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# Innovative "Attract & kill" strategy for controlling wireworms in sunflower

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# BACKGROUND

# IT IS ALL ABOUT WIREWORMS

- ✓ Wireworms, larvae of Coleopteran family Elateridae
- ✓ One of the most DEVASTATING and economically most important soil-dwelling pests of all row crops – SUNFLOWER


**WHY?**

A. High infestation levels may lead to obligatory re-sowing

B. Lack of efficient insecticides registered for seed and soil treatmetns

The use of insecticides as soil and seed treatments has been predominant practice for wireworm control in many regions of the world, including Serbia

- ✓ Negative effects (pollinators, aquatic invertebrates and fish, beneficial species, the environment and human health) have initiated legislative changes at the EU level.

- ✓ European Union issued the Directive 2009/128/EC, supporting IPM and promoting sustainable and biorational alternatives to synthetic pesticides including biological control.

- ✓ EU banned and/or restricted a number of chemical compounds

- ✓ Moratoriums imposed by many countries on neonicotinoid seed treatments as well as restrictions 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 involve the use of entomopathogenic fungi (EPFs) as environmentally friendly control agents.
- ✓ Naturally occurring soil microorganisms: genera Metarhizium and Beauveria - well studied and proven to be effective against wireworms

Achallenge remains: HOW TO ENHANCE the efficacy of these entomopathogens?

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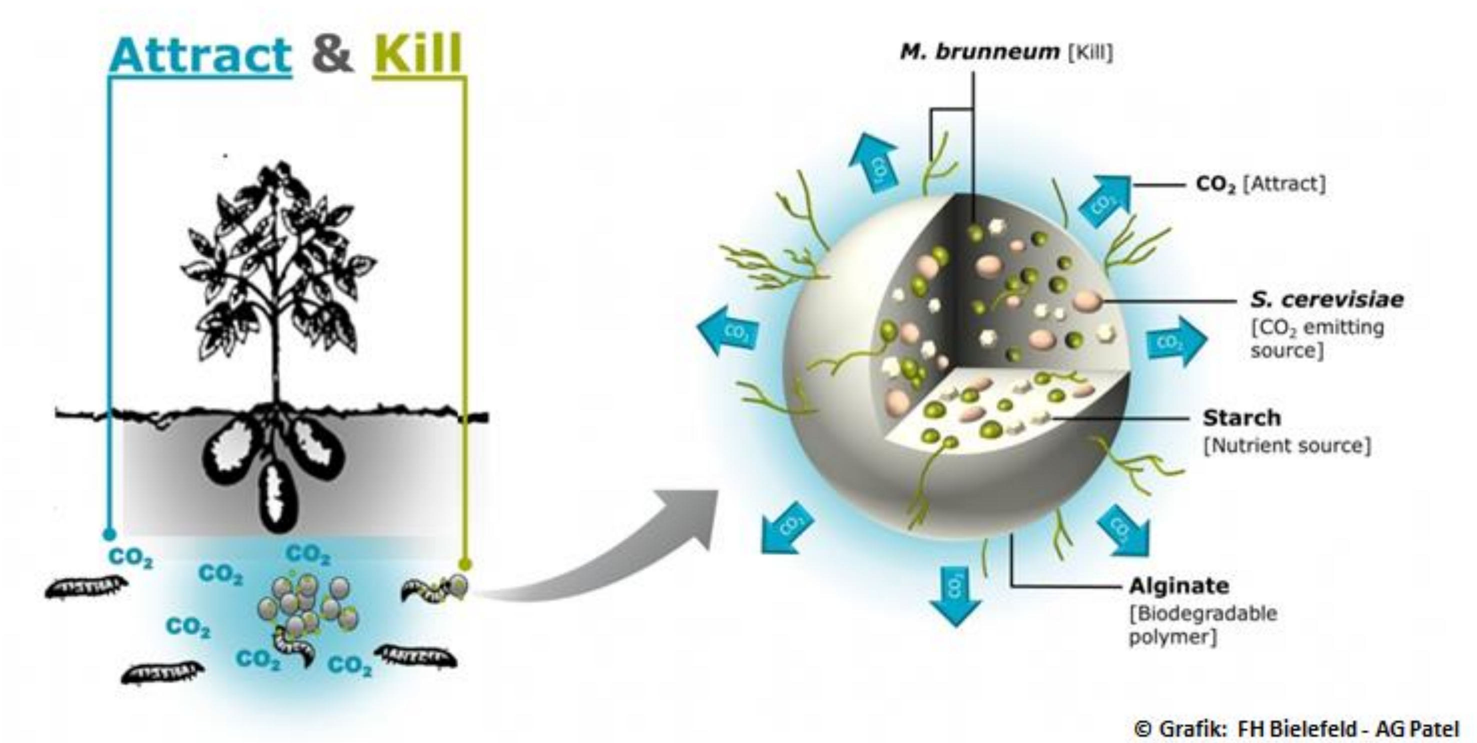
The development of an “Attract and Kill” strategy (A&K)




