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Comparison of Chemical and Biological Wireworm Control Options in Serbian Sunflower Fields and a Proposition for a Refined Wireworm Damage Assessment

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Abstract: Recent European Union regulations aim at reducing the use of chemical pesticides in agriculture. In sunflower production in Serbia, the use of synthetic insecticides for soil and seed treatments has become a prevailing practice for wireworm (Coleoptera: Elateridae) control. However, a number of compounds efficiently used as seed treatments have been phased out. This work aimed at assessing the potential of an environmentally friendly "Attract and Kill" strategy (A&K) for controlling wireworms in sunflower in comparison to conventional insecticides. The experiments were carried out in 2018, 2019 and 2021 involving (a) soil treatments with ATTRACAP® and a Force 1.5 G; (b) seed treatments with Sonido, Force 20 CS, Lumiposa and Buteo Start 480 FS; (c) controls (i.e., untreated seeds). The efficacy of the treatments was assessed based on the plant density and emerging plant damage (%). A damage rating scale (levels 0–5) was created, aiming for a more reliable and concrete interpretation of the results. Data were processed using binomial and multinomial regressions, followed by modelling of the damage and calculating the odds of damage occurrence, depending on the applied insecticide. In all experimental years and at all localities, the Force 20 CS and/or Force 1.5 G treatments resulted in the highest plant density and the lowest percentage of plant damage. ATTRACAP® showed good effectiveness, since plant density and percentage of damaged plants were at the same level of significance as the commonly used conventional insecticides in sunflower production (Sonido for seed treatment and Force 1.5 G for soil treatment). Thus, the A&K strategy was efficient in controlling wireworms at conditions of low abundance, based on three-year experimental results. Although the damage rating scale enabled a clear differentiation of plant damage caused by wireworms responsible for reduced plant density, more reliable models were obtained by binomial regressions, classifying plants as damaged or undamaged.

Keywords: "Attract and Kill" strategy; ATTRACAP®; *Metarhizium brunneum*; CO_2 attraction; Elateridae; binomial regression

1. Introduction

Sunflower production is often limited by a number of harmful insects, with wireworms (i.e., larvae of different species of the Coleopteran family Elateridae) being one of the economically most important and widespread soil-dwelling pests in all cropping systems [1–3]. Due to the fact of their polyphagous feeding mode, wireworms are specifically destructive for both cultivated and wild plant species including weeds [3–5]. In major arable crops, such as potato, maize and sunflower, these pests often cause severe economic damage across Europe and North America [6], while in Serbia, the highest damage is recorded in maize, sunflower and sugar beet [7,8]. High infestation levels may lead to obligatory re-sowing [9]. Thus, as mentioned by Hanitzsch et al. (2015) [10], research efforts aiming to develop successful strategies for controlling these pests have significantly increased over the last few decades.

Sunflower plants are most susceptible to wireworm attack during the early stages of development. Thus, prevention of damage occurrence is crucial at this stage. The use of chemical pesticides, especially for seed treatments, has become an important and inevitable cultivating practice for a number of reasons [11]. Different classes of chemical compounds were used over the past two decades, including pyrethroids, phenyl pyrazoles, and neonicotinoids [12], most of which function via pest repellence rather than resulting in the death of the wireworm larvae [13,14]. In many regions of the world, including Serbia, the use of insecticides as soil and seed treatments was thus the predominant practice of wireworm control [15,16]. However, the negative effects on bees and other pollinators [17], aquatic invertebrates and fish [18], beneficial spiders and mites [19] as well as social awareness of the harmful effects of chemical pesticides on the environment and human health have increased [20–22], initiating legislative changes at the EU level. In this regard, the European Union issued the Directive 2009/128/EC [23], supporting integrated pest management (IPM) and promoting sustainable agricultural production systems and the use of biorational alternatives to synthetic pesticides including biological control. This directive also made the implementation of integrated pest management principles obligatory and banned and/or restricted different chemical compounds with proven side effects, e.g., neonicotinoids for their severe impact on pollinators [24,25]. Moratoriums imposed by many countries on neonicotinoid seed treatments as well as restrictions and deregistration of several active substances (in Serbia: neonicotinoids and fipronil in 2014 and bifenthrin in 2018), initiated the search for alternative environmentally friendly solutions for wireworm pest control. Innovative biorational wireworm control strategies are stipulated by two European Union (EU) directives: European Commission regulation No 1107/2009 [26] and Directive 2009/128/EC [23]. These strategies aim at reducing wireworm densities below the economic threshold and require a combination of agronomic practices with a partial impact and can be achieved through long-term management [5,27]. One of the promising alternatives is the use of entomopathogenic fungi (EPFs) as environmentally favourable microbiological control agents [28–31]. Several naturally occurring soil inhabiting microorganisms, such as species from the genera *Metarhizium* and *Beauveria*, have been well studied and proven to be effective against wireworms [32–39]. However, a challenge remains: how to enhance the effectiveness of these entomopathogens? The development of an "Attract and Kill" strategy (A&K) that involves the combination of an attracting compound that lures the wireworms and a killing agent was described comprehensively in [5]. Carbon dioxide (CO_2) is a well-known attractant for wireworms [40,41] and other soil-dwelling insects [42–44], because plant roots, while growing, continuously emit CO_2, thus establishing a gradient for wireworm orientation towards this food source. Providing an alternative CO_2 source (i.e., the "Attract" component) combined with a "Kill" component could become the solution for efficient wireworm control, especially since insecticides in susceptible crops, such as sunflower, are on the brink of being phased out. The development of a refined "Attract and Kill" strategy to control wireworm infestations could thus contribute to reduced pesticide inputs in sustainable agricultural production systems and could offer potential savings for growers [45,46].

Although experiments for assessing insecticides' efficacy for wireworm control were defined by the European and Mediterranean Plant Protection Organization (EPPO) standards PP 1/46 (3) [47], in a set of field trials, we noticed that it was difficult to precisely define the differences between the efficacy of different insecticide treatments because of high data variability. Seed germination is influenced by many biotic and abiotic factors, and assessing only plant density (plant stand), especially on fields with low wireworm infestation, is not sufficient to provide reliable results on the treatment's efficacy. Due to the aforementioned problems, the authors here suggest introducing an additional damage assessment category by using a rating scale as a supplemental assessment for a more precise differentiation among treatments. In this work, a damage rating scale was proposed, aimed at a more reliable interpretation of the results on the efficacy of insecticides against wireworms. Its feasibility and potential for calculating the odds of wireworm damage occurrence, depending on the insecticide applied, was modelled using binomial and multinomial regressions.

The first aim of this work was to assess the efficacy and potential of an "Attract and Kill" strategy for controlling wireworms in sunflower in comparison with conventional insecticides. This strategy involved the use of the product ATTRACAP® (*Metarhizium brunneum* CB15) in relation to currently registered insecticides, insecticides waiting for approval (Buteo Start 480 FS), those registered for other crops (Lumiposa, Force 20 CS and Force 1.5 G) and the one withdrawn in Serbia (Sonido) under the conditions of low and high wireworm infestations. The second aim was to assess the reliability of the analyses of the proposed rating scale for wireworm damage compared to the standard EPPO procedure.

2. Materials and Methods

2.1. Wireworm Abundance Assessment

The number of wireworms was assessed based on the number of collected specimens in soil pits. At the beginning of spring, soil samples, according to a standard square method (50 × 50 cm, to a layer depth of approximately 40 cm), were collected (10 probes on each experimental field). The number of collected wireworms in the soil probes was multiplied by four to obtain the abundance per m^2 [47].

2.2. The Experimental Sites and Treatments

Field experiments were carried out at the Institute of Field and Vegetable Crops (IFVCNS) at Rimski šančevi (45°19′ N, 19°50′ E), in the vicinity of Novi Sad, Serbia, over a three-year period (2018, 2019 and 2021). Experiments were set-up using a randomised block design, according to the EPPO PP 1/46 (3) methodology [48], in five to nine replications, depending on the year and site (Table 1). The size of the basic experimental plots was 42 m^2 (10 m long, 4.2 m wide with 6 rows). In all experimental years, sunflower variety Duško (IFVCNS variety) was used. The growing technology applied in the experiments followed local agricultural practices for sunflower production. Mechanical sowing was performed with a row-to-row distance of 70 cm and 23.5 cm spacing between plants within rows.

Table 1. Experimental sites, years and treatments.

