 2020). In this context, organic seed treatment is rapidly emerging and likely to be crucial in promoting sustainable crop production. Also, its popularity is further enhanced by its biological active ingredients, which are often easier to register due to their lower toxicity (Sharma et al. 2015).

The selection of suitable sugar beet genotypes (or varieties) plays a central role in effective integrated pest management (IPM). Different sugar beet genotypes have different levels of resistance or tolerance to common sugar beet diseases (Francis & Luterbacher 2003; Grimmer et al. 2008; James et al. 2012; Francis et al. 2022). Sugar beet genetic lines resistant to nematodes, aphids and root maggots have been identified and integrated into sugar beet breeding programmes (Zhang et al. 2008). By choosing genotypes that naturally possess traits that are resistant to pests, farmers can significantly reduce the need for chemical control measures. Some sugar beet varieties may have traits that deter insect pests through altered leaf chemistry or physical characteristics. Integrating pest-resistant genotypes into cultivation practices reduces dependence on pesticides and contributes to sustainable and environmentally friendly agricultural systems (Francis et al. 2022). Therefore, a comprehensive understanding of sugar beet genotypes and their interaction(s) with insect pests is essential for developing IPM strategies that promote crop productivity and ecological balance in an agricultural ecosystem (Francis et al. 2022).

In addition to their direct resistance to pests, sugar beet genotypes with differences in germination time could also influence the dynamics of insect pest control. The most common pests that attack sugar beet are in the early stages of leaf development or the youth stage (BBCH 10–19) and cause major damage (Maceljski 2002; Bažok 2006). Genotypes with different germination times may be exposed to different pest pressures due to shifts in the time of susceptibility to pests. For example, genotypes with early germination may have increased exposure to certain pests during their susceptible seedling stage. In contrast, genotypes with delayed germination may encounter pests at different stages of development (Reed et al. 2022). This temporal variability in pest interactions could potentially influence the effectiveness of pest control strategies.

Farmers could use such differences in germination time to implement targeted pest control measures, such as adjusting planting dates or optimising the timing of pesticide applications. Understanding the nexus of sugar beet genotypes, germination timing, and pest dynamics can help develop precise and efficient IPM strategies that improve crop health and overall yield (Zhang et al. 2008).

This study aimed to evaluate the efficacy of different sugar beet varieties and insecticidal seed treatments against Croatia's two main sugar beet pests (flea beetles and weevils).

MATERIAL AND METHODS

Location. The experimental fields were located in Vukovar-Sirmium County, which included two different but geographically proximate sites characterised by similar climatic conditions: Bogdanovci (45.3440798°N, 18.9598707°E) and Ovčara (45.2992350°N, 19.0357410°E). Historically, Bogdanovci is known for its high population of flea beetles, while Ovčara is known for its occurrence of weevils (producer's historical data).

Trial experiment. Three non-commercially available sugar beet varieties [from KWS SAAT SE & Co. KGaA (KWS)] were sown in four replicates. The varieties in the trial comprised three different genotypes, which differed in the type of germination after sowing (1 – fast, 2 – medium, 3 – slow). The experimental design included the following variants: (i) control without insecticides, seed treated with (ii) thiamethoxam + tefluthrin, (iii) cyantraniliprole, (iv) flupyradifurone and (v) *Beauveria bassiana* + *Metarhizium anisopliae*. The quantity and formulation of the active ingredients in the seed-treated variant are listed in Table 1, and the details of the variants are described in the following section. These variants were systematically grown in four replicates, each occupying a 20 m² plot

(consisting of four 10 m rows spaced 0.5 m apart) at an appropriate plant density. A total of 60 plots were sown at each of the two sites (with 3 varieties, 5 variants and 4 replicates). Throughout the trial period, herbicides and fungicides were applied uniformly according to standard production practice.

Insecticide variants. *Thiamethoxam* (TMX), is a second-generation neonicotinoid insecticide developed for both foliar/soil and seed applications and is widely used on agricultural crops worldwide. Tefluthrin, referred to by its ISO Common Name (BCPC 2023), is an organic pesticide. Tefluthrin belongs to the class of pyrethroids, a group of synthetic insecticides that mimic the structure and properties of the naturally occurring insecticide pyrethrin. Due to its cost-effectiveness and long-lasting efficacy, tefluthrin is a popular active ingredient for insecticides in agriculture (McDonald et al. 1986).

Cyantraniliprole belongs to the class of ryanoid insecticides and is specifically categorised as a diamide insecticide (IRAC MoA Group 28). Its registration covers the United States, Canada, China and India. Cyantraniliprole, a ryanoid, has efficacy against sucking insect pests that show resistance to alternative insecticide classes (Vojvodić & Bažok 2021).

Flupyradifurone is an organic heterocyclic compound that is a breakthrough butenolide insecticide (PRI 2015). Its application provides robust crop protection and has the notable advantage of being much gentler on non-target organisms than other commercial insecticides. The EU approved this organic compound in 2015, highlighting its safety and efficacy. As an agonist of insect nicotinic acetylcholine receptors (nAChRs), flupyradifurone shows commendable efficacy in curbing the proliferation of sucking insects (Bass et al. 2014) while showing positive results in toxicological and ecotoxicological assessments.

Beauveria bassiana and *Metarhizium anisopliae*, two prominent entomopathogenic fungi (EPFs), are key active ingredients in pest control (Liu et al. 2022).

