

Supplementary Material for:

Trap crops enhance the control efficacy of *Metarhizium brunneum* against a soil-dwelling pest

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2. Material and methods

2.1. Study site and field cultivation

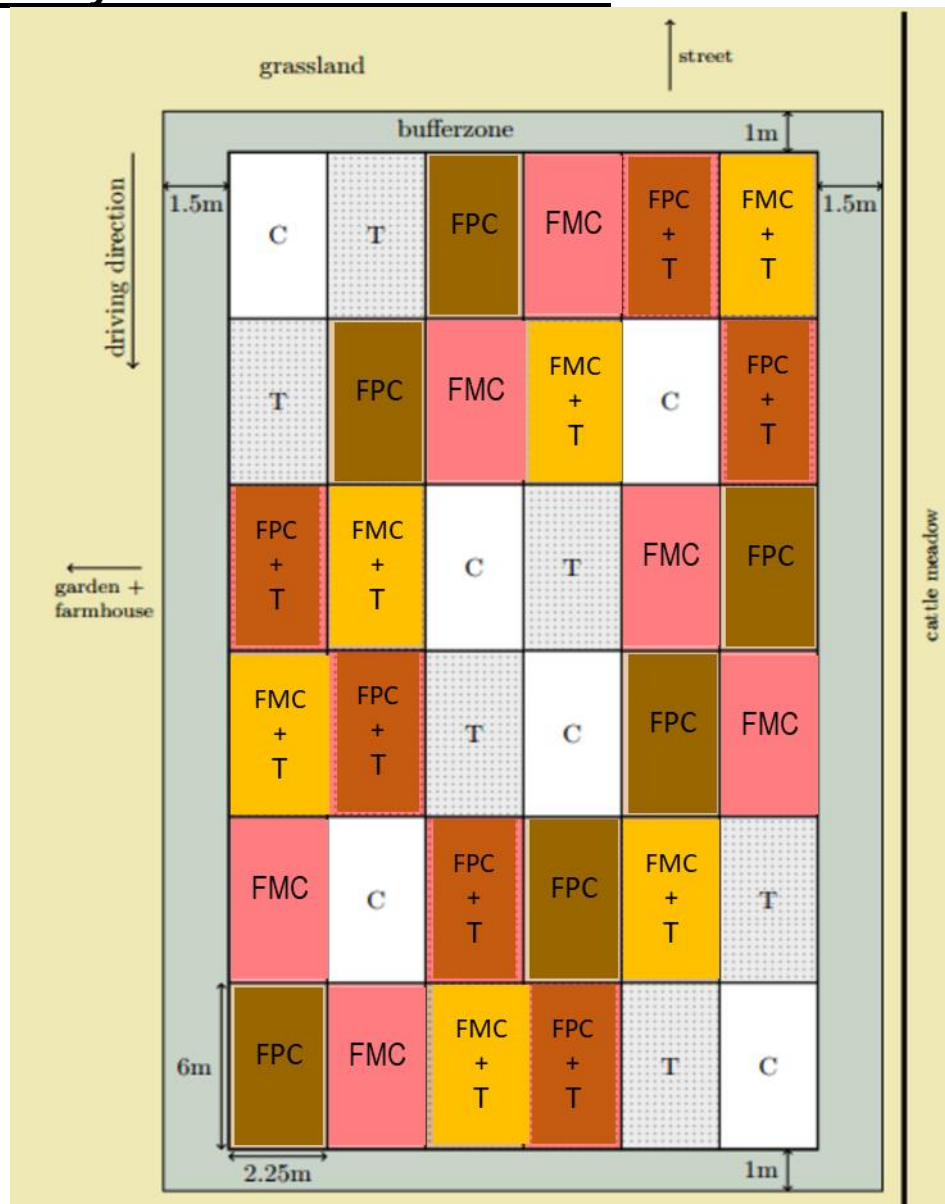


Figure 1: Study field setup with the distribution of the different treatments, C = Control treatment (white), T = trap crops (grey dotted), FPC = fungus applied in previous crop (brown), FPC + T = fungus applied in previous crop with trap crops (red), FMC = fungus applied in the main crop (pink) and FMC + T = fungus applied in the main crop with trap crops (yellow). Around the study field 1 m wide buffer zone was kept untreated. Each plot measured 2.25m*6m.