„A&K“ is the combination of an **attracting compound** that lures the wireworms and **a killing agent**

Carbon dioxide (CO2) is a well-known attractant for wireworms

EPFs as efficient killing agent

# AIM OF THE WORK

1. TO assess the efficacy and potential of "Attract and Kill" strategy for controlling wireworms in sunflower in comparison with conventional insecticides using **ATTRACAP** (*Metarhizium brrunei*)

2. TO improve assement of insecticide efficacy by introducing additional observation – wireworm damage rating scale

***WHY?***

Experiments for assessing insecticides' efficacy for wireworm control were defined by the EPPO standards PP 1/46 (3)

European and Mediterranean Plant Protection Organization
Organisation Européenne et Méditerranéenne pour la Protection des Plantes

PP 1/46(3)

## Efficacy evaluation of insecticides

### *Wireworms*

**Specific scope**
This standard describes the conduct of trials for the efficacy evaluation of insecticides against wireworms (larvae of *Elateridae*) on sugarbeet, cereals, potato, sunflower or maize.

**1. Experimental conditions**

*1.1 Test organisms, selection of crop and cultivar*

Test organisms: larvae of wireworms (*Elateridae*), especially *Agriotes* spp. (AGRISP), *Athous* spp. (ATHOSP).
Crops: sugarbeet, *Beta vulgaris* var. *altissima* (BEAVA) or fodder beet, *Beta vulgaris* var. *crassa* (BEAVC), winter or spring cereals (NNNGG), potato, *Solanum tuberosum* (SOLTU), maize, *Zea mais* (ZEAMX) or sunflower, *Helianthus annuus* (HELAN).
Any cultivar may be used, but the name of the cultivar should always be recorded in view of the risk of phytotoxicity. The trial should be performed on the test organism(s) and crop(s) specified for the intended use.
For seed treatment, it is useful to know the germination rate of the seed. The seeds for all treatments in the trial should come from the same batch of seeds. This standard may also be used for other crops, e.g. carrots.

*1.2 Trial conditions*

The trial should be set up in the field. Cultural conditions (e.g. soil type, fertilization, tillage) should be uniform for all plots of the trial and should conform with local agricultural practice. Sowing rate, and seed and row spacing, should be recorded. The presence of all stages of wireworms can generally be ensured by using land which was under permanent grass two years before, and has received no insecticide treatments since, or else by using land which was under any crop damaged by wireworms in the preceding year. In some countries, land may to have been under permanent grass for a significantly longer period to establish wireworm populations, e. g. 5-10 years permanent grass will give a 60-70% chance of infestation. Appendix I gives useful methods for determination of the infestation potential of wireworms.
The trial should form part of a trial series carried out in different regions with distinct environmental conditions and preferably in different years or growing seasons (see EPPO Standards PP 1/181 Conduct and reporting of efficacy evaluation trials and PP 1/226 Number of efficacy trials).

The type of application (e.g. a seed treatment, a granular soil application or a spray) should be as specified for the intended use.

*2.3.2 Type of equipment*
Application(s) should be made with equipment which provides an even distribution of product on the whole plot or accurate directional application where appropriate. Factors which may affect efficacy (such

and the action of the plant protection product. This normally includes data on precipitation and temperature. All data should preferably be recorded on the trial site, but may be obtained from a nearby meteorological station. For seed and tuber treatments, meteorological data at the time of sowing or planting is relevant.
On the date of application, meteorological data should be recorded which are likely to affect the quality and persistence of the treatment. This normally includes at ...on (type and amount in mm) and ...rage, maximum, minimum in °C). Any ...e in weather should be noted, and in ...e relative to the time of application. ...e trial period, extreme weather ...as severe or prolonged drought, heavy hail, etc., which are likely to influence ...d also be reported. All data concerning ...be recorded as appropriate.

...ta
...l products especially, the following ...f the soil should be recorded: pH, ...content, soil type (according to a ...l or international standard), moisture ...aterlogged), seed bed quality (tilth) and

...and frequency of assessment
...wth stage of the crop at each date of ...f assessment should be recorded.

...at emergence (about 75 % emerged), ...re counted (number per m of row) on ...sly marked lengths of row in each plot ...of plants per m length is calculated. ...ould be made whether wireworms are ...hether other soil pests (*Blaniulus* ...NGU), *Atomaria linearis* (ATOMLI), ...(SCUTSP), *Clivina fossor* (CLIVFO), ...(ONYCSP)) may be causing similar ...lix II).