Year	Locality	Infestation	Treatments	Application Type	Dose
2018	RŠ T-12	low	ATTRACAP® (*M. brunneum*)	soil	30 kg/ha
			Force 1.5 G (a.i. tefluthrin)	soil	5 kg/ha
2019	RŠ T-12	low	ATTRACAP® (*M. brunneum*)	soil	30 kg/ha
			Sonido (a.i. thiacloprid)	seed	25 mL/kg
			Force 1.5 G (a.i. tefluthrin)	soil	5 kg/ha
			Force 20 CS (a.i. tefluthrin)	seed	250 mL/100 kg
			Buteo Start 480 FS (a.i. flupyradifurone)	seed	11.3 mL/kg
2021	RŠ T-12	low	ATTRACAP® (*M. brunneum*)	soil	30 kg/ha
			Force 20 CS (a.i. tefluthrin)	seed	250 mL/100 kg
			Force 1.5 G (a.i. tefluthrin)	soil	5 kg/ha
			Buteo Start 480 FS (a.i. flupyradifurone)	seed	11.3 mL/kg
			Lumiposa (a.i. cyantraniliprole)	seed	0.8 l/100 kg
	RŠ Field 1	high	ATTRACAP® (*M. brunneum*)	soil	30 kg/ha
			Force 20 CS (a.i. tefluthrin)	seed	250 mL/100 kg
			Force 1.5 G (a.i. tefluthrin)	soil	5 kg/ha
			Buteo Start 480 FS (a.i. flupyradifurone)	seed	11.3 mL/kg
			Lumiposa (a.i. cyantraniliprole)	seed	0.8 L/100 kg
			Sonido (a.i. thiacloprid)	seed	25 mL/kg

RŠ—Rimski Šančevi; a.i.—active ingredient. Low: 0–1 wireworm per m^2; high: >1 wireworm per m^2.

The number of treatments varied, depending on the year and experimental site (Table 1). In 2018, there were four treatments including a control at the only one locality (RŠ T-12); in 2019, there were five treatments and the control (RŠ T-12), while in 2021, there were five treatments and a control at RŠ T-12 and six treatments and a control at RŠ Field 1. Insecticides were applied as seed treatments (i.e., Force 20 CS, Sonido and Lumiposa) using a Hege 11 machine, while soil insecticides (i.e., Force 1.5 G and ATTRACAP®) were applied manually immediately before sowing.

2.3. Field Observations

According to EPPO standard PP 1/46 (3) [48], plant density and the number of damaged plants per plot (transformed to %), were recorded at two growth stages, from the first to the fourth pairs of leaves, according to the BBCH scale at the BBCH 02 and BBCH 04/05 growth stages (Meier, 2018) [49]. These observations were carried out in all three experimental years (i.e., 2018, 2019 and 2021).

The rating of plant damage with a five-level scale was used as an additional assessment to the standardised EPPO 1/46 (3) method [48] for testing the efficacy of insecticides for wireworm control in the last experimental year (2021). We created a damage rating scale (0–5) and selected 10 plants from each plot for which damage on the roots was assessed and rated as follows:

0—Plant with no damage;
1—Hardly visible damage on the plant roots;
2—Clearly visible damage on the plant roots;
3—Severely damaged plant, recovery possible;
4—Heavily damaged plant, recovery impossible;
5—Plant totally destroyed and/or wilted.

Since damage level 5 was recorded only for a few plants, for the purpose of statistical analyses, damage levels 4 and 5 were merged. Therefore, they were referred to as damage level 4/5.

These additional assessments allowed a more precise interpretation of the efficacy of insecticides, since calculations based only on field emergence and plant density are not always sufficiently meaningful. This proposed additional rating scale allows for confirming damage caused by wireworms and affecting field emergence and/or plant density more accurately.

2.4. Data Analysis

In statistical analyses, quantitative data are presented using mean values and standard deviations. For qualitative data, frequencies and percentages were calculated. Graphically, data are presented by bar charts and line diagrams.

Repeated measures ANOVA was used to analyse statistical differences in plant density, and the percentage of plant damage between different treatments in all three years, involving two factors (main effects): the treatments and the BBCH growth stage as well as their interaction. Effect size was calculated by partial eta square. The Bonferroni pairwise comparison post hoc test was applied and homogenous groups were determined, denoted by combinations of small letters (a, b, c and d).

For analysing the feasibility of the newly created damage rating scale, as an additional observation to the standardised EPPO 1/46 (3) method [48], modelling the occurrence of damage and calculating the odds for certain damage level occurrence was performed by using binominal and multinomial regressions. In the binomial regressions, damage levels were grouped into two categories: (i) undamaged—for the 0 and 1 damage levels; (ii) damaged—for the 2–4 damage levels.

In the obtained model, the odds ratios were determined with their 95% confidence intervals. The Hosmer–Lemeshow goodness of fit test, the likelihood ratio test for multinomial regressions, the Nagelkerke R2, the ROC curve and area under the ROC curve were determined to examine the quality of the obtained model.

In the multinomial regressions, all five levels were used. Statistical analyses were performed using a trial version of the SPSS 23 statistical package. Differences at the 0.05 level of significance are denoted by * and at the 0.01 level of significance by **.

3. Results

3.1. Wireworm Abundance

At the locality RŠ T-12, during all three years (i.e., 2018, 2019 and 2021), zero or one specimen per m^2 were collected in soil samples, indicating a low wireworm abundance. However, in 2021, at the locality RŠ Field 1, three wireworm specimens per m^2 were sampled on average in the soil samples. This number indicates a high abundance of wireworms at this locality, since the threshold level for wireworms in sunflower is specified as two specimens per m^2 [47].

3.2. Plant Density

In 2018, two insecticides were applied as a soil treatment: the chemical insecticide Force 1.5 G, the prevailing standard in Serbia, and the biological insecticide ATTRACAP®.

For the plant density, repeated measures ANOVA showed that both main effects, treatment, and plant growth stage, were significant, with high effect sizes, while their interaction was not significant ($F(2,57) = 2.84$, $p = 0.067$, $\eta 2p = 0.091$; Figure 1). As the main effect of the treatment was significant ($F(2,57) = 5.97$ **, $p = 0.004$, $\eta 2p = 0.173$), a post hoc Bonferroni pairwise comparison was used to analyse the overall differences (regardless of the plant growth stage) between treatments. Significant differences in plant density were recorded between Force 1.5 G and the control ($p = 0.003$), whereas there was no significant difference between ATTRACAP® and Force 1.5 G treatments ($p = 0.124$) or between ATTRACAP® and the control ($p = 0.558$). Homogeneous groups are presented in Figure 1. However, it must be mentioned, again, that RŠ T-12 had low natural wireworm abundance.

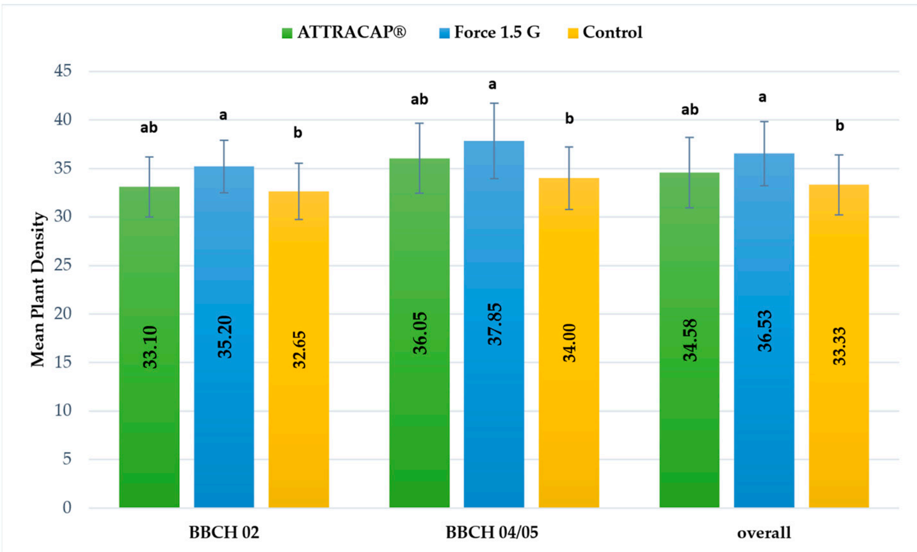

Figure 1. Plant density depending on the insecticidal treatments in 2018.

Since plant density increased over time, the effect of plant growth stage was significant ($F(1,57) = 63.28$ **, $p < 0.001$, $\eta 2p = 0.526$); thus, there was a significant difference between mean densities at the BBCH 02 and BBCH 04/05 stages (overall, regardless of treatment).

When analysing the first and the second plant growth stage (BBCH stage) separately, a significant difference in plant density was only detected between the Force 1.5 G treatment and the control at both stages ($p = 0.022$). Furthermore, for each treatment the mean values of plant density increased significantly from the BBCH 02 to BBCH 04/05 stages ($p < 0.001$).

In 2019, the experiment encompassed three insecticides applied as seed treatments (Force 20 CS, Buteo Start 480 FS and Lumiposa) and two insecticides incorporated into the soil, during the sowing (Force 1.5 G and ATTRACAP®, Dassel-Markoldendorf, Germany).

The main effect of the treatment was significant ($F (5,162) = 9.51$ **, $p < 0.001$, $\eta 2p = 0.227$). Post hoc analysis of the treatments showed that there was no statistical difference in the plant density between the chemical insecticides and ATTRACAP® (Figure 2). However, there was a significant difference in plant density between all insecticidal treatments and the control ($p < 0.001$). The same result was obtained when analysing the BBCH 02 and BBCH 04/05 stages separately. Furthermore, for all insecticidal treatments and the control, there was a highly significant increase in plant density from the BBCH 02 to BBCH 04/05 stages ($F (1,162) = 101.82$ **, $p < 0.001$, $\eta 2p = 0.386$). The highest change was observed in the Force 20 CS treatment. The interaction of the main effects was not significant ($F (5,162) = 2.23$, $p = 0.054$, $\eta 2p = 0.067$).