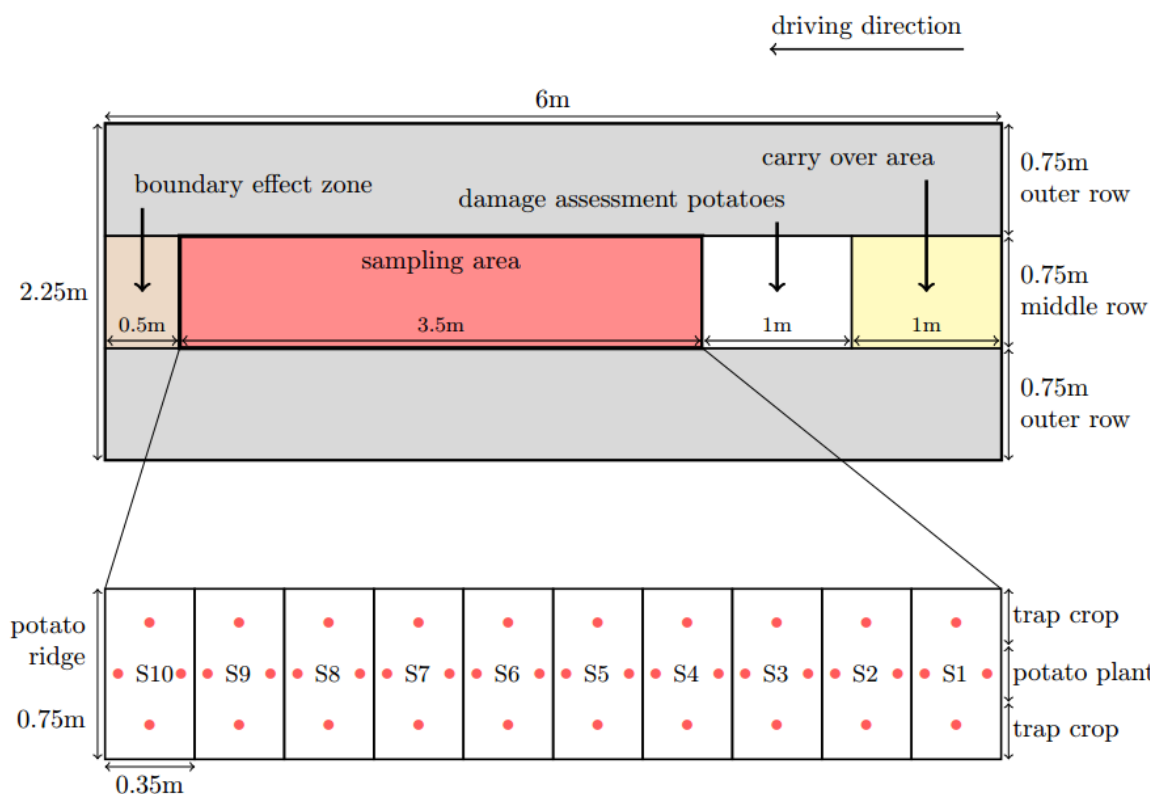


Figure II: Sampling design for each plot from the experimental field. Soil samples for wireworm collection were taken with driving direction. No samples were taken in the yellow area to avoid contamination from adjacent plots. The white area was used for damage assessment of potato tubers, no samples were taken there. In the red area soil samples were taken. The sampling area was divided into smaller sections with a length of 35 cm for each sampling date. The red dots represent where samples were taken. The brown area was used as a boundary effect zone where no samples were taken to avoid contamination.