Table 1. Details of seed treatments/variants

Variant	Concentration of active ingredient (a.i.)	Formulation of a.i.
Control	untreated	–
Thiamethoxam + tefluthrin	45 g/U + 6 g/U	liquid (WG)
Cyantraniliprole	60 g/U	liquid (WG)
Flupyradifurone	20 g/U	liquid (WG)
<i>Beauveria bassiana</i> + <i>Metarhizium anisopliae</i>	20 g/U	powder (SP)

U – unit = 100 000 seeds of sugar beet; WG – water dispersible granules; SP – soluble powder

Recent studies have highlighted their role in stimulating plant growth following targeted inoculation. *B. bassiana* and *M. anisopliae* can colonise various plants, including wheat, soybean, rice, beans, onion, tomato, palm, grape, potato and cotton (Vega 2018). Their colonisation can be local or systemic, mainly in plants' roots, stems, leaves and internal tissues (Behie et al. 2015). Endophytic colonisation by these fungi has been shown to increase plant growth through seed treatment, foliar spraying, and soil irrigation (Jaber & Enkerli 2016; Jaber 2018; Jaber & Ownley 2018).

Data collection and analysis. In the two experimental fields in Ovčara and Bogdanovci, an assessment of pest infestation and damage to sugar beet was carried out during a single growing season. This assessment focused on weevils and flea beetles and was limited to the two inner rows of each plot (to mitigate edge effects), spanning a length of 10 m. Weekly observations were made, synchronised with the developmental stage of the plants according to the BBCH scale (Bleholder et al. 2001).

Flea beetle damage was assessed by direct visual inspection. The observed plants were divided into six classes, labelled as follows: 0 (no holes); 1 (damage up to 3% of leaf area); 2 (damage 4–10%); 3 (damage 11–20%); 4 (damage 21–40%); 5 (more than 40 % leaf area damaged) (Čamprag 1973).

Damage caused by the weevil was assessed using 1 m² plots that were randomly assigned (using a random number generator) four times within each treatment plot. Within each 1 m² plot, all plants are visually assessed into one of five damage categories: 0 (no damage); 1 (up to 25% of plant parts damaged); 2 (26–50% of plant parts damaged); 3 (51–75% of plant parts damaged); 4 (more than 75% plant parts damaged) (Čamprag 1973).

The percentage of damage (%) of flea beetles and sugar beet weevil was calculated based on the frequency distribution of plants within each pest category:

$$D(\%) = \left(\frac{\sum (fxn)}{axN} \right) \times 100 \quad (1)$$

where: D (%) – percentage of damage; f – number of plants in particular class; n – class value; a – number of classes; N – number of assessed plants (Townsend & Heuberger 1943).

Damage caused by flea beetles and sugar beet weevils was investigated via analysis of variance (ANOVA) using the statistical program ARM 9 (GDM 2019). Where appropriate, data were arc. sin \sqrt{x} transformed, this transformation was used to stabilise variances and meet the assumptions of paramet-

Table 2. Sugar beet flea beetle damage on sugar beet plants in different developmental stages (BBCH) at Bogdanovci

Variety	Variant	Damage (%)				
		BBCH 12	BBCH 14	BBCH 16	BBCH 19	BBCH 31
1	no insecticide	30.2 ± 0.3 ^{ab*}	20.1 ± 0.1 ^{ab}	21.0 ± 3.5 ^{abc}	15.6 ± 8.5 ^{ns}	3.4 ± 1.2 ^{ns}
	thiamethoxam + tefluthrin	0.3 ± 0.1 ^c	1.2 ± 0.1 ^{ab}	1.4 ± 0.6 ^c	1.9 ± 0.7 ^{ns}	1.3 ± 0.3 ^{ns}
	cyantraniliprole	35.2 ± 8.9 ^a	28.1 ± 0.1 ^a	32.6 ± 8.5 ^{ab}	8.3 ± 2.2 ^{ns}	3.1 ± 0.4 ^{ns}
	flupyradifurone	10.2 ± 7.3 ^{abc}	18.8 ± 0.3 ^{ab}	36.5 ± 15.8 ^a	32.1 ± 17.7 ^{ns}	12.1 ± 9.3 ^{ns}
	<i>Beauveria bassiana</i> + <i>Metarhizium anisopliae</i>	16.6 ± 7.7 ^{ab}	4.5 ± 0.2 ^{ab}	8.4 ± 5.8 ^{bc}	12.9 ± 8.5 ^{ns}	4.9 ± 2.9 ^{ns}
2	no insecticide	18.0 ± 1.0 ^a	15.2 ± 0.1 ^{ab}	19.2 ± 6.7 ^{abc}	6.5 ± 1.7 ^{ns}	3.9 ± 0.8 ^{ns}
	thiamethoxam + tefluthrin	3.5 ± 0.3 ^{abc}	2.0 ± 0.0 ^{ab}	2.1 ± 0.3 ^c	0.3 ± 0.2 ^{ns}	0.9 ± 0.4 ^{ns}
	cyantraniliprole	23.5 ± 13.3 ^{ab}	15.3 ± 0.4 ^{ab}	36.4 ± 19.7 ^a	31.7 ± 21.3 ^{ns}	7.1 ± 3.0 ^{ns}
	flupyradifurone	6.8 ± 0.3 ^{abc}	3.3 ± 0.3 ^{ab}	7.6 ± 5.4 ^{bc}	28.7 ± 23.1 ^{ns}	9.1 ± 7.0 ^{ns}
	<i>B. bassiana</i> + <i>M. anisopliae</i>	34.3 ± 8.6 ^a	16.1 ± 0.3 ^{ab}	32.7 ± 16.5 ^{ab}	26.3 ± 11.3 ^{ns}	8.4 ± 3.0 ^{ns}
3	no insecticide	22.8 ± 0.4 ^{ab}	13.8 ± 0.3 ^{ab}	34.5 ± 18.4 ^{ab}	14.1 ± 8.2 ^{ns}	6.5 ± 3.3 ^{ns}
	thiamethoxam + tefluthrin	0.9 ± 0.2 ^{bc}	0.5 ± 0.1 ^b	0.6 ± 0.2 ^c	0.7 ± 0.3 ^{ns}	0.7 ± 0.3 ^{ns}
	cyantraniliprole	2.4 ± 0.1 ^{abc}	9.3 ± 0.1 ^{ab}	11.0 ± 3.4 ^{abc}	5.9 ± 4.5 ^{ns}	2.8 ± 1.1 ^{ns}
	flupyradifurone	12.5 ± 0.3 ^{abc}	9.2 ± 0.2 ^{ab}	15.1 ± 7.8 ^{abc}	11.6 ± 6.8 ^{ns}	4.7 ± 1.4 ^{ns}
	<i>B. bassiana</i> + <i>M. anisopliae</i>	37.1 ± 0.3 ^a	19.0 ± 0.2 ^{ab}	28.1 ± 11.5 ^{abc}	16.7 ± 8.4 ^{ns}	4.8 ± 3.1 ^{ns}
HSD $P = 0.05$		7.0	23.8	23.1		