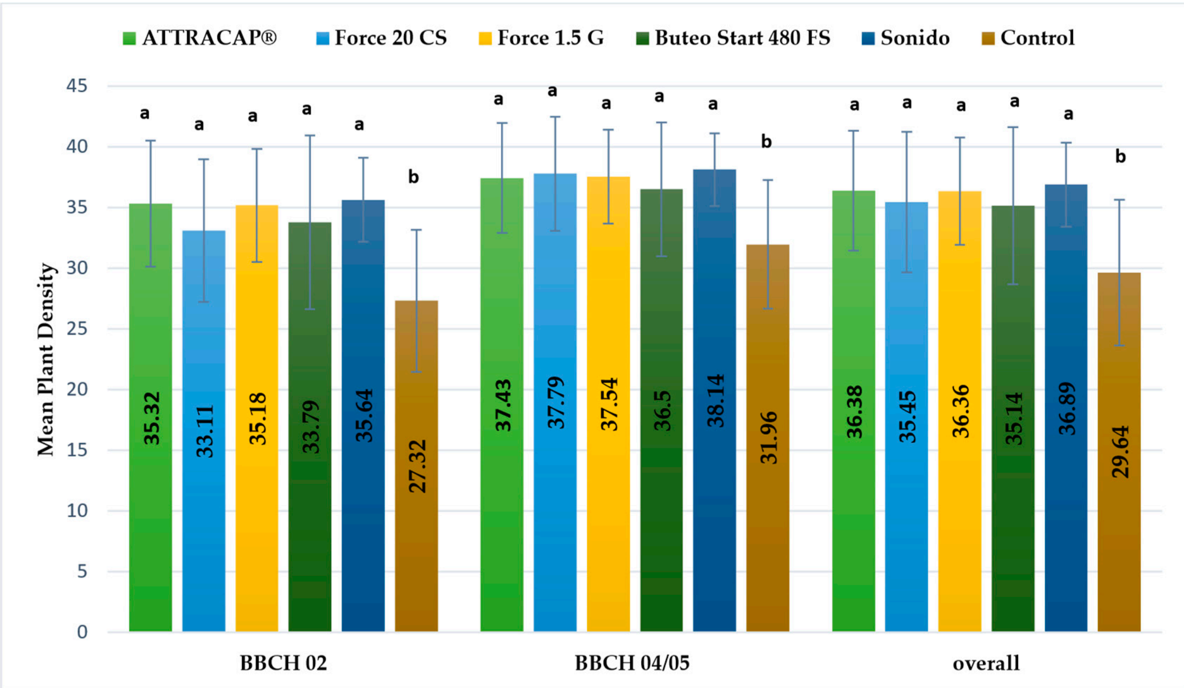

Figure 2. Plant density depending on the insecticidal treatment in 2019.

In 2021, at RŠ Field 1, the effect of the treatments was significant, ($F (5,210) = 59.17$ **, $p < 0.001$, $\eta 2p = 0.585$), regardless of the plant growth stage, and a significant difference was found between all insecticide treatments and the control ($p < 0.001$). Furthermore, the plant density significantly differed between the insecticides applied. The highest plant density was recorded in Force 20 CS and Lumiposa (no significant difference between, $p = 0.273$), and these two insecticide treatments were significantly different from the other insecticide treatments, ($p < 0.001$). In addition, there were no significant differences between ATTRACAP®, Force 1.5 G and Buteo Start 480 FS ($p = 0.899$). Differences were also observed in the first and the second BBCH phases, and the homogeneous groups are presented in Figure 3.

The increase in the plant density from the BBCH 02 to BBCH 04/05 stages was significant ($F (1,210) = 172.45$ **, $p < 0.001$, $\eta 2p = 0.451$) and was recorded for all insecticide and control treatments.

The interaction of the main effects was significant ($F (5,210) = 3.48$ **, $p = 0.005$, $\eta 2p = 0.076$) with low effect size, implying that the increase in plant density from BBCH 02 to BBCH 04/05 was not the same for all treatments. The highest increase was recorded in Buteo Start 480 FS and Force 1.5 G ($p < 0.001$). In the ATTRACAP®, Lumiposa ($p < 0.001$) and Force 20 CS ($p = 0.001$) treatments, the increase was lower.

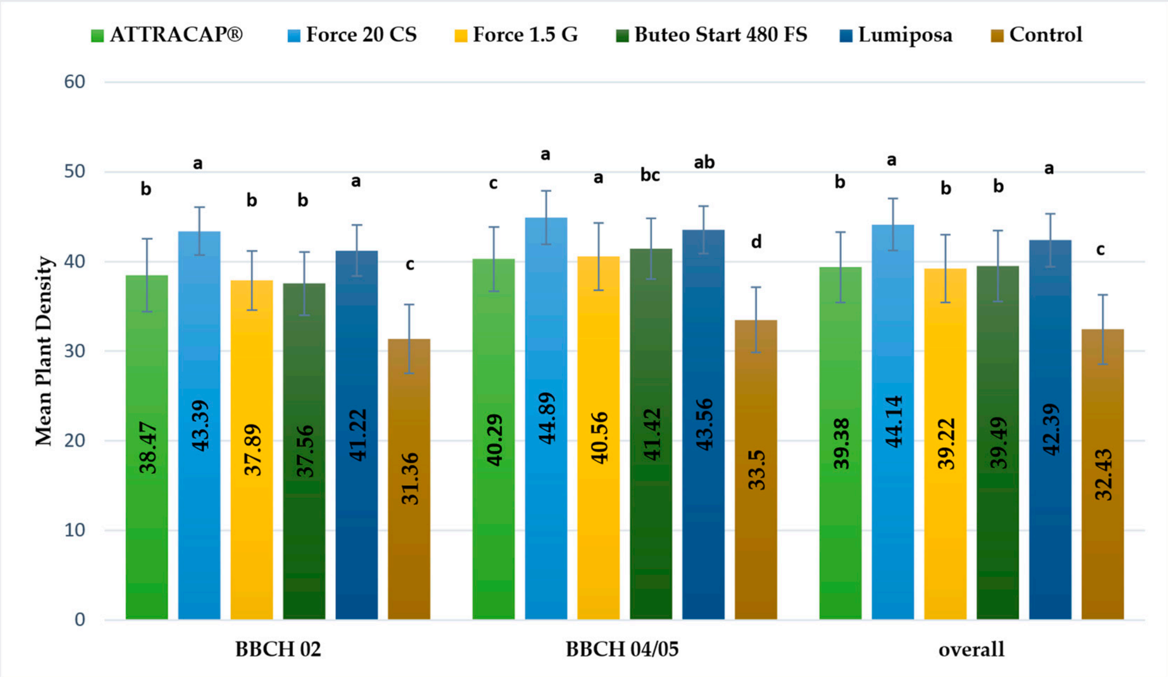

Figure 3. Plant density depending on the insecticidal treatment at RŠ Field 1 in 2021.

At RŠ T-12 in 2021, the main effect of the treatment was significant (F (6,133) = 8.35 **, $p < 0.001$, η2p = 0.274; Figure 4) for plant density. The highest was recorded when Force 20 CS or Sonido were applied. Force 20 CS was significantly different compared to the ATTRACAP® ($p < 0.001$), Buteo Start ($p = 0.003$), Force 1.5 G ($p = 0.022$) and Lumiposa ($p = 0.13$) treatments and the control ($p < 0.001$), while Sonido was significantly different from ATTRACAP® ($p = 0.007$) and the control ($p < 0.001$). Similar differences were observed in both growth stages and homogeneous groups are presented in Figure 4.

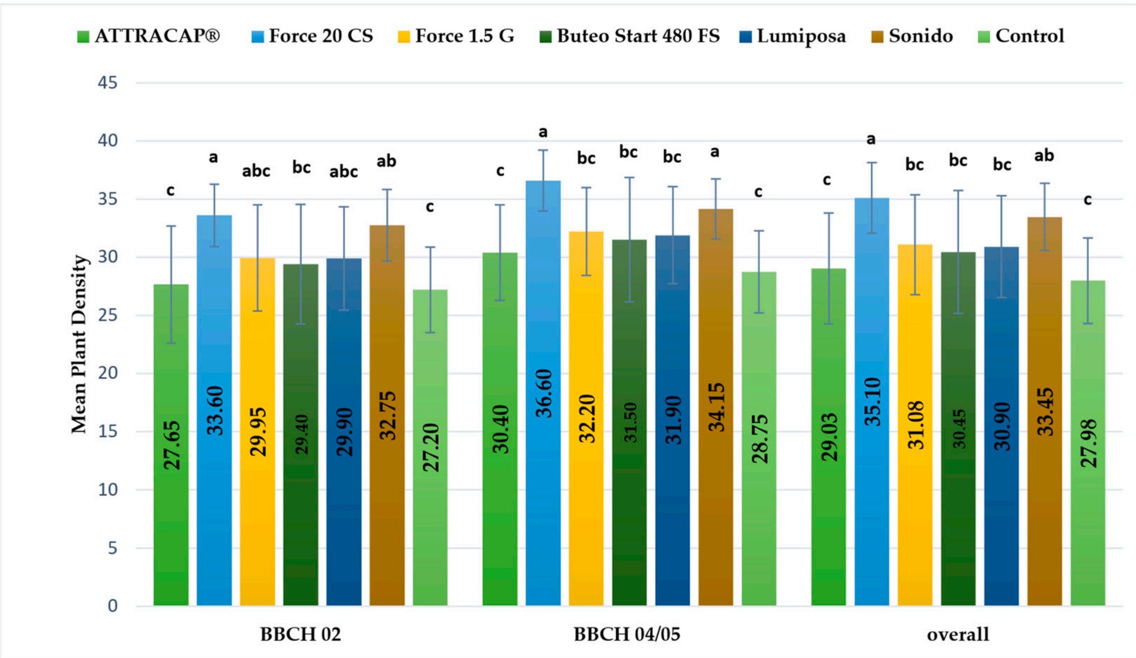

Figure 4. Plant density depending on the insecticidal treatment at RŠ T-12 in 2021.