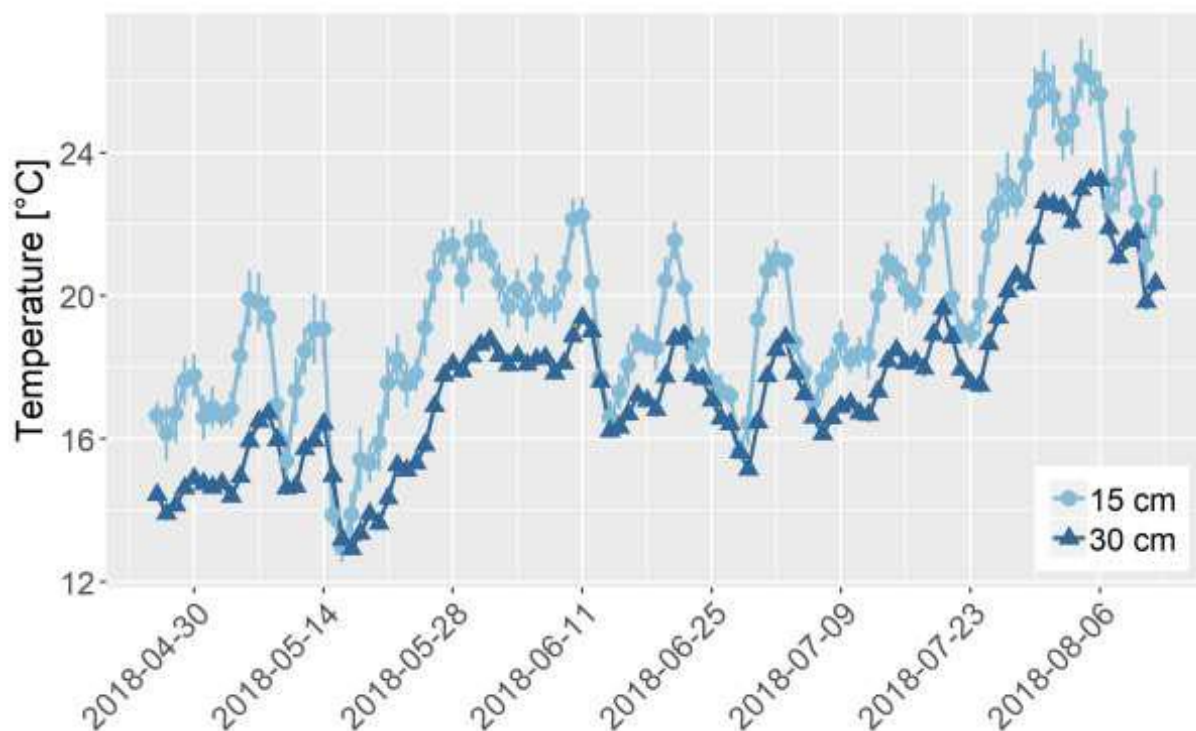


Figure III: Soil temperature in 15 cm (light blue) and 30 cm (dark blue) soil depth in the experimental field site during the investigation period from 26 April to 12 August 2018. The x-axis depicts the time span, while the y-axis pictures the soil temperature (°C).



Figure IV: Seasonal development of the trap crop. From left to right: Seed germination one week after planting on June 20, growing state during mid-season on July 26, trap crop during harvest time on August 24.

2.6. Determination of species and larval stage, and gut content analysis

PCR protocol species determination

PCR was carried out in 10 μ l reactions, each consisting of 2 μ l PCR-grade RNase-free water (Qiagen), 5 μ l of 2x Reaction mix (MP-Mix Qiagen), 0.5 μ l of each primer (10 μ M), 0.5 μ l bovine serum albumin (BSA, 10 mg/ml) and 1.5 μ l sample DNA. The thermocycling protocol, was carried out on the Mastercycler® Nexus (Eppendorf), consisted of an initial activation phase of 15 min at 95°C, followed by 35 cycles of 30 s at 94°C, 90 s at 62.5°C, 1 min at 72°C and a 10 min final elongation step at 72°C. All PCR products were stored at -28°C and later size-separated and visualized using the screening cartridge within the QIAxcel capillary electrophoretic system.

PCR protocol molecular gut content analysis

Amplifications were performed in a 15 μ L assay consisting of 1 μ L RNase-free water (Qiagen), 7.5 μ L TypeIT Mutation™ Detect PCR Kit (Qiagen), primer with respective concentration (see Table i below), 0.5 μ L BSA (concentration: 10 μ M/ml), 0.5 μ L 5x Q-Solution (Qiagen) and 4 μ L sample DNA. The thermocycling protocol contained an initial step of 5 min at 95 °C, 35 cycles of 92 °C for 20 s, 30 s annealing at temperatures as shown in Table i, 70 °C for 30 s and a final elongation step of 5 min at 70 °C, keeping the PCR at 4°C afterwards.

Solanum tuberosum primers were designed and tested for their functionality with newly extracted *S. tuberosum* samples as well as samples from all the attraction plants and *Solanum nigrum* to exclude amplification of DNA from non-target species.

Optimal annealing temperatures were tested in gradient PCRs.

Table i: Multiplex PCR Protocols applied for gut content analysis. Tested plants and their corresponding primers with sequences and fragment size as well as their concentration in the reaction and respective annealing temperature.

Multiplex	Target Species	Sense Primer Antisense Primer	Sequence 5'-3'	Fragment size	conc. [µM]	Annealing Temperature [°C]
FLPS	<i>Fagopyrum esculentum</i>	Fag-sp-S519 Fag-sp-A523	GAAAACGAAAGGAAAGGTTTCAT CAGGATTACCCGTTTTTTGA	380	0.2	56
	<i>Lolium perenne</i>	Lol-per-S528 Lol-per-A535	GCATTTTTCTATATAGAATGGAT TTTTTGTTCTAACCTCATGCTAT	254	0.4	
	<i>Phaseolus coccineus</i>	Pha-sp-S525 Pha-sp-A531	ATCCTTTCACAAAAATTCCAG TGGATCAGTTCCTCAAGGGT	235	0.2	
LF	<i>Lupinus angustifolius</i>	Lup-sp-S522 Lup-sp-A527	GAATCCATTCAACAGTTCTG GAACTTTTCTTTGTTTTGCG	244	0.2	53
	<i>Fagopyrum esculentum</i>	Fag-sp-S519 Fag-sp-A524	GAAAACGAAAGGAAAGGTTTCAT TATTACCCCTTTCATACCGCAT	206	0.2	
TAT	<i>Taraxacum officinale</i>	Tar-sp-S546 Tar-sp-A554	CGGTTCAAAACTCCTTTATG TTCCTCATGTCTCATCCTT	194	0.2	58
	<i>Trifolium repens</i>	Tri-sp-S550 Tri-sp-A558	CAGTAGGAAAGGAATCGTTCT AATCTTTTCATTTGTGATAGAAAAG	172	0.2	
	<i>Trifolium pratense</i>	Tri-sp-S550 Tri-sp-A558	CAGTAGGAAAGGAATCGTTCT AATCTTTTCATTTGTGATAGAAAAG	151	0.2	
TS	<i>Triticum aestivum</i>	Tri-aes-S536 Tri-aes-A545	GCTATTAAGTAGTTCTAAATTTGAAGTTA CCTCCCGTCTTACTTTTTTAT	306	0.2	54
	<i>Solanum tuberosum</i>	/	/	112	0.2	