*Means followed by the same letter do not significantly differ ($P = 0.05$, Tukey's HSD)

Table 3. Sugar beet flea beetle damage on sugar beet plants in different developmental stages (BBCH) at Ovčara

Variety	Variant	Damage (%)	
		BBCH 19	BBCH 31
1	no insecticide	12.7 ± 3.6 ^a	7.2 ± 3.1 ^{ab}
	thiamethoxam + tefluthrin	0.3 ± 1.3 ^c	1.1 ± 3.1 ^b
	cyantraniliprole	5.4 ± 4.4 ^{abc}	18.4 ± 9.5 ^{ab}
	flupyradifurone	4.8 ± 2.5 ^{abc}	16.9 ± 2.6 ^{ab}
	<i>Beauveria bassiana</i> + <i>Metarhizium anisopliae</i>	3.6 ± 3.7 ^{abc}	10.8 ± 12.6 ^{ab}
2	no insecticide	5.1 ± 4.7 ^{abc}	29.3 ± 6.8 ^{ab}
	thiamethoxam + tefluthrin	0.4 ± 0.2 ^{bc}	3.7 ± 1.6 ^{ab}
	cyantraniliprole	4.9 ± 2.4 ^{abc}	10.7 ± 2.2 ^{ab}
	flupyradifurone	2.3 ± 1.6 ^{abc}	3.8 ± 1.9 ^{ab}
	<i>B. bassiana</i> + <i>M. anisopliae</i>	4.5 ± 3.2 ^{abc}	22.7 ± 6.6 ^{ab}
3	no insecticide	7.7 ± 3.6 ^{abc}	40.3 ± 8.6 ^a
	thiamethoxam + tefluthrin	0.8 ± 0.4 ^{bc}	0.7 ± 1.7 ^b
	cyantraniliprole	3.0 ± 3.6 ^{abc}	12.1 ± 9.8 ^{ab}
	flupyradifurone	5.3 ± 2.3 ^{abc}	11.4 ± 4.3 ^{ab}
	<i>B. bassiana</i> + <i>M. anisopliae</i>	10.3 ± 2.6 ^{ab}	18.1 ± 13.0 ^{ab}
HSD $P = 0.05$		9.2	36.8

*Means followed by the same letter do not significantly differ ($P = 0.05$, Tukey's HSD)

ric statistical tests (which require data to be normally distributed). The interpretation was based on the transformed data. After achieving significant results in the test procedure ($P < 0.05$), a Tukey post hoc test was applied to identify specific mean variant values that showed statistically significant differences.

RESULTS

Efficacy on flea beetles. In most variants, the flea beetle infestation was more pronounced in the early stages of vegetation (BBCH 12–16) (Table 2). In the early stages of development, when sugar beet plants are most susceptible to flea beetle infestation (BBCH 2–14), the least damage occurred in all variants treated with thiamethoxam + tefluthrin (0.3–1.2). As expected, the untreated variants showed considerable damage (up to 30%). In this context (BBCH stages 12 and 14), no statistically significant differences existed between the untreated variants and the

variants of variety 1 treated with other alternative active ingredients (Table 2). Efficacy was observed in varieties 2 and 3 among the variants treated with flupyradifurone, resulting in low damage (3–12%). In addition, some variants of 2 and 3 and all variants of 1 displayed significantly higher damage levels. Notably, *B. bassiana* + *M. anisopliae* seed treatment showed the lowest efficacy against flea beetles in all three varieties, with no differences between the control variants. At BBCH 19–31, all varieties showed varying degrees of damage, although no statistically significant differences were found. Variety 1, treated with flupyradifurone (32%) and variety 2, treated with cyantraniliprole (32%), showed the highest damage. The incidence of damage at BBCH 31 remained negligible throughout the trial, with no significant differences between varieties and variants (Table 2). It is important to note that sugar beet at this stage of development is naturally resistant to flea beetle infestation.

At the Ovčara site, the flea beetle population was observed towards the end of May, when the sugar beet had already reached the BBCH 19 growth stage (Table 3). Their presence was noted until the beginning of June, which coincided with the development stage of BBCH 31. During this period, the impact of flea beetles on sugar beet leaves was relatively benign. The BBCH 31 stage, which occurs ten weeks after sowing, is characterised by a decrease in the efficacy of most seed insecticides. Nevertheless, nuanced differences were recognised. Similar to the Bogdanovci site, variants treated with thiamethoxam + tefluthrin seed treatment showed the highest efficacy at the Ovčara site.

