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Final report on Colorado potato beetle and wireworm control strategies

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ABSTRACT (FOR DISSEMINATION)	The document provides information on different control strategies against Colorado potato beetle (CPB) and wireworms. For CPB five bioinsecticides based on different substances and microorganisms and one repellent compound were evaluated. Possible resistance of 65 varieties against CPB was tested. For wireworm control different formulations based on entomopathogenic fungi (EPF) *Metarhizium brunneum* and *Metarhizium robertsii* preparations were evaluated. Treatments with spinosad resulted in significantly better control of the CPB larval population compared to all other insecticide treatments. A significant reduction in the number of CPB larvae was also achieved by the application of NeemAzal T/S and its combination with EPF. The repellent Biomit was not efficient in CPB damage control. ATTRACAP (full and half dose) together with potatoes soaked in fungal suspension showed the best results for wireworm control.
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Executive summary

The document provides information on different control strategies against Colorado potato beetle (CPB) and wireworms. The Colorado potato beetle (*Leptinotarsa decemlineata*; Coleoptera: Chrysomelidae) and wireworms (Coleoptera: Elateridae) are among the most important insect pests of potatoes worldwide.

Colorado potato beetle

In 2020 and 2021 two field experiments were carried out at Agricultural institute of Slovenia (KIS) to test five bioinsecticides based on different substances and microorganisms with proven or potential toxic effects against CPB: azadirachtin (Neemazal), spinosad (full and reduced dosage – 0,2), conidial suspension of entomopathogenic fungi *Beauveria bassiana* (Bals.-Criv.) Vuill. (KIS isolates 2300 and 2121), entomopathogenic bacteria *Bacillus thuringiensis* var. *tenebrionis* (Novodor) and RNA interference (RNAi). The biological control agents were applied against the larval population individually and in combination to explore the effectiveness and potential synergistic interactions against CPB larvae: spinosad, spinosad + *B. bassiana*, azadirachtin, azadirachtin + *B. bassiana*, *B. bassiana* and RNAi. Eight different treatments were applied on potato ('KIS Kokra' variety) plots infested with CPB larvae using a randomised complete block design with six replicates. Applications of bioinsecticides were made with a backpack sprayer in the evening hours to take advantage of overnight favourable conditions of high humidity and the absence of solar radiation. Effectiveness of individual bioinsecticides was expressed as a reduction in number of larvae and differences in plant defoliation.

At the Hungarian University of Agricultural Sciences (MATE) field experiment was set up comparing the effectiveness of Laser Duplo, active ingredient 480 g/l spinosad (0,075 l/ha) and Biomit (5 l/ha) with proven or potential toxic effects against CPB by natural infection. The variety Botond was used. Both protective chemicals were applied two times during the vegetation period in 2021 against the L2 CPB larvae, while due to the limited effect of Biomit in 2021 its application rate was increased five-fold in 2022 with the aim of covering all new developing leaves. Biomit as a dolomit based plant conditioner is enriched with several plant extracts and therefore has the potential to work as a repellent and an antinutritive substance for CPB.

In order to evaluate possible differences in genotype resistance 65 potato varieties from the Ecobreed working collection were tested in 2021 and 2022 at Plant Breeding and Acclimatization Institute (IHAR).

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Results of the study revealed that among the tested bioinsecticides all treatments with spinosad (Laser plus, Laser plus 0.2 dose and *B. bassiana*+Laser plus) provided significantly better control of larval population compared to all other insecticide treatments. The best results from the tested products showed Laser plus with significantly increased mortality rate and reduced feeding activity of CPB larvae when used at full and reduced dosage (0.2 of full dosage). Significant reduction in the number of CPB larvae was achieved also by the application of NeemAzal T/S and its combination with entomopathogenic fungi (*B. bassiana* + Neemazal – T/S). In addition to the direct impact and increase in mortality rate of different treatments, the indirect effect on the reduction of feeding is also important when evaluating their efficiency against CPB larvae. Results indicate that the bacterial based insecticide Novodor FC did not have a significant effect on CPB larvae mortality but could contribute to a successful strategy for CPB control by reducing plant defoliation caused by CPB larvae. However, insufficient efficacies were obtained with application of *B. bassiana* which showed low insecticidal activity in field conditions and was significantly less effective against CPB larvae which means that it can't be recommended as a ready-to-use bioinsecticide for CPB larvae control.

Based on the experimental results at MATE in Keszthely, the insecticide Laser Duplo prevented the defoliation of plants by CPB better than the natural repellent Biomit, which even following five times application could only reduce defoliation to 52%. Consequently, an even more frequent e.g. once a week application would be needed to prevent severe leaf damage, but that would make its application economically not viable.

The two-year research at IHAR revealed three varieties (Salome, Edony, Noblesse) that might have a strong level of resistance towards CPB. Incorporation of host plant resistance into potato varieties may be an effective tool in the management of CPB and is likely to be a valuable tool for integrated pest management approaches to CPB control.