3. Results

3.1. *Metarhizium spp.* densities

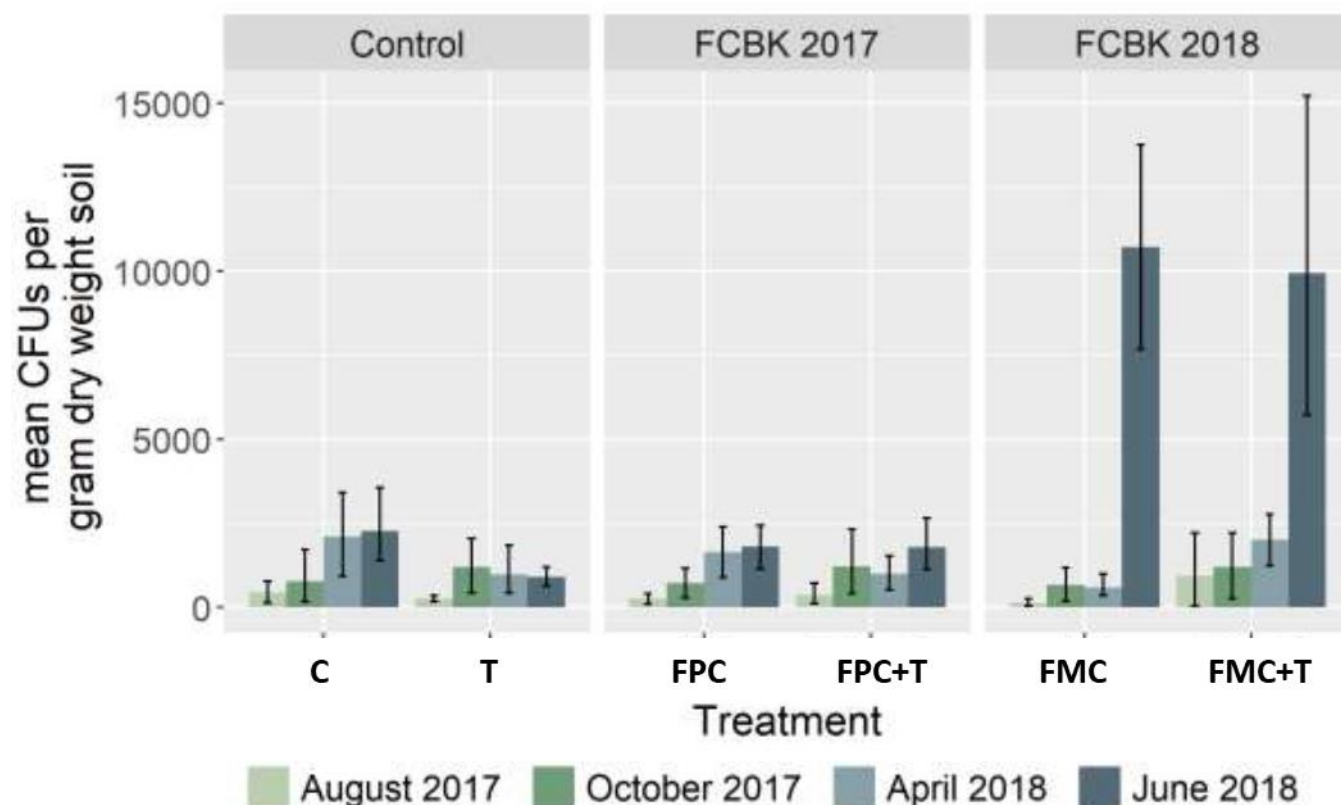


Figure V: Mean colony-forming units per gram of dry weight soil of the fungus *Metarhizium brunneum* in one with 95% bootstrapped confidence intervals (y-axis) displayed for the six treatments C = Control treatment, T = trap crops, FPC = fungus applied in previous crop, FPC + T = fungus applied in previous

crop with trap crops, FMC = fungus applied in the main crop and FMC + T = fungus applied in the main crop with trap crops (x-axis) for the four different timepoints between August 2017 and June 2018 (color).