Variety 1 showed particularly low damage due to this treatment. Conversely, variants treated with different active ingredients showed similar damage values, ranging 2–18 %. However, this damage is only of minor importance at this stage of sugar beet development. The greatest damage, which reached 40 %, was found in variety 3 on an untreated variety. Similar to Bogdanovci, variants 2 and 3 also showed a low effectiveness of seed treatment with *B. bassiana* + *M. anisopliae*.

Efficacy on weevils. At Bogdanovci during the susceptible BBCH 12 phase, weevil infestation remained minimal and ranged from 1.5% (thiamethoxam + tefluthrin in variety 2) to the highest recorded damage of 8.3% (*Bauveria bassiana* + *Metarhizium anisopliae* variant in variety 3) (Table 4). Plants of variety 1 showed the highest damage, which was at-

<https://doi.org/10.17221/8/2024-PPS>

Table 4. Sugar beet weevil damage (according to Townsend-Heuberger) on sugar beet plants in different developmental stages (BBCH) at location Bogdanovci

Variety	Variant	Damage (%)		
		BBCH 12	BBCH 14	BBCH 16
1	no insecticide	81.2 ± 11.1 ^{bc*}	88.7 ± 6.5 ^{ab}	4.6 ± 0.8 ^f
	thiamethoxam + tefluthrin	78.1 ± 2.4 ^c	7.8 ± 1.7 ^{def}	9.6 ± 0.1 ^{def}
	cyantraniliprole	100 ± 0.0 ^a	17.1 ± 1.8 ^{cd}	21.1 ± 3.1 ^{cd}
	flupyradifurone	87.6 ± 7.2 ^{abc}	69.4 ± 5.3 ^{ab}	13.7 ± 1.6 ^{cde}
	<i>Beauveria bassiana</i> + <i>Metarhizium anisopliae</i>	98.1 ± 1.1 ^{ab}	33.6 ± 7.5 ^{bc}	6.3 ± 1.5 ^{ef}
2	no insecticide	100 ± 0.0 ^a	16.7 ± 1.5 ^{cde}	100 ± 0.0 ^a
	thiamethoxam+ tefluthrin	50.3 ± 2.8 ^d	1.5 ± 0.5 ^{gh}	14.2 ± 2.9 ^{cde}
	cyantraniliprole	98.4 ± 0.0 ^{ab}	5.8 ± 0.8 ^{ef}	16.3 ± 0.9 ^{cd}
	flupyradifurone	72.5 ± 0.0 ^c	4.8 ± 1.7 ^{fg}	22.2 ± 1.1 ^{bc}
	<i>B. bassiana</i> + <i>M. anisopliae</i>	100 ± 0.0 ^a	9.0 ± 2.1 ^{def}	62.5 ± 7.2 ^a
3	no insecticide	98.9 ± 1.0 ^{ab}	54.2 ± 16.5 ^{ab}	21.3 ± 1.4 ^{cd}
	thiamethoxam + tefluthrin	47.8 ± 0.0 ^d	0.5 ± 0.5 ^h	23.1 ± 1.1 ^{bc}
	cyantraniliprole	100 ± 0.0 ^a	95.8 ± 2.4 ^a	47.3 ± 2.6 ^{ab}
	flupyradifurone	73.2 ± 0.0 ^c	7.4 ± 2.0 ^{def}	17.9 ± 1.2 ^{cd}
	<i>B. bassiana</i> + <i>M. anisopliae</i>	97.3 ± 0.0 ^{ab}	54.0 ± 10.7 ^{ab}	23.0 ± 2.8 ^{bc}
HSD $P = 0.05$		18.2	2.2	6.3

*Means followed by the same letter do not significantly differ ($P = 0.05$, Tukey's HSD)

tributed to the activity of the weevils, though this was not statistically significant (Table 4).

At BBCH 14, an intense weevil infestation led to pronounced leaf damage. As expected, the highest

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

Variety	Variant	Damage (%)		
		BBCH 12	BBCH 14	BBCH 16
1	no insecticide	4.5 ± 0.1 ^{ab*}	59.6 ± 2.8 ^a	10.2 ± 0.6 ^{bcd}
	thiamethoxam+tefluthrin	4.5 ± 0.1 ^{ab}	2.5 ± 0.3 ^e	1.1 ± 0.6 ^d
	cyantraniliprole	1.6 ± 0.2 ^{bc}	3.6 ± 0.8 ^{de}	6.8 ± 0.5 ^{bcd}
	flupyradifurone	8.1 ± 0.1 ^a	56.3 ± 2.2 ^{ab}	8.5 ± 3.3 ^{bcd}
	<i>Beauveria bassiana</i> + <i>Metarhizium anisopliae</i>	3.5 ± 0.1 ^{abc}	37.8 ± 5.8 ^c	6.9 ± 1.9 ^{bcd}
2	no insecticide	0.8 ± 0.1 ^{cd}	11.4 ± 1.7 ^d	1.6 ± 0.5 ^{cd}
	thiamethoxam+tefluthrin	1.5 ± 0.2 ^d	1.1 ± 2.0 ^e	3.7 ± 1.5 ^{cd}
	cyantraniliprole	3.5 ± 0.1 ^{abc}	41.9 ± 2.7 ^{bc}	6.2 ± 1.7 ^{cd}
	flupyradifurone	2.9 ± 0.2 ^{abc}	46.5 ± 1.2 ^{abc}	8.9 ± 2.4 ^{bcd}
	<i>B. bassiana</i> + <i>M. anisopliae</i>	4.9 ± 0.1 ^{ab}	33.4 ± 1.1 ^c	39.8 ± 2.4 ^a
3	no insecticide	1.8 ± 0.1 ^{bc}	46.5 ± 3.3 ^{abc}	16.5 ± 1.5 ^b
	thiamethoxam+tefluthrin	1.9 ± 0.2 ^{bc}	2.1 ± 0.9 ^{de}	11.3 ± 2.9 ^{bc}
	cyantraniliprole	1.9 ± 0.2 ^{bc}	3.4 ± 1.1 ^{de}	3.6 ± 1.1 ^{cd}
	flupyradifurone	1.7 ± 0.1 ^{bc}	6.5 ± 0.2 ^e	10.9 ± 3.8 ^{bc}
	<i>B. bassiana</i> + <i>M. anisopliae</i>	8.3 ± 0.1 ^a	1.7 ± 1.2 ^e	9.8 ± 2.1 ^{bcd}
HSD $P = 0.05$		7.4	6.0	9.8