...at the 4-6 leaf stage, plants remaining ...arked rows are counted and classified ...akened or dead. The possibility of ...l by fungal pathogens or other ...ld be noted. If this assessment cannot ...field, plants for which the cause of ...r should be examined using laboratory

...at emergence (about 75 % emerged), ...re counted (number per m of row) on ...sly marked lengths of row in each plot ...of plants per m length is calculated. ...ould be made to check whether wireworms are present and whether other soil pests (frit fly, cutworm, white grubs) may be causing similar damage (Appendix II).
2nd assessment: at the 5-6 leaf stage, plants remaining in the 4 x 5 m marked rows are counted. On at least 25

## Beet, sunflower

1st assessment: at emergence (about 75 % emerged), emerged plants are counted (number per m of row) on 4 x 5 m previously marked lengths of row in each plot and the number of plants per m length is calculated. Observations should be made whether wireworms are present and whether other soil pests (*Blaniulus guttulatus* (BLANGU), *Atomaria linearis* (ATOMLI), *Scutigerella* spp. (SCUTSP), *Clivina fossor* (CLIVFO), *Onychiurus* spp. (ONYCSP)) may be causing similar damage (Appendix II).

2nd assessment: at the 4-6 leaf stage, plants remaining in the 4 x 5 m marked rows are counted and classified as healthy, weakened or dead. The possibility of damage caused by fungal pathogens or other arthropods should be noted. If this assessment cannot be done in the field, plants for which the cause of damage is unclear should be examined using laboratory methods.

- ✓ Efficacy is DEDUCED based on NUMBER OF PLANTS PER METER OF ROW

- ✓ NUMBER OF PLANTS (plant stand) is influenced by many biotic and abiotic factors

- ✓ Assessing only plant stand, especially on fields with low wireworm infestation, is not sufficient to provide reliable results on the treatment's efficacy

# MATERIAL AND METHODS

Wireworm Abundance Assessment

- ✓ standard square method (50 x 50 cm, to a layer depth of approximately 40 cm)
- ✓ the number of collected specimens in soil pits per m2
- ✓ the beginning of spring
- ✓ 10 probes on each experimental field

The Experimental Sites and Treatments

- ✓ Field experiments were carried out at the Institute of Field and Vegetable Crops at Rimski šančevi,Novi Sad, Serbia,
- ✓ a randomised block design, according to the EPPO PP 1/46 (3) methodology
- ✓ 5 - 9 replications, depending on the year and site
- ✓ basic experimental plots was 42 m2 (10 m long, 4.2 m wide with 6 rows).
- ✓ sunflower variety Duško (IFVCNS variety)
- ✓ Mechanical sowing with a row-to-row distance of 70 cm and 23.5 cm within rows.

# MATERIAL AND METHODS

**Table 1.** Experimental sites, years and treatments.

| Year | Locality | Infestation | Treatments | Application Type | Dose |
|---|---|---|---|---|---|
| 2018 | RŠ T-12 | low | [illegible] | [illegible] | 30 kg/ha |
| | | | [illegible] | [illegible] | 5 kg/ha |
| 2019 | RŠ T-12 | low | [illegible] | [illegible] | 30 kg/ha |
| | | | [illegible] | [illegible] | 25 mL/kg |
| | | | [illegible] | [illegible] | 5 kg/ha |
| | | | [illegible] | [illegible] | 250 mL/100 kg |
| | | | [illegible] | [illegible] | 11.3 mL/kg |
| 2021 | RŠ T-12 | low | [illegible] | [illegible] | 30 kg/ha |
| | | | [illegible] | [illegible] | 250 mL/100 kg |
| | | | [illegible] | [illegible] | 5 kg/ha |
| | | | [illegible] | [illegible] | 11.3 mL/kg |
| | | | [illegible] | [illegible] | 0.8 l/100 kg |
| | RŠ Field 1 | high | [illegible] | [illegible] | 30 kg/ha |
| | | | [illegible] | [illegible] | 250 mL/100 kg |
| | | | [illegible] | [illegible] | 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 $m^2$.

## Field Observations

- ✓ Counting number of plants per row – calculate final plant stand
- ✓ two growth stages, from the first to the fourth pairs of leaves (BBCH 02 and BBCH 04/05)
- ✓ The rating of plant damages in 2021 - DAMAGED or NOT DAMAGED

This proposed additional rating allows for confirming damage caused by wireworms and affecting field emergence and/or plant stand more accurately.

## Statistical analysis

- ✓ Repeated measures ANOVA and the Bonferroni pairwise comparison post hoc test analysed statistical differences in plant stand
- ✓ Modelling the occurrence of damage i.e. calculating the odds for damage occurrence was performed using binominal regression.