3.2. Wireworm abundance

Of all larvae, 88.7% could be molecularly identified and only the two species *Agriotes sputator* (77%) and *Agriotes obscurus* (23%) were present among all samples. Species ratio did not significantly differ between treatments or sampling dates. When partitioning the sampled larvae by soil sample location, 69% of all individuals were caught in the ridge center in between potato plants and 31% at the slope of the ridge. The location ratio varied between treatments, with the highest proportion of 40% larvae caught in the ridge slope in the Control treatment when trap crops were present and the lowest proportion of 19% larvae caught in the ridge slope in treatment FPCC combined with trap crops. Species ratio did not significantly differ between treatments, except for the FPCC treatment without trap crops, where 8% less *A. obscurus* could be found compared to the other treatments. *Agriotes sputator* was present in all eight larval instars, *A. obscurus* larvae could only be collected in the instars L3 to L8. For both species, the largest fraction of individuals belonged to L6 (25% for *A. sputator* and 32% for *A. obscurus*), followed by L5 and L7. Larval stage distribution showed a very similar pattern for both species and no difference between sampling location or treatments could be detected. Species and larval stage distribution between trap crop and potato rows did not show a significant difference between treatments.

Table ii: Total wireworm numbers for each treatment (C (control), T (trap crop), FPC (fungus previous crop), FMC (fungus main crop), FPC + T (fungus previous crop & trap crop), FMC + T (fungus main crop & trap crop)), divided by species affiliation (*Agriotes sputator* or *Agriotes obscurus*) and by the location of the collected soil sample. The numbers in the parentheses represent relative numbers.

	Species		Soil sample location	
	<i>A. sputator</i>	<i>A. obscurus</i>	ridge center	ridge slope
C	47 (78%)	13 (22%)	54 (75%)	18 (25%)
T	38 (76%)	12 (24%)	33 (60%)	22 (40%)
FPC	41 (72%)	16 (28%)	42 (68%)	20 (32%)
FMC	50 (86%)	8 (14%)	40 (62%)	24 (38%)
FPC + T	39 (75%)	13 (25%)	48 (81%)	11 (19%)
FMC + T	21 (72%)	8 (28%)	21 (64%)	12 (36%)
Σ	236 (77%)	70 (23%)	238 (69%)	107 (31%)