Significant differences were recorded between plant growth stages (F (1,133) = 94.89 **, $p < 0.001$, η2p = 0.416) with a high effect size. A significant increase in plant density from BBCH 02 to BBCH 04/05 was recorded for all insecticides: ATTRACAP®, Buteo Start 480 FS, Force 1.5 G, Force 20 CS ($p < 0.001$), Lumiposa ($p = 0.001$), Sonido ($p = 0.18$) and the control ($p = 0.009$). The highest increase was found in the Force 20 CS, ATTRACAP® and Force 1.5 G treatments, respectively. The interaction of the main effects was not significantly different (F (6,133) = 0.994, $p = 0.430$, η2p = 0.043).

3.3. Plant Damage

Repeated measures ANOVA was used to analyse plant damage, with two factors: the treatment and plant growth stage. For all years and fields, the interaction between two main effects was significant, implying that the increase in plant damage from BBCH 02 to BBCH 04/05 was not the same for all treatments and depended on the treatment applied. Moreover, the main effect of the plant growth stage was always significant, as the plant damage did increase over time.

In 2018, the main effect of treatment was not significant, (F (2,12) = 1.62, $p = 0.238$, η2p = 0.213), as the percentage of damaged plants did not significantly differ between treatments. The same was observed in each of the BBCH stages (Table 2). However, the percentage of damaged plants between BBCH 02 and BBCH 04/05 differed significantly, indicating a significant effect of the plant growth stage (F (1,12) = 52.81 **, $p < 0.001$, η2p = 0.815). The interaction between the treatments and the BBCH stages was also significant (F (2,12) = 4.68 *, $p = 0.031$, η2p = 0.438), indicating that the increase in the percentage of damaged plants from the first to the second BBCH stage was not the same for all treatments. The highest increase was recorded in the control ($p < 0.001$) and Force 1.5 G ($p = 0.001$), the lowest in the ATTRACAP® treatment ($p = 0.060$), indicating a continuous protective action of this insecticide.

Table 2. Plant damage depending on the insecticidal treatment in 2018.

	Plant Damage (%)		
	BBCH 02	BBCH 04/05	Overall [1]
ATTRACAP®	1.37 ± 1.02 a	2.42 ± 1.59 a	1.90 ± 1.38 a
Force 1.5 G	0.75 ± 1.29 a	2.84 ± 1.60 a	1.79 ± 1.76 a
Control	1.88 ± 1.72 a	5.12 ± 2.85 b	3.50 ± 2.80 a
Treatment	F (2,12) = 1.62, $p = 0.238$, $\eta^2 p = 0.213$		
Growth stage	F (1,12) = 52.81 **, $p < 0.001$, $\eta^2 p = 0.815$		
Interaction	F (2,12) = 4.68 *, $p = 0.031$, $\eta^2 p = 0.438$		

[1] Regardless of growth stage; **—highly significant differences ($p < 0.01$); *—significant differences ($p < 0.05$).

In 2019, the main effect of the treatment was significant (F (5,36) = 3.78 **, $p = 0.007$, η2p = 0.344). Post hoc pairwise comparison revealed that the highest percentage of the damaged plants was recorded in the control and was significantly higher than in Force 20 CS and Force 1.5 G treatments ($p = 0.018$; Table 3). We analysed the first and the second BBCH stages separately, and the homogeneous groups are presented in Table 3. The main effect of the BBCH stage was also significant (F (1,36) = 21.15 **, $p < 0.001$, η2p = 0.370).

The interaction between the treatments and the growth stages, however, did again significantly differ (F (5,36) = 3.06 *, $p = 0.021$, η2p = 0.298). A significant increase was observed in the control ($p < 0.001$) and the Buteo Start 480 FS ($p = 0.008$) treatment, while in Sonido ($p = 0.845$), ATTRACAP® ($p = 0.073$), Force 1.5 G ($p = 0.446$) and Force 20 CS ($p = 0.464$), there was no significant increase in the percent of damaged plants.

Table 3. Plant damage depending on the insecticidal treatment in 2019.

	Plant Damage (%)		
	BBCH 02	**BBCH 04/05**	**Overall [1]**
ATTRACAP®	0.64 ± 0.69 a,b	1.09 ± 0.99 b	0.86 ± 0.86 a,b
Force 20 CS	0.22 ± 0.39 b	0.40 ± 0.58 c	0.31 ± 0.48 b
Force 1.5 G	0.22 ± 0.38 b	0.41 ± 0.56 c	0.31 ± 0.47 b
Buteo Start 480 FS	0.36 ± 0.94 a,b	1.05 ± 1.41 b	0.70 ± 1.21 a,b
Sonido	1.68 ± 1.95 a,b	1.72 ± 1.87 b	1.70 ± 1.84 a,b
Control	2.16 ± 2.05 a	3.35 ± 2.15 a	2.75 ± 2.11 a
Treatment	F (5,36) = 3.78 **, $p = 0.007$, $\eta^2 p = 0.344$		
Growth stage	F (1,36) = 21.15 **, $p < 0.001$, $\eta^2 p = 0.370$		
Interaction	F (5,36) = 3.06 *, $p = 0.021$, $\eta^2 p = 0.298$		

[1] Regardless of growth stage; **—highly significant differences ($p < 0.01$); *—significant differences ($p < 0.05$).

In 2021, at RŠ Field 1, the main effect of the treatment was significant (F (5,48) = 9.37 **, $p < 0.001$, η2p = 0.494 (Table 4). A significant difference in the percentage of damaged plants was recorded between the control and all insecticidal treatments ($p < 0.001$). However, there was neither a significant difference ($p > 0.05$) between insecticidal treatments overall nor in either of the plant growth stages observed separately. The main effect of the BBCH stage was significant (F (1,48) = 28.35 **, $p < 0.001$, η2p = 0.371).

Table 4. Plant damage depending on insecticidal treatments at RŠ Field 1 in 2021.

	Plant Damage (%)		
	BBCH 02	**BBCH 04/05**	**Overall [1]**
ATTRACAP®	0.48 ± 0.83 a	0.77 ± 1.44 a	0.63 ± 1.14 a
Force 20 CS	0.13 ± 0.26 a	0.26 ± 0.52 a	0.20 ± 0.41 a
Force 1.5 G	0.15 ± 0.30 a	0.29 ± 0.68 a	0.22 ± 0.52 a
Buteo Start 480 FS	0.00 ± 0.00 a	0.34 ± 0.46 a	0.17 ± 0.36 a
Lumiposa	0.21 ± 0.64 a	0.34 ± 0.62 a	0.28 ± 0.62 a
Control	1.33 ± 0.81 b	2.69 ± 1.26 b	2.01 ± 1.24 b
Treatment	F (5,48) = 9.37 **, $p < 0.001$, $\eta^2 p = 0.494$		
Growth stage	F (1,48) = 28.35 **, $p < 0.001$, $\eta^2 p = 0.371$		
Interaction	F (5,48) = 6.86 **, $p < 0.001$, $\eta^2 p = 0.417$		

[1] Regardless of growth stage; **—highly significant differences ($p < 0.01$).

A significant (F (5,48) = 6.86 **, $p < 0.001$, η2p = 0.417) interaction between the treatment and the growth stage was recorded. The increase in the percentage of damaged plants from the BBCH 02 to the BBCH 04/05 stages was not the same for all treatments). The highest increase was recorded for the control treatment ($p < 0.001$). The increase in the percentage of damaged plants from the first to the second growth stage was not statistically significant for any of the used treatments: ATTRACAP® ($p = 0.122$), Buteo Start ($p = 0.067$), Force 1.5 G ($p = 0.440$), Force 20 CS ($p = 0.473$) or Lumiposa ($p = 0.504$).