Wireworms

In order to evaluate several innovative strategies for wireworm control in potato fields, two field experiments with variety KIS Kokra were set up at KIS in 2020 and two with variety KIS Savinja in 2021. To test the bioaugmentation method, i.e. the introduction and potential multiplication of entomopathogenic fungi (EPF) in the rhizosphere, six of the most virulent KIS *Metarhizium brunneum* and *Metarhizium robertsii* isolates were formulated on rice and added to potato tubers at planting. To test the »attract and kill«

method, the commercial granular bioinsecticide ATTRACAP (Biocare GmbH, active ingredient *M. brunneum* Cb15-III) was used. The ATTRACAP granules contain EPF (*M. brunneum* Cb15-III), starch and baker's yeast (*Saccharomyces cerevisiae*). When ATTRACAP is added to moist soil, yeasts begin to produce CO_2. Wireworms are attracted to the CO_2 source and consequently come into contact with the EPF present in the granules. To test plant growth stimulation method, we soaked potato tubers in spore suspension of six KIS EPF isolates for 1 h 30 min so that the spores would get adsorbed to the surface of the potato tubers and potentially colonise the external or internal tissues of the plants. We had seven treatments in the experiments: (a) potato tubers soaked in the fungal suspension of all six isolates (Potato_fungi), (b) fungi formulated on rice (Potato_rice) and (c) a combination of both treatments (Potato_fungi_rice). In addition, two treatments with the commercial bioinsecticide ATTRACAP were made, testing either full (30 kg/ha) or half (15 kg/ha) recommended dose and the commercial insecticide Force (Syngenta, active ingredient Tefluthrin, 15 g/kg) was used as a conventional (positive) control treatment alongside the untreated control. The effectiveness of tested methods was evaluated at potato harvest on 100 randomly selected tubers per plot as the amount of tuber yield and as the number of tubers where we counted more than one wireworm hole per tuber, since tubers with more holes are less attractive for consumers.

The results of testing various preparations containing Metarhizium on wireworms, especially potatoes soaked in fungal suspension and half dose of ATTRACAP resulted in lower number of tubers with more than one hole compared to the control. Thus, the introduction of EPF in the rhizosphere as an attract-and-kill method, or as plant growth promotor by soaking tubers in fungal suspension, has the potential to reduce tuber damage.

Wireworms generally do not cause yield loss, and this is consistent with our results, as wireworms did not affect the number or mass of the tubers and mostly did not cause yield losses at any location, with the exception of the full dose of Attracap and potatoes soaked in fungal suspension together with fungi formulated on rice at Field 2 in 2020.

Overall, bioinsecticides based on entomopathogenic fungi were found to be quite effective in reducing potato damage caused by wireworms, as the effectiveness of the treatments, especially ATTRACAP half dose and potatoes soaked in fungal suspension, were comparable to that of a conventional insecticide based on tefluthrin.

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Colorado potato beetle

Introduction

The Colorado potato beetle (*Leptinotarsa decemlineata*; Coleoptera: Chrysomelidae) and wireworms (Coleoptera: Elateridae) are among the most important insect pests of potatoes worldwide. The Colorado potato beetle (CPB) is a well-known pest of potato plants. Damage is caused by larvae that feed on the leaves and stems of plants and can during their development period cause up to 80 % defoliation of the attacked plants. The above-ground destruction of potato plants can cause severe reduction in tuber size and overall yield (Alyokhin, 2009; Vincent et al., 2013). Colorado potato beetle can be controlled by classical 'chemical' insecticides which current EU agricultural policies are trying to reduce the use of (EU, 2022), or those based on plant extracts, entomopathogenic microorganisms and other substances that pose a lower risk for human and animal health and the environment. In order to evaluate several innovative CPB control strategies, we set up field experiments in 2020 and 2021 at the Agricultural Institute of Slovenia (KIS) and in 2021 and 2022 at the Hungarian University of Agricultural Sciences (MATE).

There are no commercial varieties on the market that show a high level of resistance towards CPB, although some level of avoidance is seen on the variety Dakota Diamond (Thompson et al. 2008). Ghassemi-Kahrizeh (2009) showed that cvs. Delikat and Bridjet were less favourable for CPB compared to cvs. Agria and Stima, which can be used in plant breeding programs to produce resistant varieties. In order to evaluate possible differences in genotype resistance 65 potato varieties from the Ecobreed working collection were tested in 2021 and 2022 at Plant Breeding and Acclimatization Institute (IHAR).

Materials and methods

Bioinsecticides against Colorado potato beetle

CPB experiments at KIS

In 2020 and 2021 two field experiments were carried out to test five bioinsecticides based on different substances and microorganisms with proven or potential toxic effects against CPB: azadirachtin (Neemazal), spinosad (full and reduced dosage – 0,2), conidial suspension of entomopathogenic fungi *Beauveria bassiana* (Bals.-Criv.) Vuill. (KIS isolates 2300 and 2121), entomopathogenic bacteria *Bacillus thuringiensis* var. *tenebrionis* (Novodor) and RNA interference (RNAi). The biological control agents were applied against the larval population individually and in combination to explore the effectiveness and potential synergistic interactions against CPB larvae: spinosad, spinosad + *B. bassiana*, azadirachtin, azadirachtin + *B. bassiana*, *B. bassiana* and RNAi (Table 1).

Table 1: List of all CPB control treatments tested in 2020 and 2021.