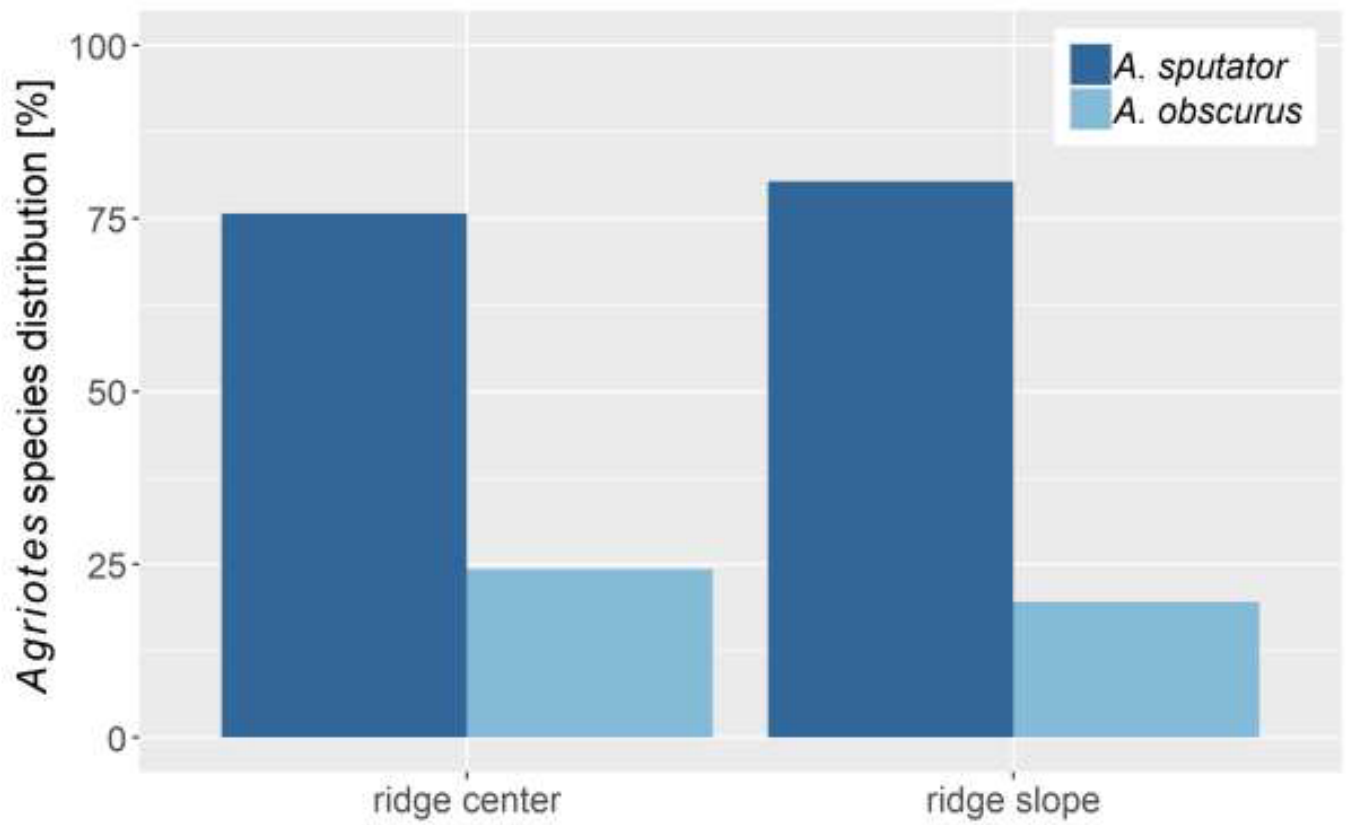


Figure VI: Distribution of the two wireworm species *Agriotes sputator* and *Agriotes obscurus*, based on the location (x-axis) of the collected soil samples in percent (y-axis).

3.3. Wireworm diet

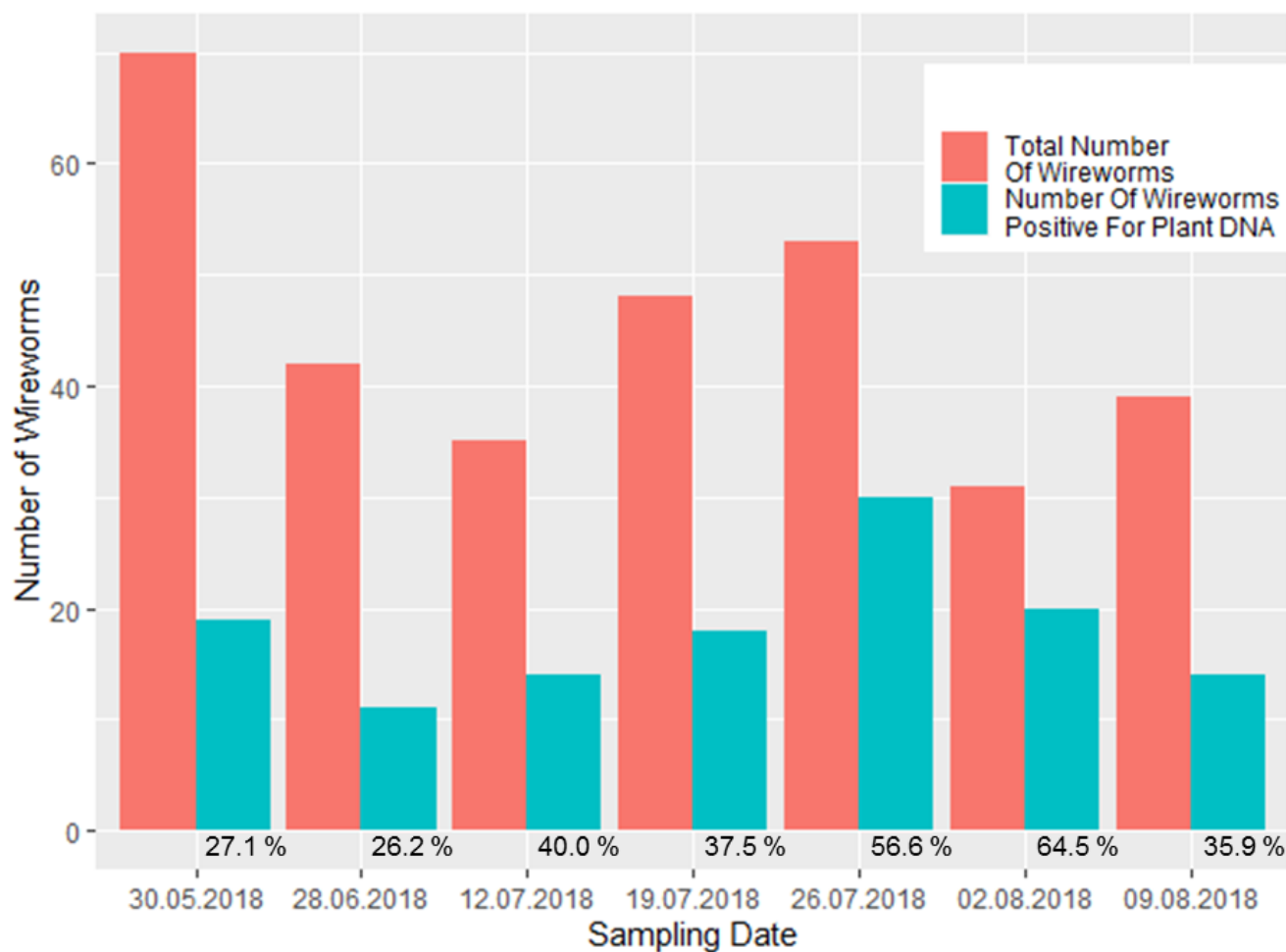


Figure VII: Comparison of the total number of wireworms (red) found on each sampling date to the number of wireworms positive for plant DNA (blue) with the resulting percentage of plant-positive wireworms.