*Means followed by the same letter do not significantly differ ($P > 0.05$, Tukey's HSD)

damage occurred in non-treated variants of variety 1 (60%) and variety 3 (47%). Notably, variety 1 showed considerable damage in the flupyradifurone-treated variant (56%). Conversely, in variety 2, the highest damage was observed in the variants treated with cyantraniliprole (42%) and flupyradifurone (46%). As vegetation progressed, a reduction in leaf damage caused by weevils was observed in all variants of each variety at the BBCH 16 development stage.

Table 5 shows the percentage of weevil damage to the sugar beet plants at the Ovčara site, which in some cases led to extensive destruction of the plants. A low percentage of plant recovery or subsequent emergence was observed in selected variants. Nevertheless, the results underline the presence of a weevil population at the Ovčara site that has the potential to decimate sugar beet crops. Weevil infestation was severe in all variants, especially during the BBCH 12 stage when the plants were at their most susceptible stage of development.

In this phase, the damage ranged from 48% (thiamethoxam + tefluthrin variant in variety 3) to total damage of 100% (cyantraniliprol in varieties 1 and 3, *B. bassiana* + *M. anisopliae* and the control variant in variety 2). All varieties showed damage that persisted throughout the season. During the sustained weevil attack, variety 3 and the thiamethoxam + tefluthrin variant provided some protection, allowing the sugar beet plants to recover from the initial attack during this trial period. However, most plants in the other variants and varieties continued to suffer significant damage, and despite some recovery, overall damage levels remained high after BBCH 16. Throughout the trial period, treatments with thiamethoxam + tefluthrin provided the most effective sugar beet protection, regardless of the variant (Table 5).

DISCUSSION

The focus of this research was to evaluate the efficacy of seed treatments in combination with different sugar beet varieties in the control of two important sugar beet pests in Croatia: the flea beetle and the sugar beet weevil. Field trials were conducted at two locations in Vukovar-Sirmia County and covered the developmental stages of sugar beet from BBCH 12 to BBCH 31. As expected, no pests were observed before BBCH 12, which is consistent with the early leaf development stage (the first pair of leaves unfolded). Accordingly, the

lower susceptibility of sugar beet to flea beetle and weevil infestation after reaching BBCH 31 was attributed to the growth of a significant leaf mass (Virić Gašparić et al. 2021).

The optimal agrotechnical period for sowing sugar beet is from March 15 to April 10 (Tot 2008), and our experiments were conducted within this time frame. Bažok et al. (2015) emphasised the usefulness of early sowing, preferably at the beginning of the optimal period. Early sowing leads to the rapid development of the cotyledon stage in sugar beet and makes it more resistant to attack by various insect pests, especially flea beetles and weevils (Virić Gašparić 2022).

No clear pattern between germination and susceptibility was identified, and the data showed different responses at different stages of development and among the three variants.

In 2022, the emergence of sugar beet coincided with a pest infestation, which had a pronounced impact on damage in the early stages of development (BBCH 12–16). This illustrates the complexity of effective plant protection in which factors such as the overwintering of pests, the duration of winter and soil temperatures in Spring and time of sowing influence the speed of germination (Maceljski 2002; Bažok et al. 2012; Poggi et al. 2018; Viric Gasparic et al. 2021).

Between 1965 and the early 2000s, the sugar beet weevil was of little concern in Croatia, in contrast to its importance in the Serbian region of Vojvodina, which borders eastern Croatia (Čamprag et al. 2006). The escalation in the sugar beet weevil as a serious pest in Croatia after 2008 is attributed to climate change (Bažok et al. 2012; Vuković et al. 2014). This trend is further seen in Poland, Austria, Hungary and other Eastern European countries, causing considerable economic damage due to the weevil's reappearance (Holy & Skuhrovec 2020). The increase in pest abundance can be linked to favourable climatic conditions and restrictions on effective insecticides (Holy & Skuhrovec 2020), such as the neonicotinoid ban in the EU (Viric Gasparic 2022).