Treatment	Active substance	Concentration	Year applied
Neemazal - T/S	azadirachtin A (1 g/L)	0,5 %	2020, 2021
Laser plus	spinosad (480 g/L)	0,040 %	2020, 2021
Laser plus 0,2 dose	spinosad (480 g/L)	0,008 %	2021
B. bassiana	*Beauveria bassiana* (KIS isolates 2300 and 2121)	10^5 conidia/mL	2020, 2021
Novodor FC	*Bacillus thuringiensis* var. *tenebrionis* (20 g/L 10000 BTTU/g)	1 %	2021
RNAi	RNAi (dsMESH)	10 µg/mL	2020, 2021
***B. bassiana* + Laser plus 0,2 dose**	*Beauveria bassiana* (KIS isolates 2300 and 2121) + spinosad (480 g/L) 0,2 dose	10^5 conidia /mL 0,002 %	2021
***B. bassiana* + Neemazal – T/S**	*Beauveria bassiana* (KIS isolates 2300 and 2121) + azadirachtin (1 g/L)	10^5 conidia /mL 0,5 %	2020, 2021

Eight different treatments were applied on potato ('KIS Kokra' variety) plots infested with CPB larvae using a randomised complete block design with six replicates. In 2020 blocks were divided into seven plots (six treatments and untreated control), each measuring 3 x 2.5 m (7,5 m^2) and comprising 3 rows of potatoes. Applications of bioinsecticides were made with a backpack sprayer in the evening hours to take advantage of overnight favourable conditions of high humidity and the absence of solar radiation. Effectiveness of individual bioinsecticides was expressed as a reduction in number of larvae and differences in plant defoliation. Before the bioinsecticide application we selected and marked 5 CPB-infested plants per plot for further assessments. CPB larvae were counted before insecticidal spraying and on the 3rd and 7th day after (bio)insecticide treatment. Level of plant defoliation was assessed visually on the same plants immediately before spraying and on the 7th day after treatment. Defoliation was assessed by visual inspection of 10 youngest compound potato leaves

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per plant using a rating scale that divided defoliation damage into 5 classes: 0 < 5 %, 1 5-10 %, 2 ≤ 50 %, 3 ≤ 75 %, 4 >75-95 % and 5 > 95 % of leaf surface eaten.

CPB experiments at MATE

A small field experiment using a randomised complete block design with three replicates, was set up comparing the effectiveness of Laser Duplo, active ingredient 480 g/l spinosad (0,075 l/ha) and Biomit (5 l/ha) with proven or potential toxic effects against CPB by natural infection at the Potato Research Station, Keszthely. The variety Botond was used with 15 tubers per plot. Both protective chemicals were applied two times during the vegetation period in 2021 against the L2 CPB larvae, while due to the limited effect of Biomit in 2021 its application rate was increased five-fold in 2022 with the aim of covering all new developing leaves. Biomit as a dolomit based plant conditioner is enriched with several plant extracts and therefore has the potential to work as a repellent and an antinutritive substance for CPB according to its manufacturer (https://biomit-world.com/hu/biomit-novenykondicionalo/#pll_switcher).

The effect of the treatments was analysed in terms of the percentage of plant defoliation in the second week following application of the final treatment.

Genotype differences and CPB at IHAR

Tubers from 65 varieties were planted in a variety trial in the field with 30 hill plots (two rows with 15 tubers in each) and with 2 replicates. Observations were conducted for two years in 2021 and 2022. The experimental field was located at IHAR in the central part of Poland in Młochów on a sandy loam soil. Colorado potato beetle damage of the potato foliage in the experiment was visually estimated as % of leaf surface destroyed.

Statistical analysis

For CPB data, statistical differences larval mortality according to Henderson-Tilton correction were calculated using ANOVA and Bonferroni's Multiple Comparison Test. Results of leaf damage increase were analysed using Dunnett's Multiple Comparison Test to assess whether the treatments significantly differ from the negative control ($p<0.05$). Results were analysed and visualised using Prism 8 software (GraphPad).

Results

Bioinsecticides against CPB

Studies have been conducted on microbial insecticides summarising that different species or isolates of entomopathogenic fungi (EPF) against different host species show different pathogenicity (Wright and Ramos, 2005; Gödel et al., 2020). The present study indicated that the *B. bassiana* isolates KIS 2300 and KIS 2121 did not increase the mortality of CPB larvae. The highest efficiency of the tested bioinsecticides on CPB larvae was achieved by Laser plus applied at full and 20% of the full recommended dose (Table 2). In both experiments conducted in 2020 and 2021, the CPB mortality after application of Laser plus (full dose) was similar and amounted to 95.7 % or 90.7 %, respectively. Such high efficacy of Laser plus resulted in the fact that the addition of Laser plus at 20 % recommended dose did not differ significantly from efficacy of Lase plus at full recommended dose (assessed only in 2021). Also, despite showing a numerical increase in the efficacy of reduction of CPB larvae, the combination of Laser plus 20% dose and *B. bassiana* was not statistically different from the Laser plus full dose rate. Application of Neemazal – T/S caused a significant increase in mortality rate of CPB larvae i.e. from 31.8 % in 2020 to 61.7 % in 2021. However, the same mortality rates for Neemazal – T/S and the mixture *B. bassiana* + Neemazal – T/ indicates no obvious synergistic effect of these bioinsecticides. We observed no difference in larval mortality between the Novodor, RNAi and control treatments.

Table 2: Mean percent mortality (Henderson-Tillton corrected) of Colorado potato beetle larvae in in 2020 and 2021. Means followed by different letters within a year denote significant differences ($p<0.05$)

Treatment	Mortality (%)	
	2020	2021
Neemazal – T/S	31,8 bc	61,7 ab
Laser plus	95,7 a	90,7 ab
Laser plus 0,2 dose	nd	81,3 ab
B. bassiana	12,2 de	0 d
Novodor FC	nd	2,6 cd
RNAi	23,1 cde	25,2 bcd
***B. bassiana* + Laser plus 0,2 dose**	nd	98,9 a
***B. bassiana* + Neemazal – T/S**	43,8 b	25,4 bcd
negative control	0 e	0 cd

Assimilatory leaf area is a key factor which determines the productivity of arable crops and reduction of potato plant assimilation area due to CPB larvae feeding may lead to a reduction of tuber yield. The extent of losses depends on the degree of defoliation and development stage at which the defoliation occurs. In our experiments the leaf area consumed by CPB larvae was significantly lower in all treatments (except the *B. bassiana* and RNAi in 2021) compared to the untreated control in the first seven days after application (Fig. 1). The average level of plant defoliation ranged from 0.17 ± 0.17 (Laser plus in 2020) to 0.74 ± 0.19 (Novodor FC in 2021) which were significantly different from the control where the level of plant defoliation was 1.67 ± 0,08 (2020) and 1.89 ± 0.26 (2021) respectively.