In 2021, at RŠ T-12 (low wireworm abundance), the main effect of treatment was significant (F (6,28) = 3.17 **, $p = 0.017$, η2p = 0.404). Homogeneous groups in both BBCH stages and overall are presented in Table 5. The main effect of the BBCH stage was significant (F (1,28) = 5.68 **, $p = 0.024$, η2p = 0.169).

Repeated measures ANOVA showed that the interaction between the main effects was marginally significant (F (6,28) = 2.344, $p = 0.058$, η2p = 0.334). For all insecticidal treatments, the percentage of damaged plants in the insecticidal treatments did not significantly differ between the BBCH 02 and BBCH 04/05 stages ($p > 0.05$), whereas in the control treatment, a significant increase in the percentage of damaged plants was recorded ($p < 0.001$).

Table 5. Plant damage depending on the insecticidal treatment at RŠ T-12 in 2021.

	Plant Damage (%)		
	BBCH 02	**BBCH 04/05**	**Overall** [1]
ATTRACAP®	1.81 ± 1.81 a	1.96 ± 1.73 a,b	1.89 ± 1.67 a,b
Force 20 CS	0.00 ± 0.00 a	0.42 ± 0.63 b	0.21 ± 0.47 b
Force 1.5 G	1.00 ± 1.31 a	0.90 ± 1.16 b	0.96 ± 1.17 b
Buteo Start 480 FS	0.63 ± 1.06 a	0.60 ± 1.00 b	0.62 ± 0.97 b
Lumiposa	0.00 ± 0.00 a	0.34 ± 0.47 b	0.17 ± 0.36 b
Sonido	0.15 ± 0.34 a	0.44 ± 0.64 b	0.29 ± 0.51 b
Control	1.14 ± 1.61 a	3.35 ± 2.67 a	2.25 ± 2.19 a
Treatment	$F\ (6{,}28) = 3.17$ **, $p = 0.017$, $\eta^2 p = 0.404$		
Growth stage	$F\ (1{,}28) = 5.68$ **, $p = 0.024$, $\eta^2 p = 0.169$		
Interaction	Not significant, $p = 0.058$		

[1] Regardless of growth stage; **—highly significant differences ($p < 0.01$).

3.4. Plant Damage Rating

3.4.1. Damage Level Depending on the Treatments Applied

Damage level was analysed using binomial regression. The percentage of undamaged and damaged plants is presented for both experimental fields and localities (Figures 5 and 6).

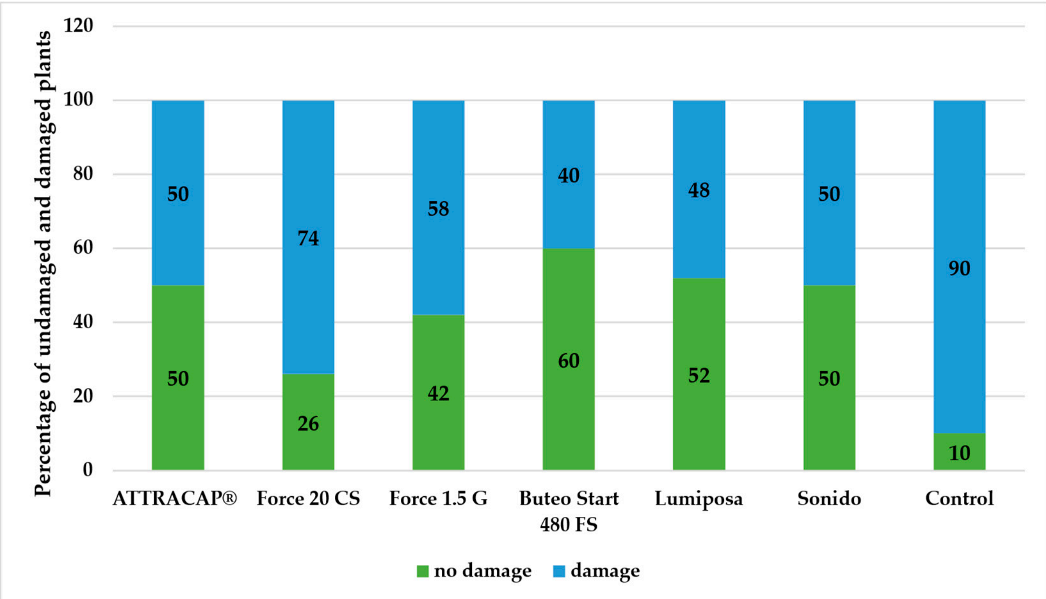

Figure 5. Percentage of undamaged and damaged plants in different insecticidal treatments in the sunflower field RŠ Field 1 in the year 2021.

At RŠ Field 1 (high wireworm abundance), the highest percentage of damaged plants was recorded in the control (90%). In the ATTRACAP® treatment (50%), the percentage of damage was equivalent to treatments Sonido and Lumiposa (50% and 48%, respectively), while in the Force 20 CS treatment, it was the lowest (26%; Figure 5).

At RŠ T-12, the highest percentage of damaged plants was, again, recorded in the control (54.4%), whereas the percentages in the ATTRACAP® treatment (17.8%) and the chemical treatments (16.7–23.3%) were at the same level, except in Buteo Start 480 FS (34.4%), which had the highest damage level (Figure 6).

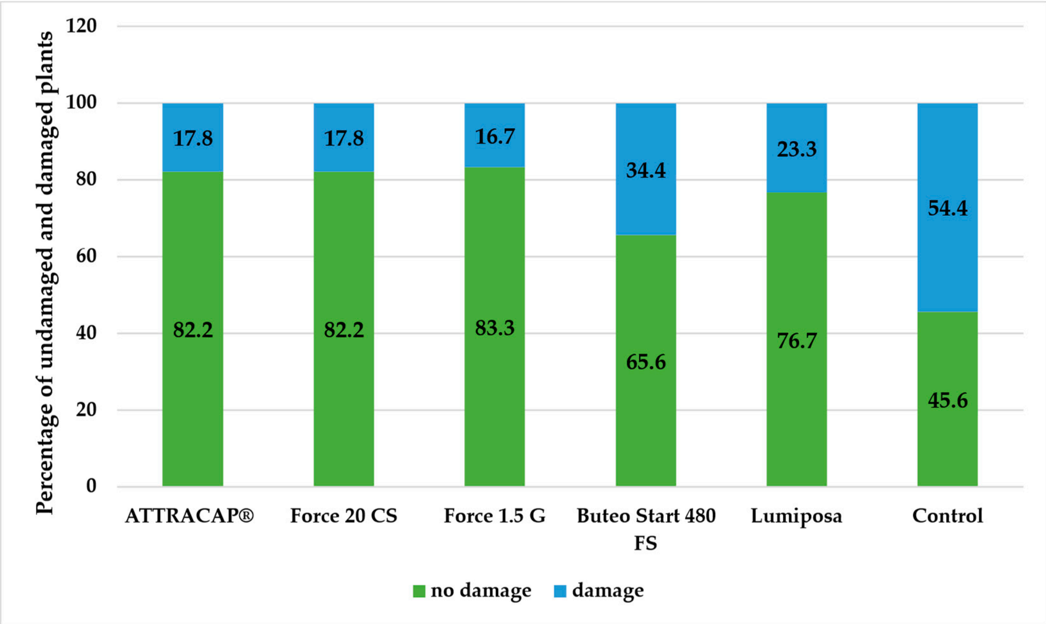

Figure 6. Percentage of undamaged and damaged plants in different insecticidal treatments in the sunflower field RŠ T-12 in the year 2021.

The percentage of the five damage levels is presented for both experimental fields: RŠ Field 1 and RŠ T-12 (Figures 7 and 8).

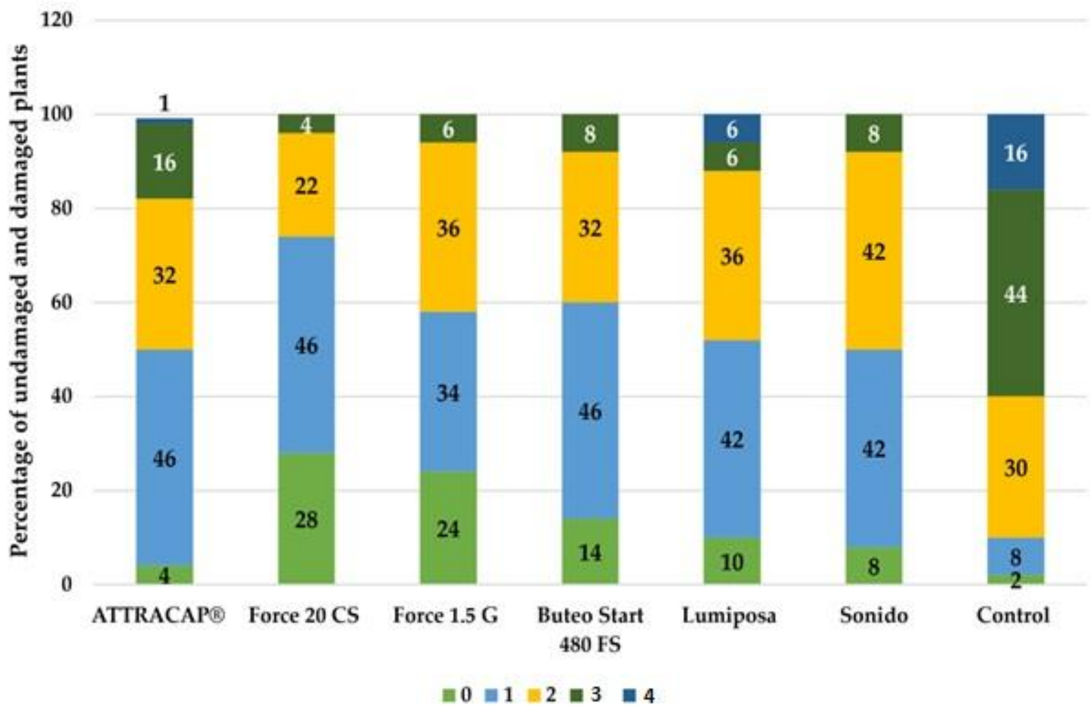

Figure 7. Percentage of plant damage levels 0-4 in different insecticidal treatments in the sunflower field RŠ Field 1 in the year 2021.