Our study found that treatment with neonicotinoids effectively protected crops and curbed flea beetles and weevil incursion and damage. However, the extent of the damage varied between locations due to different climatic conditions and the pests' preference for certain cropping practices (i.e. crop rotation). In particular, seed treatment with thia-

methoxam + tefluthrin mitigated flea beetle damage, with observed damage at the Bogdanovci site reaching almost 40% at the BBCH 14 and BBCH 16 stages. This underlines the severity of flea beetle infestation in Croatia and corroborates the data of other published studies (Kereši et al. 2006; Bažok et al. 2012). Similarly, this treatment reduced weevil damage at the Ovčara site, often reaching 100% in most variants. The insecticide treatments had an impact on plant damage at all three stages of early plant development (BBCH 12–19), emphasising the protective role of neonicotinoid and pyrethroid seed treatments against flea beetle and weevil infestations.

The effectiveness of the seed treatment with neonicotinoids and pyrethroids was particularly evident, as it reduced flea beetle damage, which exceeded 30% in untreated plots during BBCH 12–16.

Notably, the average percentage of damage at the Bogdanovci site, at 11.4% and 35%, was significantly higher than at the Ovčara site, where no damage occurred at the same stages of sugar beet development. Plant damage at the Bogdanovci site was influenced by insecticide treatments at all three stages of plant development (BBCH 12–16). They are confirming that neonicotinoid seed treatments provide optimal plant protection against flea beetle infestation. Non-chemical alternatives for flea beetle control in sugar beet remain difficult to find, with pyrethroid-based foliar spraying remaining the main control agent; its use has intensified following the 2018 neonicotinoid ban (Bažok et al. 2022).

Seed coating with neonicotinoids and pyrethroids effectively controlled weevils at the most susceptible developmental stages of sugar beet at low population pressure, resulting in significantly less damage to untreated control plants. During the study, the trial at the Ovčara site showed significantly higher weevil infestation rates. However, the damage on the plots treated with neonicotinoids was significantly lower than on the other variants. These results underline the success of seed treatment in protecting sugar beet at critical stages of development. However, the efficacy of neonicotinoids and pyrethroids against higher weevil populations remains limited, indicating the need for testing alternative strategies.

Even with varying population pressure, the recurrence of pests necessitates diversifying pest control approaches. With the ban on neonicotinoids and emerging reports of resistance, researching and testing alternative insecticides is becoming increasingly

important (Furlan & Kreutzweiser 2015; Hauer et al. 2017; Veres et al. 2020). Researching and validating the efficacy of these alternatives under field conditions is imperative, given their potential to replace neonicotinoid treatments. This study highlights the need for a multipronged pest control approach that integrates established and innovative strategies to protect sugar beet crops effectively.

In light of the data presented in this study, a re-evaluation of neonicotinoid seed treatment as a safe and effective crop protection measure is warranted. While synthetic insecticides should be allowed, they must be accompanied by strict application regulations and regular ecotoxicological assessments to minimise environmental risks and beneficial insects. In light of recent EU decisions to ban the use of neonicotinoids in the field, the development of alternative approaches to seed dressing has become essential. Current research suggests numerous alternatives have already been formulated, some showing promising efficacy values. However, many of these alternatives provide important but potentially inadequate control when used alone. Future strategies may, therefore, require their combined integration as part of an integrated pest management approach to protect sugar beet yields while preserving human health, pollinators and other ecosystems. To further advance these approaches, trials should be conducted to investigate possible additive or synergistic effects.

CONCLUSION

This comprehensive field study highlights the role of both neonicotinoid (+ pyrethroid) seed treatments and alternative active ingredients in protecting sugar beet crops against the significant threat of flea beetles and weevils. The reduction in damage at different stages of development underlines the effectiveness of these treatments, especially in the critical early stages. The promising results of thiamethoxam + tefluthrin and other alternative active ingredients suggest their potential as valuable components in integrated pest management strategies. As the prevalence of these insect pests continues to increase in certain regions and resistance to traditional control measures grows, these results underscore the need for sustainable and diversified approaches to pest control. Furthermore, the changes in pest dynamics caused by climate change emphasise the need for adaptive methods to ensure the resilience and viability of sugar beet cultivation.