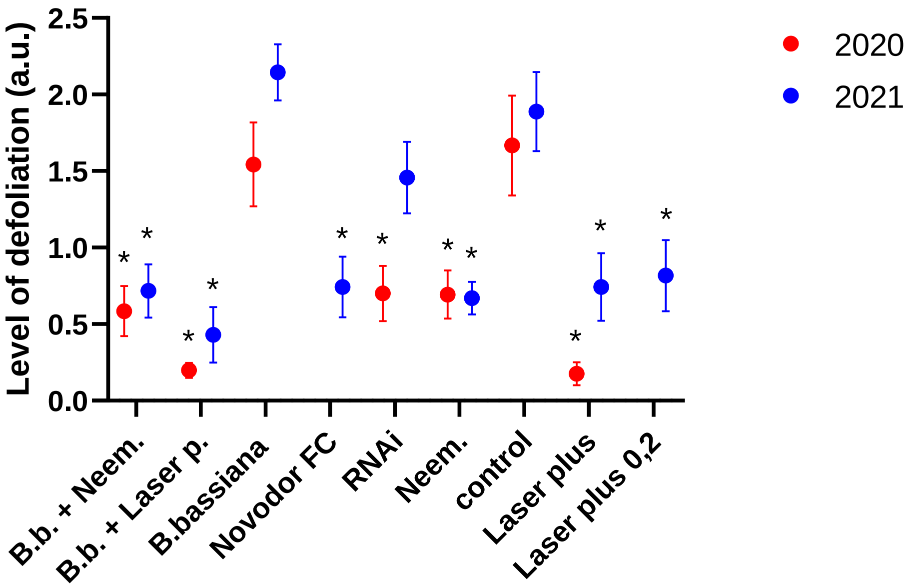

Fig. 1: Potato leaf damage due to CPB herbivory expressed as leaf defoliation increase. Means marked with * are significantly different from the control treatment.

The insecticide Laser Duplo which is permitted for use in organic potato production was more effective in controlling CPB compared to the repellent Biomit. The application of Laser Duplo at two timings resulted in rather low 21 % level of defoliation of plants when averaged across the two growing seasons. The use of Biomit in 2021 (applied twice) resulted in high 70 % level of defoliation, while in 2022 (applied five times) resulted in a lower level of foliar defoliation at 52 %. The repellent effect of Biomit to prevent damage by CPB was not high enough for practical use to protect the plants and was not economically justifiable especially when applied five times (at 2 week intervals) during the season.

Table 3: Percentage of leaf damage by Colorado potato beetle on the variety Botond in experiments at MATE in 2021 and 2022

	2021			**Average**	**2022**			**Average**
	I	II	III		I	II	III	
Spinozad*	27	15	18	20,0	17	28	22	22,3
Biomit**	80	75	56	70,3	57	48	52	52,3

*Spinosade was used two times in each season.

**Biomit was applied two times in 2021 and five times in 2022.

Genotype differences and CPB

In the season 2021 the level of damage caused by Colorado potato beetle ranged from 0% to 90% (Table 4). In 2022 the damage caused by CPB was much lower than in 2022 (Fig 2.). The ANOVA revealed a highly significant effect for year (Y) on the % damage caused by CPB, while the effect of variety was not significant (Table 5).

Table 4: Varieties the highest % damage caused by CPB in Poland in 2021.

Variety	% of damage in 2021
12-LHI-6	40
Colomba	40
Goldmarie	40
Triplo	45
Valor	45
Ditta	50
Lilly	50
Casablanca	55
Denar	55
Charlotte	60
Colleen	60
Gatsby	60
Agria	70
Belmonda	70
Caprice	70
Twinner	90

Table 5: Sources of variation and ANOVA results for percentage damage caused by CPB (IHAR 2021 and 2022).

	ANOVA results					
Sources of variation	**Sum of squares**	**Degrees of freedom**	**Mean square**	**F statistic**	**p-value**	**Significance**
Varieties (V)	15065.48	64	235.40	0.47491	0.998369	ns
Year (Y)	18696.01	1	18696.01	83.7089	0.000000	***
Maturity (M)	82.57	2	41.28	0.11107	0.894959	ns

ns=not significant
*significant at P < 0.05
**significant at P < 0.01
*** significant at P < 0.001

The highest percentage of damage averaged across 2021 and 2022 (Fig. 2) was recorded in varieties: Twinner, Caprice, Belmonda, Agria, Gatsby, Coleen, Charlotte, Denar, Casablanca, Lilly and Ditta. For the varieties Salome, Edony and Noblesse there were no damage caused by CPB in both years of the experiment.