## RESULTS

### Plant stand

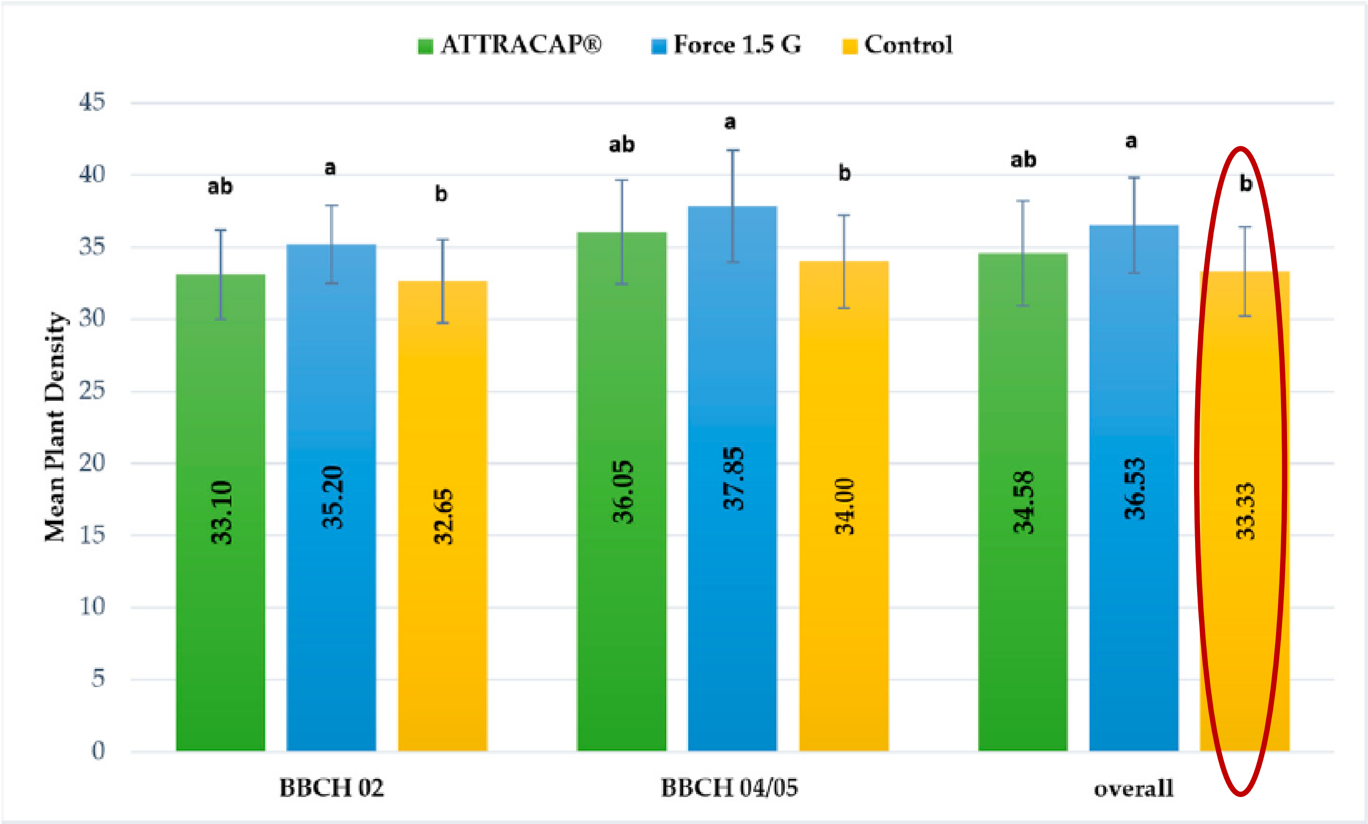



**Figure 1.** Plant density depending on the insecticidal treatments in 2018.

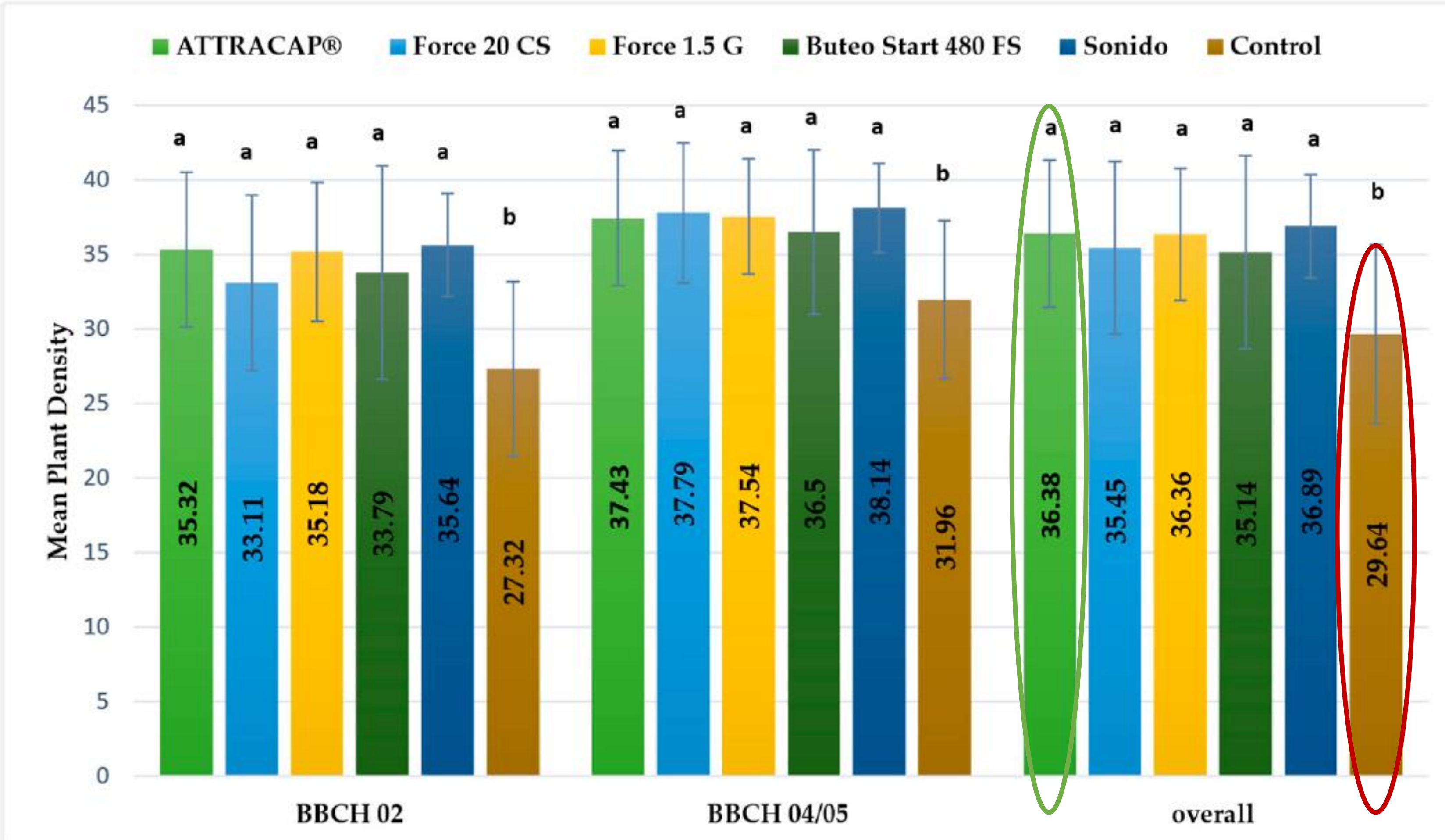



**Figure 2.** Plant density depending on the insecticidal treatment in 2019.

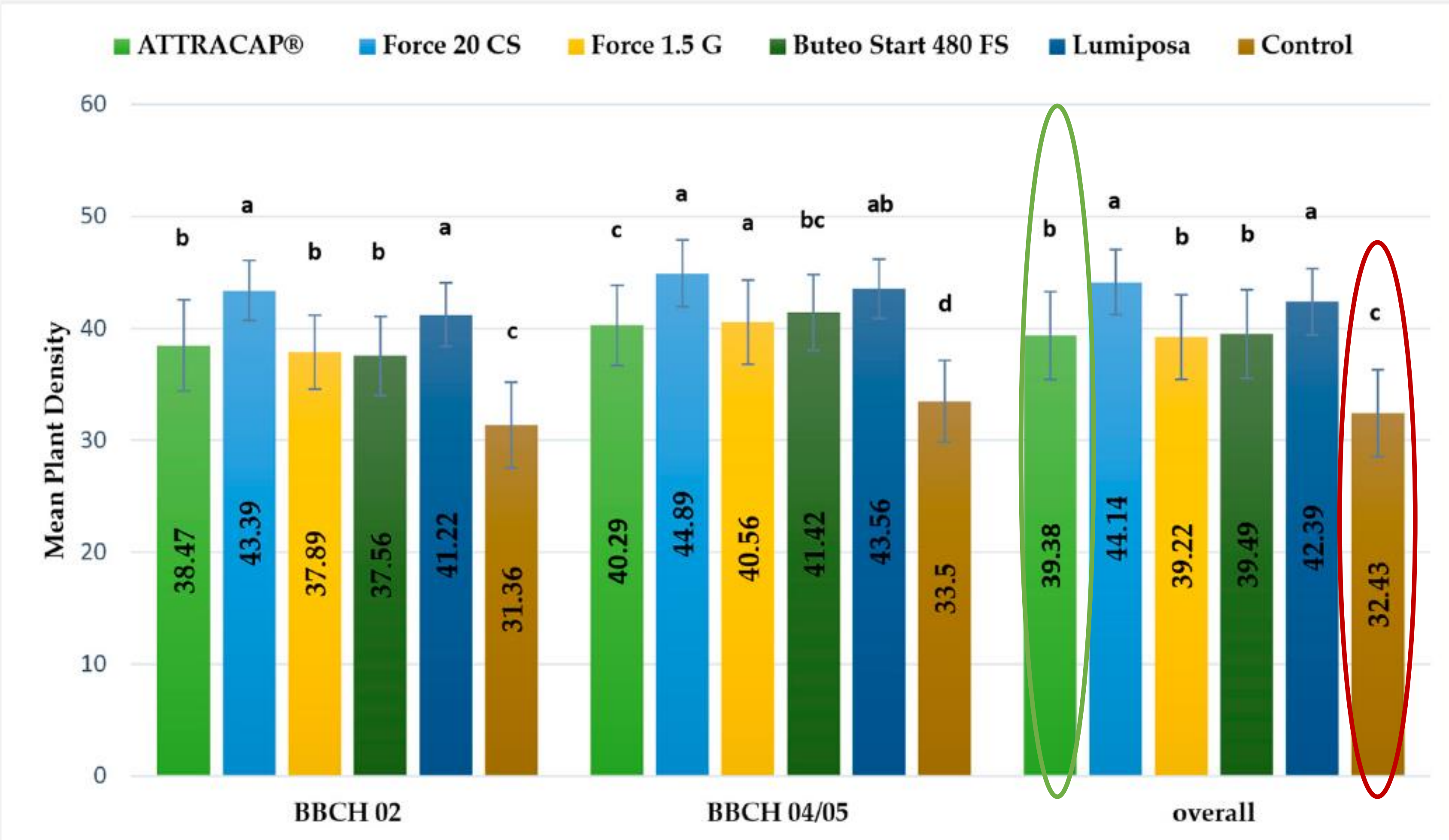