3.4. Potato yield

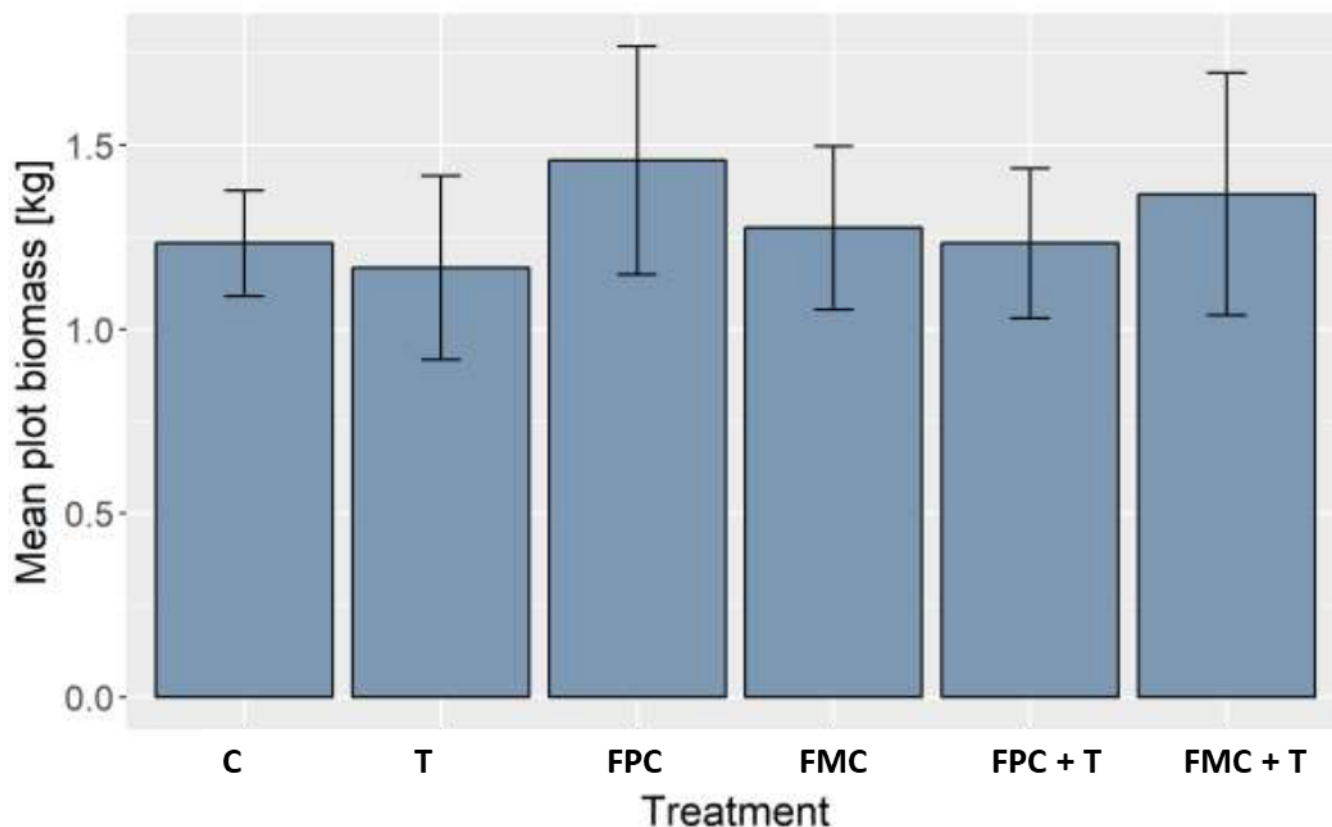


Figure VIII: Mean plot biomass in kilogram (y-axis) with 95 % confidence intervals displayed for each treatment: C = Control treatment, T = trap crops, FPC = fungus applied in previous crop, FPC + T = fungus applied in previous crop with trap crops, FMC = fungus applied in the main crop and FMC + T = fungus applied in the main crop with trap crops (x-axis).