<https://doi.org/10.17221/8/2024-PPS>

REFERENCES

- Barić B., Pajač Živković I. (2020): Načela Integrirane Zaštite Bilja; University of Zagrebu, Faculty of Agriculture, Zagreb, Croatia. (in Croatian)
- Bass C., Puinean A.M., Zimmer C.T., Denholm I., Field L.M., Foster S.P., Gutbrod O., Nauen R., et al. (2014): The evolution of insecticide resistance in the peach potato aphid, *Myzus persicae*. *Insect Biochemistry and Molecular Biology*, 51: 41–51.
- Bažok R. (2006): Žičnjaci – Važni štetnici ratarskih kultura. *Glasilo Biljne Zaštite*, 6: 3–10. (in Croatian)
- Bažok R. (2010): Suzbijanje Štetnika u Proizvodnji Šećerne Repe. *Glasilo Biljne Zaštite*, 10: 153–165. (in Croatian)
- Bažok R., Buketa M., Lopatko D., Ljekar K. (2012): Suzbijanje Štetnika Šećerne Repe Nekad i Danas. *Glasilo Biljne Zaštite*, 12: 414–428. (in Croatian)
- Bažok R., Gotlin Čuljak T., Grubišić D. (2014): Integrirana Zaštita Bilja Na Primjerima Dobre Prakse. *Glasilo Biljne Zaštite*, 14: 357–390. (in Croatian)
- Bažok R., Barić K., Čačija M., Drmić Z., Dermić E., Gotlin Čuljak T., Grubišić D., et al. (2015): Šećerna repa: zaštita od štetnih organizama u sustavu integrirane biljne proizvodnje. In: Bažok, R. (ed.), University of Zagreb, Faculty of Agriculture: 143. (in Croatian)
- Bažok R., Šatvar M., Radoš I., Drmić Z., Lemić D., Čačija M., Virić Gašparić H. (2016): Comparative efficacy of classical and biorational insecticides on sugar beet weevil, *Bothynoderes punctiventris* Germar (Coleoptera: Curculionidae). *Plant Protection Science*, 52: 134–141.
- Bažok R., Lemić D., Čačija M., Kadoić Balaško M., Virić Gašparić H., Skendžić S., Šutić A., Glušić P., et al. (2022): Smanjena osjetljivost repina buhača na piretroide- još jedna ozbiljna prijetnja održivom uzgoju šećerne repe. *Zbornik Sažetaka 65. Seminara Biljne Zaštite*, 52–53. (in Croatian)
- BCPC (2023): Compendium of Pesticide Common Names: tefluthrin. BCPC. Available at <http://www.bcpcepesticide-compendium.org/tefluthrin.html>
- Behie S.W., Jones S.J., Bidochka M.J. (2015): Plant tissue localisation of the endophytic insect pathogenic fungi *Metarhizium* and *Beauveria*. *Fungal Ecology*, 13: 112–119.
- Bleiholder H., Weber E., Lancashire P., Feller C., Buhr L., Hess M., Wicke H., Hack H., et al. (2001): Growth Stages of Mono- and Dicotyledonous Plants. BBCH Monograph. Federal Biological Research Centre for Agriculture and Forestry: Berlin and Braunschweig.
- Byrne F.J., Toscano N.C. (2006): Uptake and persistence of imidacloprid in grapevines treated by chemigation. *Crop Protection*, 8: 831–834.
- Castle S.J., Byrne F.J., Bi J.L., Toscano N.C. (2005): Spatial and temporal distribution of imidacloprid and thiamethoxam in citrus and impact on *Homalodisca coagulata* populations. *Pest Management Science*, 61: 75–84.
- CBS (2022): Croatian Bureau of Statistics Croatian Bureau of Statistics. Areas and Production of Cereals and Other Crops. Provisional Data. Available at <https://podaci.dzs.hr/2021/en/10683>
- Čamprag D. (1973): Najvažnije štetočine šećerne repe u Jugoslaviji, Mađarskoj, Rumuniji i Bugarskoj, sa Posebnim osvrtom na važnije štetne vrste; Poljoprivredni Fakultet, Institut za Zaštitu Bilja Novi Sad: Novi Sad, Serbia: 343–352. (in Serbian)
- Čamprag D. (1983): Štetočine i paraziti ratarskih kultura. Priručnik Izvještajne i Prognozne Službe Zaštite Poljoprivrednih Kultura. Belgrade, Serbia: 168–216. (in Serbian)
- Čamprag D.S., Sekulić R.R., Kereši T.B. (2006): Forecasting of major sugarbeet pest occurrence in Serbia during the period 1961–2004. *Zbornik Matice srpske za prirodne nauke*, 110: 187–194.
- Dobrinčić R. (2002): Prednosti i nedostaci tretiranja sjemena ratarskih kultura insekticidima. *Glasilo Biljne zaštite*, 2: 37–41. (in Croatian)
- EFSA (2018a): European food safety authority peer review of the pesticide risk assessment for bees for the active substance imidacloprid considering the uses as seed treatments and granules. *EFSA Journal*, 16. doi:10.2903/j.efsa.2018.5178.
- EFSA (2018b): European food safety authority peer review of the pesticide risk assessment for bees for the active substance clothianidin considering the uses as seed treatments and granules. *EFSA Journal*, 16: e05177. doi:10.2903/j.efsa.2018.5177.
- EFSA (2018c): European food safety authority peer review of the pesticide risk assessment for bees for the active substance thiamethoxam considering the uses as seed treatments and granules. *EFSA Journal*, 16. doi:10.2903/j.efsa.2018.5179.
- EC (2013): European Commission Implementing Regulation (EU) No. 485/2013 amending implementing regulation (EU) No. 540/2011, as regards the conditions of approval of the active substances clothianidin, thiamethoxam and imidacloprid, and prohibiting the use and sale of seeds. *Official Journal of the European Union*, 139: 12–26.
- Francis F., Then C., Francis A., Gbangbo Y.A.C., Iannello L., Ben Fekih I. (2022): Complementary strategies for biological control of aphids and related virus transmission in sugar beet to replace neonicotinoids. *Agriculture*, 12: 1663.
- Francis S.A., Luterbacher M.C. (2003): Identification and exploitation of novel disease resistance genes in sugar beet. *Pest Management Science*, 59: 225–230.
- Furlan L., Kreutzweiser D. (2015): Alternatives to neonicotinoid insecticides for pest control: case studies in agriculture and forestry. *Environmental Science and Pollution Research*, 22: 135–147.