**Figure 3.** Plant density depending on the insecticidal treatment at RŠ Field 1 in 2021.

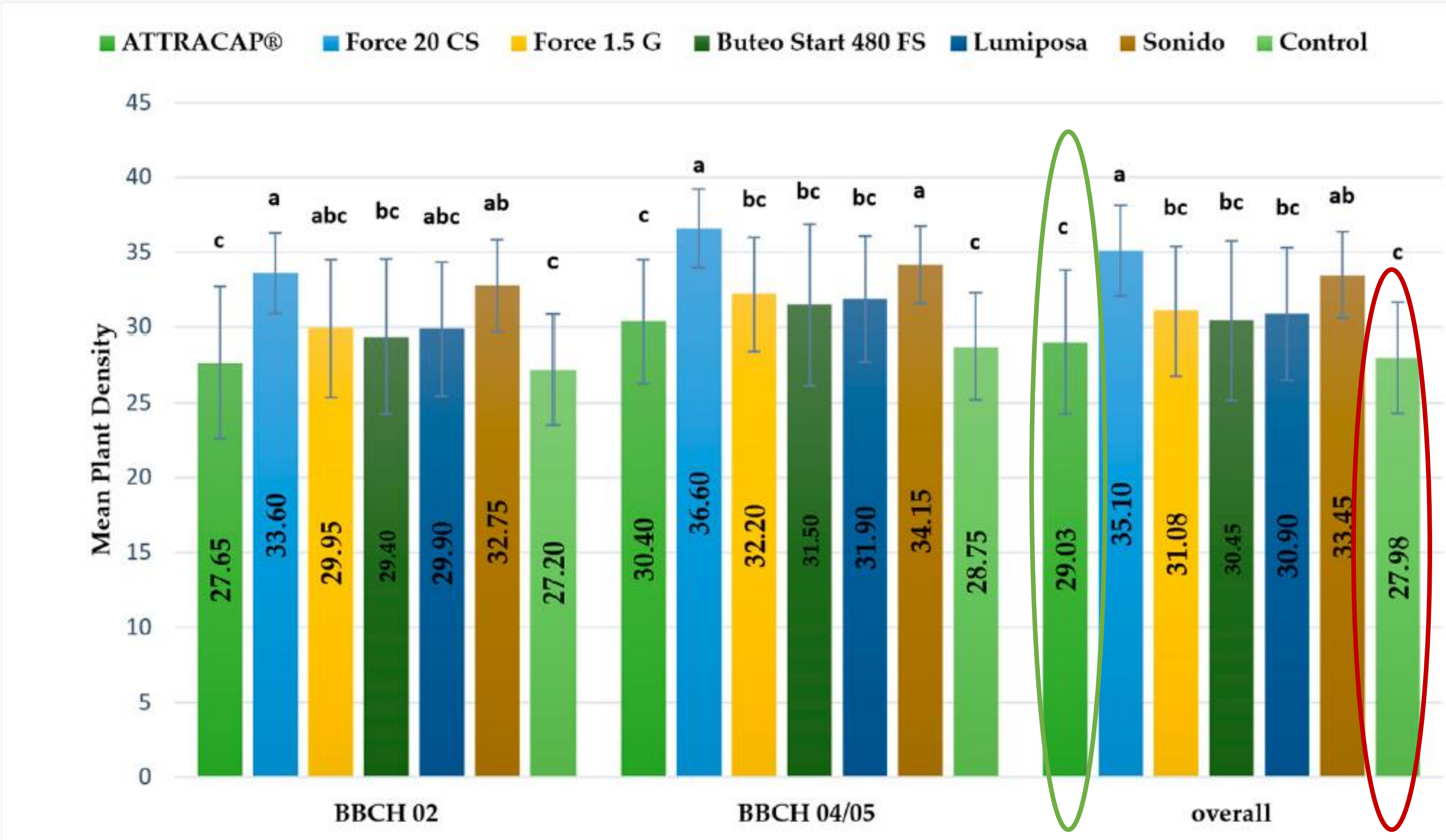



**Figure 4.** Plant density depending on the insecticidal treatment at RŠ T-12 in 2021.