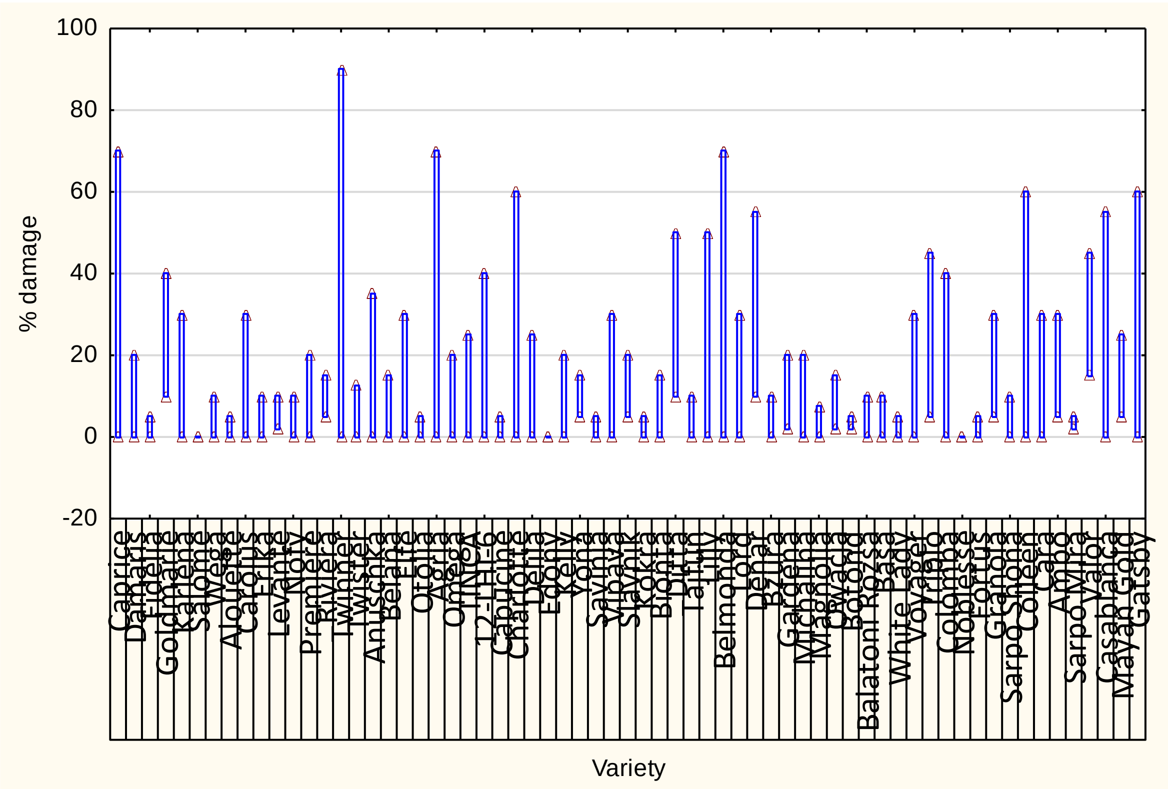

Fig. 2: Mean % damage caused by CPB for 65 potato varieties averaged across 2021 and 2022.

Conclusions on CPB

Entomopathogenic fungi, especially from the genus *Beauveria* (Ascomycota: Hypocreales), have been shown to be a promising solution for the control of CPB in potato (Brandl et al. 2017; Wraight et al. 2008). *Beauveria* sp. naturally occurs in the soil and is therefore associated with soil-inhabiting insects like CPB larvae undergoing pupation. *Beauveria* sp. has also been effectively tested for CPB larvae control in the form of a foliar spray (Lacey et al. 1999).

Results of the study revealed that among the tested bioinsecticides all treatments with spinosad (Laser plus, Laser plus 0.2 dose and *B. bassiana*+Laser plus) provided significantly better control of larval population compared to all other insecticide treatments. The best results from the tested products showed Laser plus with significantly increased mortality rate and reduced feeding activity of CPB larvae when used at full and reduced dosage (0.2 of full dosage). Significant reduction in the number of CPB larvae was achieved also by the application of NeemAzal T/S and its combination with entomopathogenic fungi (*B. bassiana* + Neemazal – T/S). In addition to the direct impact and increase in mortality rate of different treatments, the indirect effect on the reduction of feeding is also important when evaluating their efficiency against CPB larvae. Results indicate that the bacterial based insecticide Novodor FC did not have a significant effect on CPB larvae mortality but could contribute to a successful strategy for CPB control by reducing plant defoliation caused by CPB larvae. However, insufficient efficacies were obtained with application of *B. bassiana* which showed low insecticidal activity in field conditions and was significantly less effective against CPB larvae which means that it can't be recommended as a ready-to-use bioinsecticide for CPB larvae control. Although in some studies RNAi has shown a positive effect against CPB larvae even when tested under field conditions (Petek et. Al. 2020) in our study the use of RNAi didn't show a significant effect on larval mortality and resulted in a significantly lower rate of plant defoliation only in 2020.

Based on the experimental results at MATE in Keszthely, the insecticide Laser Duplo prevented the defoliation of plants by CPB better than the natural repellent Biomit, which even following five times application could only reduce defoliation to 52%. Consequently an even more frequent e.g. once a week application would be needed to prevent severe leaf damage, but that would make its application economically not viable.

The two-year research at IHAR revealed three varieties (Salome, Edony, Noblesse) that might have a strong level of resistance towards CPB. Incorporation of host plant resistance into potato varieties may be an effective tool in the management of CPB and is likely to be a valuable tool for integrated pest management approaches to CPB control.

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Wireworms

Introduction

Wireworms (Coleoptera: Elateridae) are the larvae of click beetles and they are also known as "elater larvae". They can be found in the soil where they feed on the underground parts of plants, such as the roots and tubers of potatoes. Damage from wireworms can include wilting, stunted growth and even plant death. Because wireworms can survive for several years in the soil, infestations can be difficult to control. Entomopathogenic fungi (EPF), particularly from the genus *Metarhizium* (Ascomycota: Hypocreales), have been shown to be a promising solution for controlling wireworms in potatoes (Reinbacher et al. 2021; Brandl et al. 2017; Kabaluk et al. 2005). With this in mind, we tested different formulations based on EPF *Metarhizium brunneum* and *Metarhizium robertsii* preparations, thus evaluating different modes of action such as plant growth stimulation, bioaugmentation and the »attract and kill« method.