At RŠ Field 1, having high natural wireworm abundance, the highest percentage of undamaged plants (damage level 0) was recorded in the treatment with Force 20 CS (28%) and Force 1.5 G (24%), followed by Buteo Start 480 FS (14%), Lumiposa (10%), Sonido (8%) and ATTRACAP® (4%). The percentage of plant damage rated as 1 ranged from 34% to 46%

in all treatments, while in the control, it was the lowest, 8%. When specifically considering the percentage of damage rated as level 2, the ATTRACAP® (32%) provided the same level of protection as the chemical treatments (22–42%). The percentage of damage level 3 was higher in ATTRACAP® (16%) compared to the chemical insecticides (4–8%) but lower compared to the control (44%). The highest percentage of damage rated as level 4/5 was recorded in the control treatment (16%), followed by Lumiposa and ATTRACAP® (6% and 2%, respectively) (Figure 7). The abovementioned implies that regardless of the treatment, damage occurred and neither biological nor chemical insecticides can provide absolute protection from wireworms.

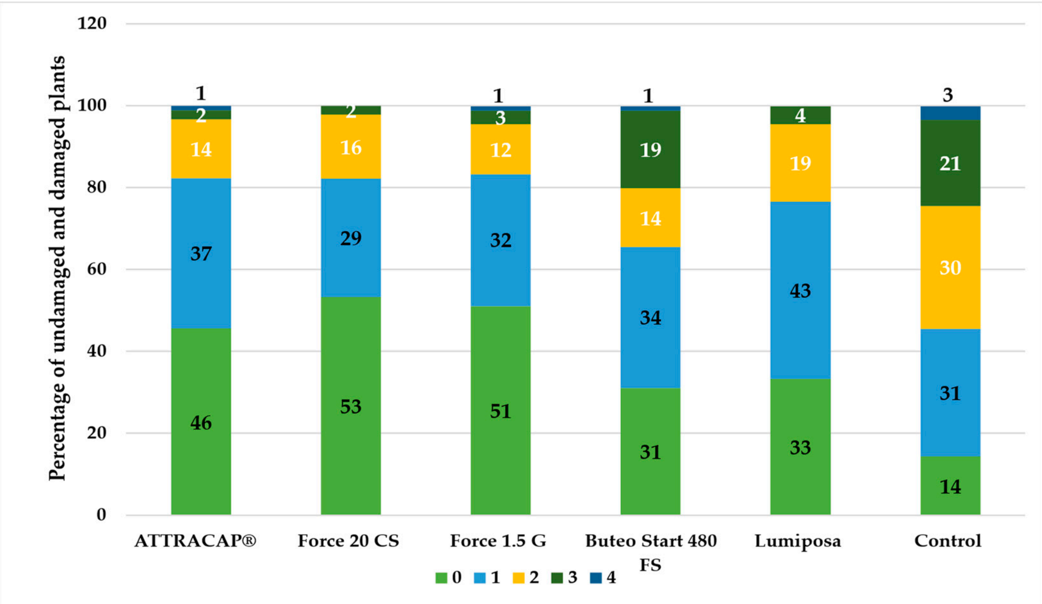

Figure 8. Percentage of plant damage levels 0–4 in different insecticidal treatments in the sunflower field RŠ T-12 in the year 2021.

At RŠ T-12, the locality with low natural infestation, the situation was slightly different (Figure 8). The best protection, based on the percentage of plants with no damage (damage level 0), was provided by the Force 20 CS (53%), Force 1.5 G (51%) and ATTRACAP® (46%), followed by Lumiposa (33%) and Buteo Start 480 FS (31%). In the group rated as damage level 1, the treatments were (sorted from highest to lowest): Lumiposa (43%) > ATTRACAP® (37%) > Buteo Start 480 FS (34%) > Force 1.5 G (32%) > control (31%) > Force 20 CS (29%). The percentage of plant damage rated as 2 was the highest in the control (30%) followed by other treatments, sorted from highest to lowest: Lumiposa (19%) > Force 20 CS (16%) > ATTRACAP®/Buteo Start 480 FS (14%) > Force 1.5 G (12%). The percentage of plants rated as damage level 3 was the highest in the control (21%) followed by the Buteo Start 480 FS (19%), while in the ATTRACAP® treatment and others (2–8%). The highest relative percentage of the plant damage rated as 4 was found in the control (3%), followed by the ATTRACAP®, Force 1.5 G and Buteo Start 480 FS (2% in all three treatments). The occurrence of higher damage levels (3 and 4/5) in the ATTRACAP® treatment was the same as in the Force 20 SC and Force 1.5 G treatments, implying a protective role against wireworms.

3.4.2. Binary Logistic Regression

A binary logistic regression was performed for modelling the occurrence of damage as a dependent variable with independent variables being locality and treatment (Table 6). For this purpose, the damage level ratings were simplified and divided in two groups: undamaged (including levels 0–1) and damaged (including levels 2–4) plants.

Table 6. Odds of plant damage occurrence compared to the control, depending on insecticidal treatments.

	B	OR Exp (B)	*p*-Value	95% CI	1/OR
Locality	1.061	2.890	0.000	(2.109, 3.961)	-
ATTRACAP®	−1.695	0.184	0.000	(0.109, 0.310)	5.4
Force 20 CS	−2.181	0.113	0.000	(0.065, 0.197)	8.8
Force 1.5 G	−1.885	0.152	0.000	(0.089, 0.259)	6.6
Buteo Start 480 FS	−1.350	0.259	0.000	(0.156, 0.431)	3.9
Lumiposa	−1.553	0.212	0.000	(0.126, 0.355)	4.7
Sonido	−1.445	0.236	0.000	(0.117, 0.457)	4.2
Constant	0.384	1.468	0.042	-	-

OR—odds ratio; 95%CI—95% confidence interval for OR; 1/OR—reciprocal value of OR.

Locality had a significant influence on the occurrence of wireworm damage on the sunflower plants, since the coefficient for the variable locality was positive and significantly different from zero ($p < 0.001$), with RŠ T-12 taken as the reference category. The odds ratio (OR) (RŠ Field 1) = 2.89, which implied that the odds for the occurrence of damage on RŠ Field 1 was 2.89 times higher than on field RŠ T-12. Considering that the natural wireworm abundance was much higher at RŠ Field 1, the obtained results were close to the real conditions.

The results of the binary logistic regression also confirmed that the application of all insecticides had an influence on the occurrence of damage, taking the control as the reference category. Since the coefficients for all treatments were negative and significantly different from zero ($p < 0.001$), all insecticides reduced the odds of plant damage occurrence compared to the control. The odds ratio for the ATTRACAP®: OR (Attracap) = 0.184, which implied that the odds for the occurrence of damage using ATTRACAP® were 81.6% (0.816 = −0.184) lower compared to the control, where no insecticide was applied. The odds of damage occurrence in the control were the highest (8.8 and 6.6 times, respectively) compared to the odds in the Force 20 SC and Force 1.5 G treatments.

The Hosmer–Lemeshow test of goodness of fit test ($\chi 2$ (8) = 9.627, $p = 0.292$) indicates that the obtained model adequately described the data. The model explained 18.7% (Nagelkerke R2 = 0.187) of the variance occurrence of damage and correctly classified 68.2% of cases.

To check the model quality, a receiver operating characteristic curve (ROC curve) was constructed (Figure 9). The area under the ROC curve was 0.711, with 95% CI (0.675, 0.746; $p < 0.001$), demonstrating a 71.1% chance that the model could distinguish between damaged and undamaged plants.