# Plant damage

**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 ($p < 0.05$).

**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 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 ($p < 0.05$).

**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 ($p < 0.01$).

**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 ($p < 0.01$).

# RESULTS

## Plant damage ratings (0-1)

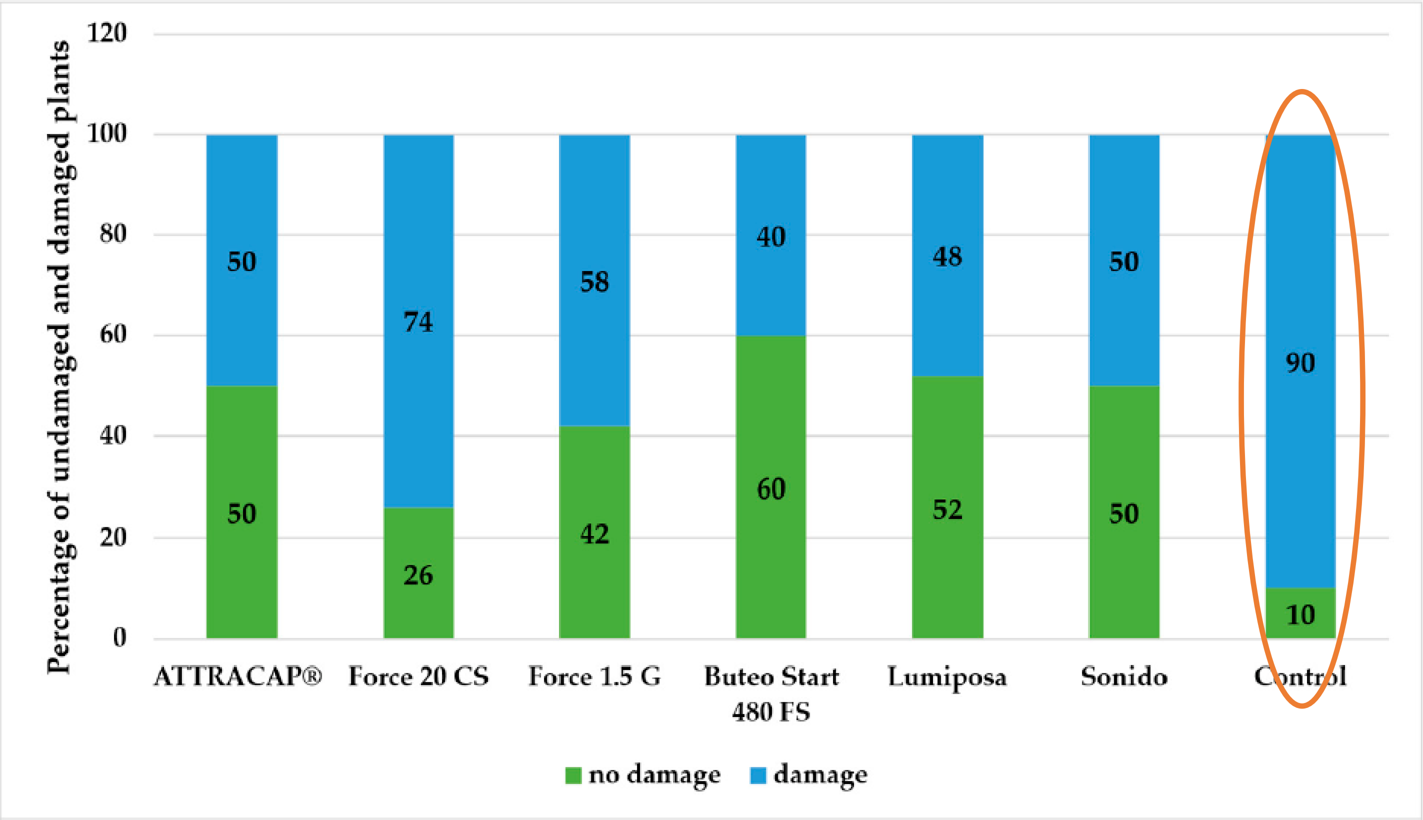



**Figure 5.** Percentage of undamaged and damaged plants in different insecticidal treatments in the sunflower field RŠ Field 1 in the year 2021.