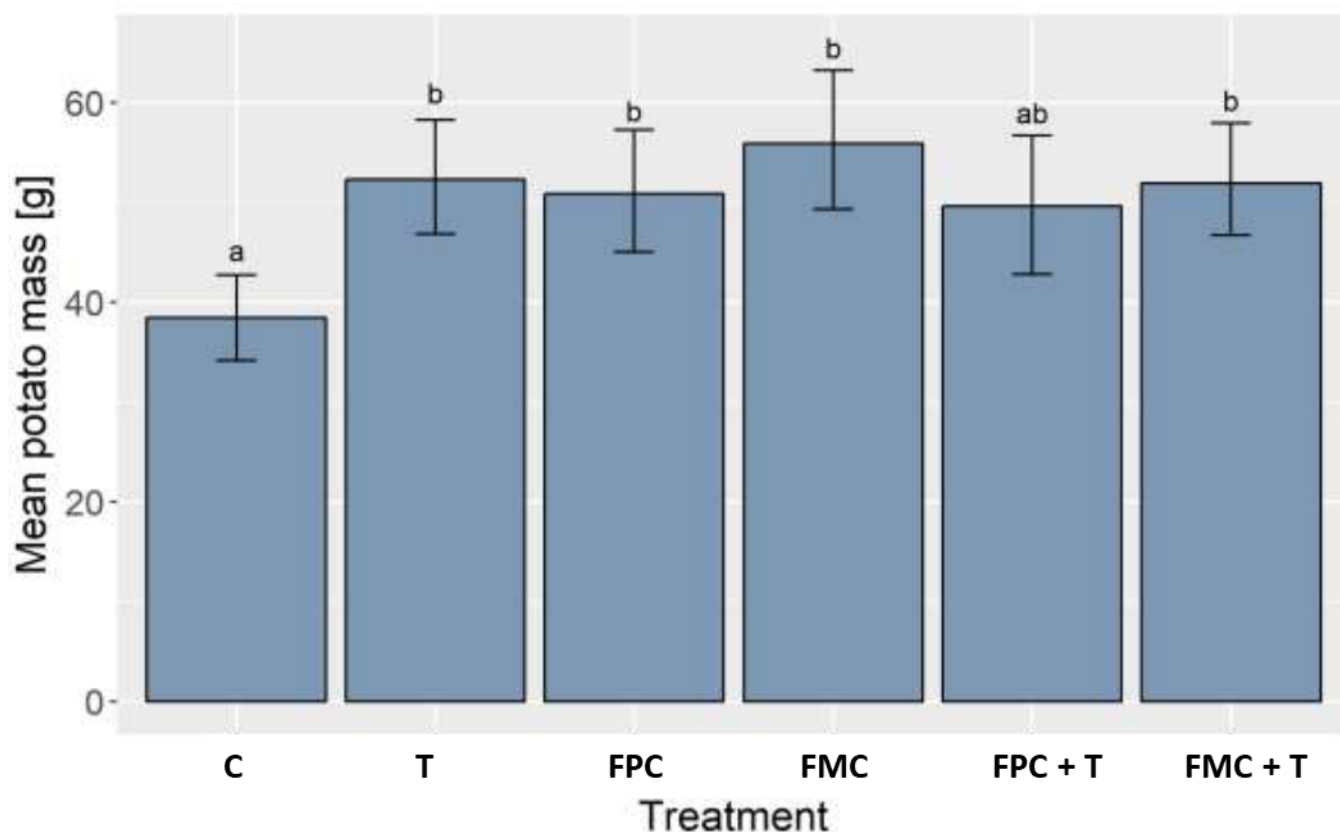


Figure IX: Mean mass of one potato tuber in gram (y-axis) with 95 % bootstrapped confidence intervals for each treatment: C = Control treatment, T = trap crops, FPC = fungus applied in previous crop, FPC +

T = fungus applied in previous crop with trap crops, FMC = fungus applied in the main crop and FMC + T = fungus applied in the main crop with trap crops (x-axis). Significant differences between treatments are illustrated by small letters and calculated by multiple comparisons Kruskal post-hoc.

Table iii: The proportion in percent of potato tubers reaching at least the required mass for the different square mesh sizes displayed for each treatment: C (control), T (trap crop), FPC (fungus previous crop), FMC (fungus main crop), FPC + T (fungus previous crop & trap crop), FMC + T (fungus main crop & trap crop).

square mesh size [mm]	C	T	FPC	FMC	FPC + T	FMC + T
35 (min. 31.8 g)	57	73	68	72	60	67

Table 4: Summary of all relevant results concerning potato damage, wireworm abundance and *Metarhizium* spp. densities compared among treatments: C (control), Trap (trap crop), FPC (fungus previous crop), FMC (fungus main crop), FPC + T (fungus previous crop & trap crop), FMC + T (fungus main crop & trap crop).

Treatment	Wireworm Holes / g potato		Wireworm number per sampling session		<i>Metarhizium</i> spp. densities in June 2018 (CFUs/g soil dry weight)		Damaged tubers (> 2 holes)	Average reduction wireworm holes / g potato (%)	Average reduction wireworm abundance (%)
	Mean	SD (±)	Mean	SD (±)	Mean	SD (±)			
Control	0.12815	0.06037868	3.50	0.30954940	2122.0	1437.243	66.67%		
Trap	0.08981	0.03526553	2.94	0.27262380	972.5	464.409	66.67%	29.92%	15.89%
FPCC	0.09954	0.02348759	3.22	0.24877150	1688.2	792.794	83.30%	22.33%	7.94%
FPCC + T	0.09365	0.05093745	2.94	0.23576240	1821.2	944.744	56.96%	26.92%	15.89%
FMCC	0.08368	0.04077474	3.33	0.25888580	8073.0	2144.485	48.23%	34.70%	4.77%
FMCC + T	0.07368	0.03387423	1.72	0.14309400	8401.0	3945.817	66.67%	42.50%	50.80%