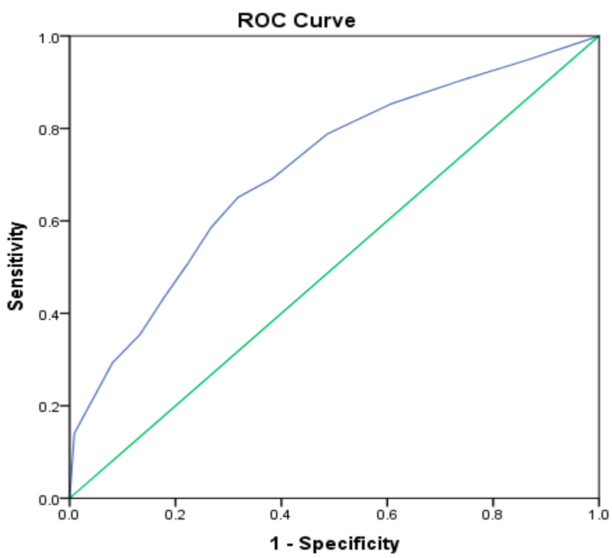

Figure 9. ROC curve for the model of plant damage occurrence in binomial regression.

3.4.3. Multinomial Logistic Regression

The multinomial logistic regression was used to model the influence of the locality and treatment on the occurrence of all five damage levels. As a reference category for dependant variable, level 0 was taken. For all damage levels, the control was used as the reference category for the variable treatment, and the field RŠ T-12 was used as the reference category for the variable locality. The coefficients for both independent variables were statistically different from zero; therefore, they had an influence on the occurrence of different damage levels (Table 7).

Table 7. Results of the multinomial regression for modelling wireworm damage levels depending on the locality and treatment.

Damage Level	Variables	B	OR = Exp(B)	*p*-Value	95% CI	1/OR
Level 1	locality	1.171	3.225	0.000	(2.121, 4.889)	
	ATTRACAP® 9	−0.662	0.516	0.084	(0.243, 1.093)	1.94
	Force 20 CS	−1.214	0.297	0.001	(0.141, 0.624)	3.37
	Force 1.5 G	−1.193	0.303	0.002	(0.144, 0.641)	3.30
	Buteo Start 480 FS	−0.470	0.625	0.228	(0.291, 1.343)	1.60
	Lumiposa	−0.369	0.691	0.342	(0.323, 1.479)	1.45
	Sonido	−0.135	0.873	0.837	(0.240, 3.179)	1.14
	Constant	0.487	-	0.405	-	-
	Locality	1.744	5.719	0.000	(3.633, 9.005)	
Level 2	ATTRACAP®	−1.661	0.19	0.000	(0.086, 0.418)	5.26
	Force 20 CS	−2.261	0.104	0.000	(0.047, 0.230)	9.61
	Force 1.5 G	−2.017	0.133	0.000	(0.061, 0.289)	7.52
	Buteo Start 480 FS	−1.419	0.242	0.000	(0.109, 0.538)	4.13
	Lumiposa	−1.238	0.29	0.002	(0.132, 0.636)	3.45
	Sonido	−0.792	0.453	0.228	(0.125, 1.644)	2.21
	Constant	−0.086	-	0.885	-	-
	Locality	1.797	6.032	0.000	(3.386, 10.744)	
Level 3	ATTRACAP®	−2.709	0.067	0.000	(0.026, 0.170)	14.92
	Force 20 CS	−4.079	0.017	0.000	(0.005, 0.056)	58.82
	Force 1.5 G	−3.577	0.028	0.000	(0.010, 0.081)	35.71
	Buteo Start 480 FS	−1.723	0.179	0.000	(0.078, 0.411)	5.59
	Lumiposa	−2.829	0.059	0.000	(0.021, 0.166)	16.97
	Sonido	−2.458	0.086	0.002	(0.018, 0.416)	11.63
	Constant	−1.797	-	0.019	-	-
	Locality	2.693	14.776	0.000	(5.022, 43.477)	
Level 4/5	ATTRACAP®	−3.113	0.044	0.000	(0.008, 0.233)	22.73
	Force 20 CS *	-	-	-	-	-
	Force 1.5 G	−4.194	0.015	0.000	(0.002, 0.131)	66.67
	Buteo Start 480 FS	−3.541	0.029	0.001	(0.003, 0.252)	34.48
	Lumiposa	−2.453	0.086	0.001	(0.020, 0.369)	11.63
	Sonido *	-	-	-	-	-
	Constant	−22.239	-	0.000	-	-

* No level 4/5 damages were recorded in treatments with Force 20 CS and Sonido. OR—odds ratio; 95% CI—95% confidence interval for OR; 1/OR—reciprocal value of OR.

Observing the influence of the locality on the plant damage level, the coefficients for all treatments were significantly different from zero and the corresponding p-values were less than 0.001, implying that the locality had an important influence on the damage levels.

When analysing locality RŠ Field 1, the odds for the occurrence of hardly visible damage on plant roots (damage level 1), compared to the undamaged plants, was 3.23 times higher when compared to the odds at field RŠ T-12, given that the odds ratio was OR = 3.23. When using the treatment as a variable, Force 20 CS and Force 1.5 G were the most effective, since the odds of the occurrence of hardly visible damages (level 1) were 3.37 and 3.30 times higher for the control. In the case of ATTRACAP®, the odds were 1.94 higher for the control.

The least effective treatment was Sonido, with the corresponding odds ratio being not significantly different from 1 (Table 7).

The odds of the occurrence of clearly visible damage on plant roots (level 2) at RŠ Field 1 were 5.72 times higher than at RŠ T-12 (OR (Field 1) = 5.72). Force 20 CS proved to be the most effective treatment with the odds of the occurrence of clearly visible damages (level 2) for the control being 9.61 times higher (OR (Force 20 CS) = 9.61). The other insecticide treatments reduced the odds of visible damages in the following order: Force 1.5 G (7.52 times), ATTRACAP® (5.26), Buteo Start 480 FS (4.13), Lumiposa (3.45) and Sonido (2.21 times).

The odds of the occurrence of visibly damaged plants having a chance to recover (damage level 3) compared to the control group, at the locality RŠ Field 1, were 6.03 times higher than at RŠ T-12. Force 20 CS proved to be the most efficient treatment, because the odds (OR = 58.8) were 58.8 times higher compared to the control treatment. Force 1.5 G (35.7 times), Lumiposa (16.9 times), ATTRACAP® (14.9 times) and Sonido (11.6 times), respectively, were ranked thereafter. Buteo Start 480 FS had the lowest efficiency in reducing the odds of the level 3 damage (5.6 times) occurrence.

The odds of the occurrence of heavily damaged plant with no chance for recovery (damage level 4/5), at RŠ Field 1 were 14.78 times higher than at RŠ T-12. As there were no level 4 damages in the Force 20 CS treatment, a large negative coefficient was obtained. This finding implies that the occurrence of complete plant damage would be many times lower when using the Force 20 CS than in the control group. Among the other treatments, Force 1.5 G (66.67), Buteo Start 480, FS 480 FS (34.48) and ATTRACAP® (22.73 times) were the most effective when compared to the untreated plants. Lumiposa (11.63) was the least efficient.

The likelihood ratio test in the multinomial regression showed that the logistic regression model was significant ($\chi2$ (4) = 239.1, $p < 0.0005$). The model explained 25.1% (Nagelkerke R2) of the variance of the damage occurrence. The percentage of correctly classified cases was 42.9%.

To check the model quality, ROC curves were constructed for each damage level (Table 8). The model was good at predicting each damage level, since p-values for each level were less than 0.001. The area under curve (AUC) indicated the extent at which the model would be able to distinguish the damage level from the other levels. The proposed model was able to distinguish damage level 0 from the other levels (1–4/5) in 73.7% of cases; damage level 1 from levels 0, 2, 3 and 4/5 in 58.7% of cases; level 2 from levels 0, 1, 3 and 4/5 in 63.7% of cases; level 3 from damage levels 0, 1, 2 and 4/5 in 75.7% of cases and damage level 4 from levels 0–3 in 82.6% of cases, respectively.

Table 8. Area under ROC curves.

Damage Level	AUC	95%CI	*p*-Value
0	0.737	(0.703, 0.772)	$p < 0.001$
1	0.587	(0.548, 0.625)	$p < 0.001$
2	0.637	(0.594, 0.680)	$p < 0.001$
3	0.757	(0.705, 0.810)	$p < 0.001$
4/5	0.826	(0.725, 0.927)	$p < 0.001$

4. Discussion

Under the Directive 128/2009, a number of efficient chemical compounds have been banned or are being phased out, leaving major crops without adequate protection from wireworms. The recently developed "Attract and Kill strategy", resulting in the product ATTRACAP®, was tested as an alternative biological control strategy for controlling wireworms in sunflower cropping systems, in comparison to currently available chemical insecticides used against these pests. Under the conditions of low wireworm abundance, ATTRACAP® exhibited good performance, comparable to the chemical pesticides used (i.e., Force 20 CS, Force 1.5 G, Sonido, Lumiposa and Buteo Start 480 FS), depending on the year, based on the plant density and percent of plant damage. Plant density and percent of plant damage did not differ between pesticides applied but were significantly different from the control at the RŠ T-12 field with low wireworm abundance. However, in 2021, when an additional locality was introduced (RŠ Field 1, high wireworm abundance), ATTRACAP® performed similar to Sonido and Lumiposa, regarding the percent of plant damage, while Force 20 CS and Force 1.5 G, in general, performed better.