Materials and methods

Bioinsecticides against wireworms

In order to evaluate several innovative strategies for wireworm control in potato fields, we set up two field experiments in 2020 and two in 2021. The Agricultural Institute of Slovenia's (KIS) mycological collection holds many isolates of entomopathogenic fungi (EPF) that have proven to be quite effective. Therefore, to test the bioaugmentation method, i.e. the introduction and potential multiplication of EPF in the rhizosphere, six of the most virulent KIS *Metarhizium brunneum* and *Metarhizium robertsii* isolates were formulated on rice and added to potato tubers at planting. To test the »attract and kill« method, the commercial granular bioinsecticide ATTRACAP (Biocare GmbH, active ingredient *M. brunneum* Cb15-III) was used. The ATTRACAP granules contain EPF (*M. brunneum* Cb15-III), starch and baker's yeast (*Saccharomyces cerevisiae*). When ATTRACAP is added to moist soil, yeasts begin to produce CO_2. Wireworms are attracted to the CO_2 source and consequently come into contact with the EPF present in the granules. To test plant growth stimulation method, we soaked potato tubers in spore suspension of six KIS EPF isolates for 1 h 30 min so that the spores would get adsorbed to the surface of the potato tubers and potentially colonise the external or internal tissues of the plants. Tubers were air dried and planted the next day (Fig. 3).

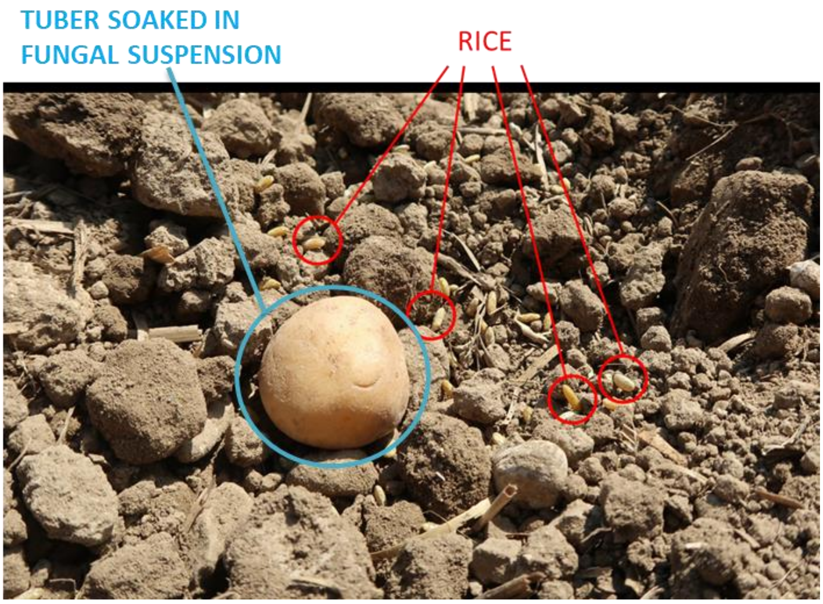

Fig. 3: Potato seed soaked in fungal suspension with fungal formulation multiplied on rice.

We had seven treatments in the experiment: (a) potato tubers soaked in the fungal suspension of all six isolates (Potato_fungi), (b) fungi formulated on rice (Potato_rice) and (c) a combination of both treatments (Potato_fungi_rice). In addition, two treatments with the commercial bioinsecticide ATTRACAP were made, testing either full (30 kg/ha) or half (15 kg/ha) recommended dose and the commercial insecticide Force (Syngenta, active ingredient Tefluthrin, 15 g/kg) was used as a conventional (positive) control treatment alongside the untreated control (Table 6).

Table 6: List of wireworm control treatments tested in 2020 and 2021

	2020	2021
Potato tubers soaked in the fungal suspension	✓	×
Fungi formulated on rice [51.3 kg/ha]	×	✓
Potato tubers soaked in the fungal suspension and fungi formulated on rice [51.3 kg/ha]	✓	✓
ATTRACAP full dose [30 kg/ha]	✓	✓
ATTRACAP half dose [15 kg/ha]	✓	✓
Negative control	✓	✓
Positive control – insecticide Force [5 kg/ha]	✓	✓

The experimental design was a complete randomised block design with six treatments and eight replicates in Field 1, Field 3 and Field 4, where we planted KIS-Savinja variety (N=576 tubers per treatment). Field 2 had six treatments and six replicates and we also planted the variety KIS-Kokra (N=252 tubers per treatment). At all locations, there were

2 rows of protection buffer zone on each side of the experiment. All fields were located at Agricultural Institute of Slovenia, Infrastructure Centre Jablje.

The effectiveness of tested methods was evaluated at potato harvest on 100 randomly selected tubers per plot as the amount of tuber yield and as the number of tubers where we counted more than one wireworm hole per tuber, since tubers with more holes are less attractive for consumers. Tubers from one row of each plot were additionally classified into four size classes (>65 mm; 65-45 mm; 45-25 mm; <25 mm) and the effect of the treatments on the number and mass of tubers in each size class was also tested.

Statistical analysis

For wireworm data, the effectiveness of treatments on the number and mass of tubers in each size class, on yield and on number of tubers with more than one hole was analysed using a one-way ANOVA, followed by Bonferroni-Holm multiple comparison test.