# RESULTS

## Plant damage ratings 0-1

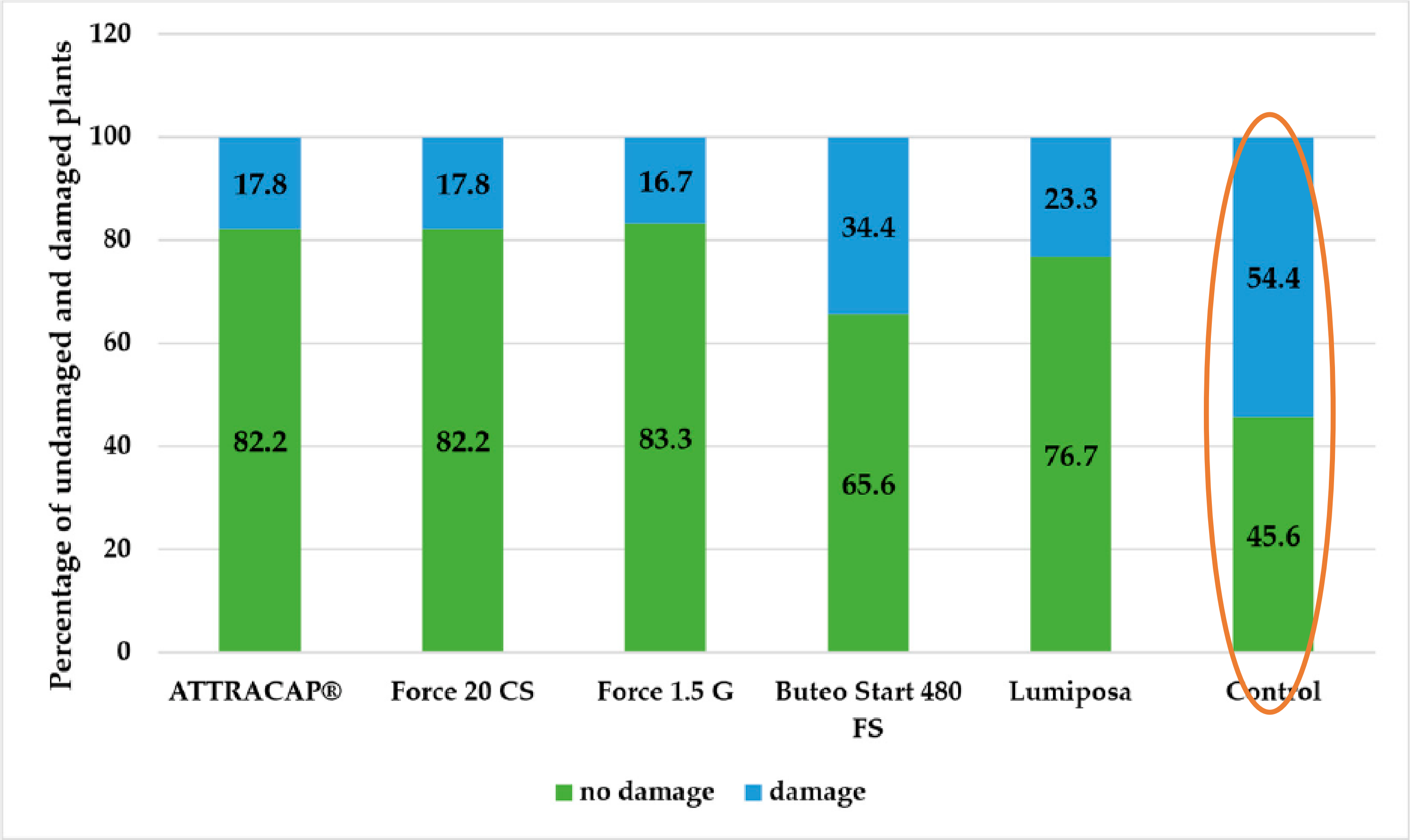



**Figure 6.** Percentage of undamaged and damaged plants in different insecticidal treatments in the sunflower field RŠ T-12 in the year 2021.

# Binary logistic regression

**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.

# CONCLUSION

- ✓ **ATTRACAP** performed similar to all chemical insecticides applied under conditions of LOW wireworm infestation
- ✓ Even under HIGH wireworm infestations it provided certain protection, and performed as severl other insecticides
- ✓ The creation of damage rating scale enabled more precise and relevant assessment of wireworm damages on sunflower plants.
- ✓ Modelling wireworm damage using binomial regression provided valuable information about the odds of wireworm 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.

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BIOLOGICAL PLANT PROTECTION

# THANK YOU FOR YOUR ATTENTION







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