4.1. Plant Density

The toxicity and efficacy of insecticides used to control wireworms is usually deduced from the protection of plant density rather than from directly observed insect responses and mortality [50]. A number of papers describe the efficacy (based on the plant density) of insecticides belonging to the groups of organophosphates, pyrethroids, neonicotinoids and diamides, while only a few so far refer to the "A&K" strategy and the use of different EPFs against wireworms, mostly in maize and potato crops [13,37,50,51]. This study presents the first report on the field performance of ATTRACAP® within the "A&K" strategy in comparison with the abovementioned insecticides in a sunflower cropping system.

In general, in our work, the tefluthrin-based insecticide Force 20 CS (pyrethroid group) was the best performing, since the highest plant density was recorded using this compound, regardless of the year and experimental locality. Van Herk et al. (2015) [50] reported a considerable repellence of wireworms when exposed to a tefluthrin-based insecticide under laboratory conditions as well as the absence of mortality. This is partially in accordance with our results, since plant density was the highest in the treatments where Force 20 CS and Force 1.5 G were used, although we did not monitor the wireworm mortality in this work.

A thiacloprid-based insecticide, Sonido (neonicotinoid group), provided a good protection of sunflower against wireworms, based on a high plant density in two of the experimental years (i.e., 2019 and 2021). Similar results were presented by Vernon et al. (2009) [14] reporting that some neonicotinoids (i.e., thiamethoxam or clothianidin) enabled excellent protection of plant density in wheat against these pest species. The role of neonicotinoids in pest control was comprehensively reviewed by Furlan et al. (2021) [52], concluding that these systemic compounds provide a good protection of different crops against wireworms, as was proven in our work for thiacloprid in sunflower as well. However, when it comes to insecticide efficacy it must be remembered that the exposure to neonicotinoid insecticides generally induces prolonged and reversible morbidity during which wireworms do not feed [53]; hence, they may protect plants from feeding damage without decreasing wireworm population densities [14,54].

A chlorantraniliprole-based insecticide, Lumiposa (diamide group), also showed a good protective potential against wireworms, based on the plant density. According to Leinfelder-Miles (2014) [55], the insecticide Lumivia™ 500 (a.i. chlorantraniliprole) enabled high plant density in wheat, which is in accordance with our results. In a review from 2021, Vojvodić, and Bažok (2021) [56] suggested that insecticides from the group of diamides should be used as a protective method to control pests during early plant growth based on their good performance in the field, as was proven in this work. However, data from van Herk et al. (2015) [50] imply the opposite. Namely, seed treatments with chlorantraniliprole did not exhibit satisfactory efficacy with regard to wireworm control in their field experiments, while in our study the treatment with Lumiposa resulted in plant densities in the same range as of the best performing Force 20 CS at both experimental sites in 2021.

Recently, several studies on the efficacy of EPFs as biological control agents targeting wireworms have been published [51,57,58]. In addition, the "A&K" strategy using EPFs as biological control agents is well reported in potatoes [5,36,37,51,58]; however, there are no published data for sunflower crops so far. In our work, in the ATTRACAP® treatment, the plant density was at the same level as in chemical treatments in 2019 (Force 20 CS, Force 1.5 G, Buteo Start 480 FS and Sonido) and 2021 (Force 1.5 G and Buteo Start 480 FS) at RŠ Field 1, which is to mention, in particular, since this was the field with high wireworm abundance. Only in 2018 was the performance of ATTRACAP® not satisfactory, since the plant density achieved was between Force 1.5 G and the control. However, we speculate that due to the low wireworm abundance at this field, their attack was also very low; hence, the differences were not significant. Several studies [59–61] claim that control efficacies achieved by biological control measures are not sufficiently high compared to the efficacy of chemical insecticides (carbosulfan, fonofos, findane, fipronil, imidacloprid, thiamethoxam or bifenthrin); however, our results imply that this is not necessarily the case, as demonstrated in our field studies.

4.2. Plant Damage

In our field trials in 2018, 2019 and 2021 (both localities), plant damage was the lowest in both Force treatments (a.i. tefluthrine). This is in accordance with the results of van Herk et al. (2008, 2015) [13,50], who reported that pyrethroid insecticides, such as tefluthrin, protect wheat and potatoes from wireworm feeding. However, these authors emphasize that tefluthrin acts by repelling wireworms, not by reducing populations, which was not studied in our work.

In all three experimental years, the percent of damaged plants in treatments with Lumiposa (a.i. chlorantraniliprole) was at the same level as of other insecticides. These findings are quite opposite to the one presented by Leinfelder-Miles (2014) [55]. Namely, this author reports that the chlorantraniliprole-based insecticide, Lumivia™ 500, provided good protection but only in combination with several other insecticides, while when applied alone, it performed similarly to the untreated control.

The ATTRACAP® treatments provided satisfactory protection of sunflower plants based on percentage of damaged plants in the second assessment (BBCH 04/05), throughout the entire experimental period (2018, 2019 and 2021). Depending on the experimental year, the percentage of damaged plants in the ATTRACAP® treatment was either on the same level as in other chemical treatments or higher but significantly lower than in the untreated control. The percent of damaged sunflower plants was proven to be dependent on the applied treatment as already reported by Gvozdenac et al. (2019) [31], which is in accordance with our results. In a 2-year study, Brandl et al. (2017a) [37] applied the "A&K" strategy using ATTRACAP® against wireworms in organic potato production systems in Lower Saxony, Germany. An application of granules within the potato rows reduced wireworm tuber damage by 37–75% in comparison to the untreated control demonstrating the feasibility of this strategy in the field. These results are supportive to ours, where the level of plant damage was reduced when the ATTRACAP® was applied as a soil insecticide. The implementation of the "A&K" strategy enhanced *M. brunneum* efficacy and significantly reduced wireworm damage but was dependent on the time and type of application [51].

Factors, such as temperature, exposure time, conidia soil concentration, and food availability, also affect mortality rates of wireworms [33,38,62] and the efficacy of EPFs as well as "A&K" strategy. While lower temperatures slow down the spread of wireworm infection by EPFs [33], drought conditions usually experienced during summer might affect the viability of EPF isolates in the soil. There are several other constraints in applying EPFs. The temperature conditions under which EPFs are applied must be in optimum for good efficacy of the EPFs as a biocontrol agent [63,64]. For early season applications, as in our experiments (optimal sowing date for sunflower under growing conditions in Serbia), a fungal isolate adapted to lower temperatures should be used [65]. In our work, the performance of ATTRACAP® differed between the years and localities, confirming the previously mentioned constraining parameters. Several studies [36,37,66–70] show that the application pattern is another important point to consider, with banded or spot applications being particularly useful under certain conditions.

4.3. New Approach—Introducing Damage Rating Scale

IPM procedures for efficient wireworm management (including damage thresholds) in Europe already exists [71] as well as a standardised method for assessing the efficacy of insecticides (EPPO PP 1/46 (3) [48]. This EPPO method determines the efficacy of an insecticide (chemical or biological) mostly based on plant density. However, the plant density is influenced by certain biotic (presence and abundance of soil dwelling pests, seed germination rates and seed pathogens) or abiotic factors (soil humidity during seed emergence, inadequate soil preparation, nutrient levels, etc). That is why plant density reduction is not necessarily caused by wireworm feeding activity. The method, recommended so far, is therefore not suitable to differentiate the underlying causal factors of plant damage and/or lower plant density. In this work, we proposed the implementation of a damage rating scale, based on different levels of damage, specifically caused by wireworm feeding activity. This newly developed approach provides a higher level of reliability of assessments in field trial protocols allowing determination of the long-term and short-term effects of any insecticide treatments or combinations on wireworm control. Additionally, the results of these observations, namely, the incidence of different treatment damage levels, enable modelling of the damage occurrence odds, depending on the applied control treatment. The proposed damage rating scale thus makes possible a clear differentiation of plant damage caused by wireworms responsible for reduced plant densities. A similar scale was introduced by Milovac et al. (2017) [72] as an additional and more precise assessment for differentiating treatment efficacies in oilseed rape stem weevils' control.

When discussing insecticide treatment efficacy, it needs to be considered that exposure to some groups of insecticides, for example, neonicotinoids, can elicit a repellent activity rather than cause mortality, thus protecting plants from feeding damage without decreasing wireworm populations [14,53,54]. The findings of these studies explain, to some extent, why plant density protection is reported without wireworm population reduction. These findings fully support our proposal to introduce a method that enables clear differentiation if plants have signs of feeding activity or not, which then can be distinguished from other damages.

5. Conclusions

The newly created damage rating scale enabled more precise and relevant assessment of wireworm damages on sunflower plants. As an addition to estimations such as plant density and percent of plant damage, it allowed undoubtable decision making on the insecticide performance against wireworms.

Modelling wireworm damage using binomial and multinomial regression provided valuable information about the odds of damage occurrence on certain localities depending on the insecticides applied. This information is useful for future choices of insecticides to be used in controlling these pests. The binomial regression approach (undamaged and damaged plants) provided more reliable results, in comparison to multinomial (five damage levels). Therefore, further bioassays are needed in this regard, providing more estimates and data for this analysis to work properly and being reliable under different field conditions.

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