Results

The effectiveness of the different treatments was evaluated based on the number and mass of tubers in each size class, yield per row (in a single plot) and the number of tubers with more than one hole. Differences between treatments were not prominent (Fig. 4). Force appeared to be most effective in reducing the number of holes per tuber, especially on Field 1, where it reduced the number of tubers with more than one hole for 75.6%. The most effective EPF treatments for reducing the number of tubers with more than one hole were with potatoes soaked in fungal suspension at Field 1 (57.2% reduction) and potatoes soaked in fungal suspension together with fungi formulated on rice at Field 2 (43.9% reduction). In 2021, we made a change to one of the treatments, namely replacing potato tubers soaked in fungal suspension with fungi formulated on rice. In that year, the most effective EPF treatment for reducing the number of tubers with more than one hole was the half dose of ATTRACAP at Field 4 (49.5% reduction), while at Field 3 all EPF treatments resulted in higher number of tubers with more than one hole compared to the control.

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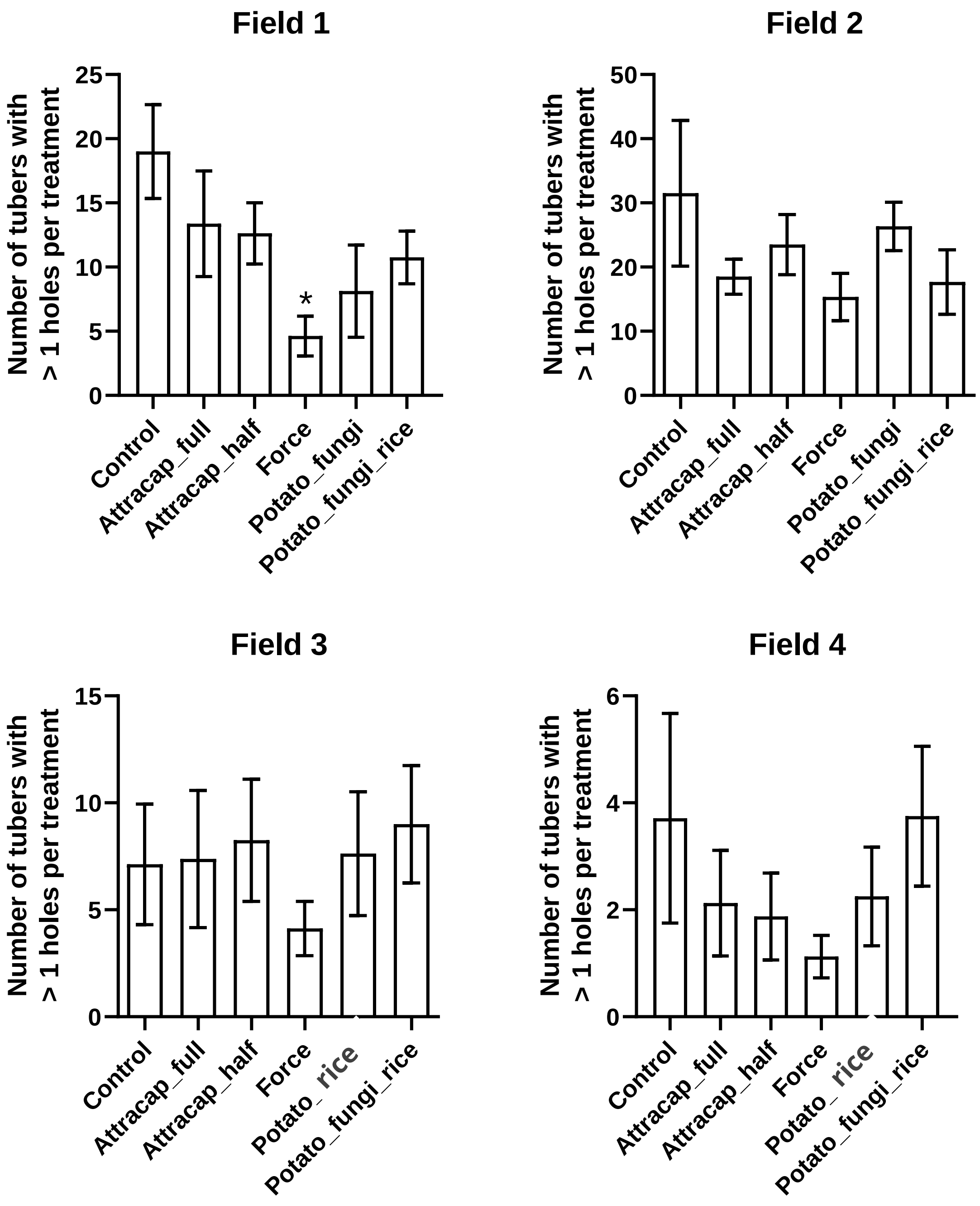

Fig. 4: Number of tubers with more than one hole in different treatments in year 2020 (Field 1 and Field 2) and 2021 (Field 3 and Field 4). Means marked with * are significantly different from the control group mean ($p<0.05$).

At all locations, tuber yield per row showed no significant differences between treatments, except a significant yield reduction from both the full dose of Attracap (21.3% reduction) and potatoes soaked in fungal suspension together with fungi formulated on rice (20.2% reduction) at Field 2. Nevertheless, full dose of Attracap increased yield by 16.7% at Field 3, but not significantly (Fig. 5). The treatments also had no significant effect on tuber number and mass in all four size classes (>65 mm; 65-45 mm; 45-25 mm; <25 mm).