- Grimmer M.K., Bean K.M.R., Qi A., Stevens M., Asher M.J.C. (2008): The action of three beet yellows virus resistance QTLs depends on alleles at a novel genetic locus that controls symptom development. *Plant Breeding*, 127: 391–397.
- Hauer M., Hansen A.L., Manderyck B., Olsson Å., Raaijmakers E., Hanse B., Stockfisch N., Märländer B. (2017): neonicotinoids in sugar beet cultivation in Central and Northern Europe: Efficacy and environmental impact of neonicotinoid seed treatments and alternative measures. *Crop Protection*, 93: 132–142.
- Harrison-Dunn A.-R. (2021): Why Are Banned 'Bee-Killer' Neonicotinoids Still Being Used in Europe? – Modern Farmer. Available at <https://modernfarmer.com/2021/03/why-are-banned-bee-killer-neonicotinoids-still-being-used-in-europe/>
- Holy K., Skuhrovec, J. (2020): Rýhonosec řepný — škůdce cukrovky z červeného seznamu (Sugar beet weevil — red-listed sugar beet pest). *Listy Cukrovarnické a Řepářské*, 136: 371–375. (in Czech)
- Jaber L.R. (2018): Seed inoculation with endophytic fungal entomopathogens promotes plant growth and reduces crown and root rot (CRR) caused by *Fusarium culmorum* in wheat. *Planta*, 248: 1525–1535.
- Jaber L.R., Enkerli J. (2016): Fungal entomopathogens as endophytes: can they promote plant growth? *Biocontrol Science and Technology*, 27: 28–41.
- Jaber L.R., Ownley B.H. (2018): Can we use entomopathogenic fungi as endophytes for dual biological control of insect pests and plant pathogens? *Biological Control*, 116: 36–45.
- James L.C., Bean K.M.R., Grimmer M.K., Barnes S., Kraft T., Stevens M. (2012): Varieties of the future: identification of 'broad spectrum' genetic resistance in sugar beet. *International Sugar Journal*, 114: 164–168.
- Kereši T., Sekulić R.R., Čačić N.J., Forgić G.D., Marić V.R. (2006): Control of sugar beet pests at early season by seed treatment with insecticides. *Zbornik Matice Srpske za Prirodne Nauke*: 195–204.
- Kristek A. (2015): Važnost šećerne repe za Republiku Hrvatsku. In: Bažok R. (ed.): Šećerna Repa - Zaštita od Štetnih Organizama u Sustavu Integrirane Biljne Proizvodnje. University of Zagreb Faculty of Agriculture, Zagreb, Croatia: 7–9.
- Liu Y., Yang Y., Wang B. (2022): Entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* play roles of maize (*Zea mays*) growth promoter. *Scientific Reports*, 12. doi:10.1038/S41598-022-19899-7.
- Maceljiski M. (2002): Poljoprivredna Entomologija; Zrinski, Čakovec, Croatia.
- McDonald E., Punja N., Jutsum A.R. (1986): Rationale in the invention and optimisation of tefluthrin, a pyrethroid for use in soil. *British Crop Protection Conference – Pests and Diseases, Proceedings*: 199–206.
- Poggi S., Le Cointe R., Riou J.B., Larroudé P., Thibord J.B., Plantegenest M. (2018): Relative influence of climate and agroenvironmental factors on wireworm damage risk in maize crops. *Journal of Pest Science*, 91: 585–599.
- Pospíšil M. (2013): Ratarstvo II. Dio–Industrijsko Bilje; Zrinski, Čakovec, Croatia. (in Croatian)
- PRI (2015): Pesticide Research Institute. Flupyradifurone: A new insecticide or just another neonicotinoid? Available at <https://www.pesticideresearch.com/site/2015/02/05/flupyradifurone-a-new-insecticide-or-just-another-neonicotinoid/>
- Reed R.C., Bradford K.J., Khanday I. (2022): Seed germination and vigor: ensuring crop sustainability in a changing climate. *Heredity*, 128: 450–459.
- Sharma K.K., Singh U.S., Sharma P., Kumar A., Sharma L. (2015): Seed treatments for sustainable agriculture-a review. *Journal of Applied and Natural Science*, 7: 521–539.
- Sur R., Stork A. (2003): Uptake, translocation and metabolism of imidacloprid in plants. *Bulletin of Insectology*, 56: 35–40.
- Tot I. (2008): Osnovni preduvjeti za uspjeh u proizvodnji šećerne repe. *Glasnik Zaštite Bilja*, 31: 76–80. (in Croatian)
- Townsend G.R., Heuberger J.V. (1943): Methods for estimating losses caused by diseases in fungicide experiments. *Plant Disease Report*, 27: 340–343.
- Vega F.E. (2018): The use of fungal entomopathogens as endophytes in biological control: a review. *Mycologia*, 110: 4–30.
- Veres A., Wyckhuys K.A.G., Kiss J., Tóth E., Burgio G., Pons X., Avilla C., Vidal S., et al. (2020): An update of the worldwide integrated assessment (wia) on systemic pesticides. part 4: alternatives in major cropping systems. *Environmental Science and Pollution Research*, 27: 29867–29899.
- Viric Gasparic H., Lemic D., Drmic Z., Cacija M., Bazok R. (2021): The efficacy of seed treatments on major sugar beet pests: possible consequences of the recent neonicotinoid ban. *Agronomy*, 11: 1277.
- Virić Gašparić H. (2022): Neonicotinoid degradation dynamics in sugar beet plants grown from treated seeds and influence on harmful and beneficial fauna [PhD thesis]. Zagreb: University of Zagreb, Faculty of Agriculture.
- Vojvodić M., Bažok R. (2021): Future of insecticide seed treatment. *Sustainability*, 13: 8792.
- Vuković S., Indžić D., Gvozdenac S., Grahovac M., Marinković B., Kereši T., Tanasković S. (2014): Comparative evaluation of insecticides in control of *Bothynoderes punctiventris* Germ. under laboratory and field conditions. *Romanian Agricultural Research*, 31: 348–355.
- Zhang C.L., Xu D.C., Jiang X.C., Zhou Y., Cui J., Zhang C.X., Chen D.F., Fowler M.R., et al. (2008): Genetic approaches to sustainable pest management in sugar beet (*Beta vulgaris*). *Annals of Applied Biology*, 152: 143–156.

Received: January 12, 2024

Accepted: April 30, 2024

Published online: July 1, 2024