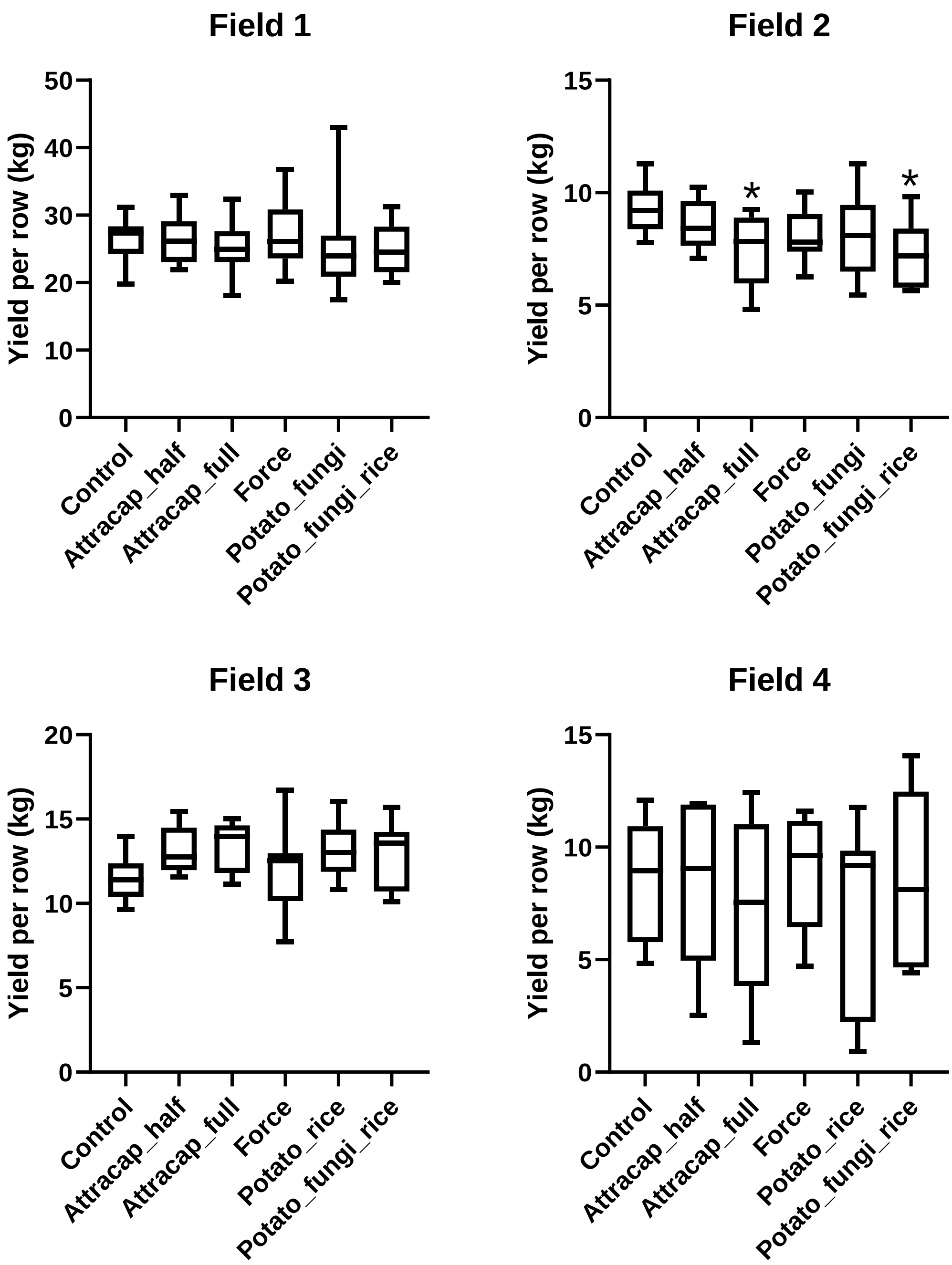

Fig. 5: Potato yield (kg per row length 6.3 m) in different treatments per row in year 2020 (Field 1 and Field 2) and 2021 (Field 3 and Field 4). Means marked with * are significantly different from the control treatment (p<0.05).

Conclusions on wireworms

Entomopathogenic fungi, especially from the genus *Metarhizium* (Ascomycota: Hypocreales), have been shown to be a promising solution for the control of wireworms in potato (Reinbacher et al. 2021; Brandl et al. 2017; Wraight et al. 2008). *Metarhizium* sp. naturally occurs in the soil and are therefore associated with soil-inhabiting insects like wireworms.

The results of testing various preparations containing *Metarhizium* on wireworms, especially potatoes soaked in fungal suspension and half dose of ATTRACAP resulted in lower number of tubers with more than one hole compared to the control. This was particularly evident at Field 1 and Field 4. Thus, the introduction of EPF in the rhizosphere as an attract-and-kill method, or as plant growth promotor by soaking tubers in fungal suspension, has the potential to reduce tuber damage.

Wireworms generally do not cause yield loss (Parker and Howard, 2001). This is consistent with our results, as wireworms did not affect the number or mass of the tubers and mostly did not cause yield losses at any location, with the exception of the full dose of Attracap and potatoes soaked in fungal suspension together with fungi formulated on rice at Field 2.

Overall, bioinsecticides based on entomopathogenic fungi were found to be quite effective in reducing potato damage caused by wireworms, as the effectiveness of the treatments, especially ATTRACAP half dose and potatoes soaked in fungal suspension, were comparable to that of a conventional insecticide based on tefluthrin (Force).

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