Deliverable 4.3

[Report on the efficacy of a lure and kill strategy against western corn rootworm larvae under semi-field conditions]

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Deliverable 4.3 Report on the efficacy of a lure and kill strategy against western corn rootworm larvae under semi-field conditions

The efficacy of the "kill" component (*Metarhizium brunneum*) for the "lure and kill" strategy was screened in a series of greenhouse trials. These screenings allowed the selection of the most suitable *M. brunneum* strain and formulation for a successful implementation of a "lure and kill" strategy against western corn rootworm larvae.

Greenhouse trial 1: Robustness of BIPESCO 5

The robustness of the commercially viable entomopathogenic fungus (EPF) *M. brunneum* strain "BIPESCO 5" (provided by P3) against western corn rootworm (WCR) larvae has already been evaluated as part of Deliverable 4.1. The application of the EPF ($2x10^7$ BIPESCO 5 spores/ plant = 1 x 10^{13} spores / ha) reduced the larval density by 46% (17 larvae/plant in the control; 9 larvae/plant with BIPESCO5 ($t = 2.21$; $P<0.05$; Fig .1)) compared to the untreated control after 3 weeks of larval development. The BIPESCO 5 strain was therefore regarded as suitable for further evaluation as a formulated product and for integration into a "lure and kill" approach against WCR larvae

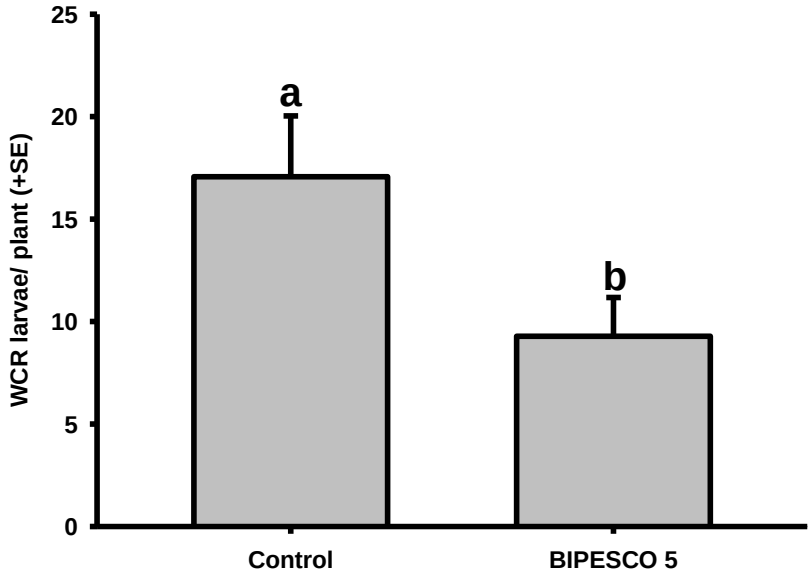

Fig. 1: Number of WCR larvae extracted from the root system of each plant 3 weeks after larval hatch with the application of BIPESCO5 spores.

Greenhouse trial 2: Screening of BIPESCO 5 formulations

A number of different potential formulations for an encapsulation of BIPESCO 5 spores were available from P2. It was therefore decided to initiate an additional study, prior to the semi field study on lure and kill, to evaluate the efficacy of different BIPESCO 5 capsule formulations. This study aimed to support the decision on the use of a specific formulation to test a “lure and kill” approach and exclude any potential harmful or negative effect for BIPESCO 5 viability through a formulation.

The following BIPESCO 5 treatments were set up:

1. Untreated control
2. Unformulated spore suspension with spores from culture (cultured by P1) (**S_E**)
3. Unformulated spore suspension with spores from spore powder (provided by P 11) (**S_O**). Spore powder will be used in capsule formulation.
4. Capsule formulation **Typ I** (Alginate) (provided by P2)
5. Capsule formulation **Typ II** (Alginate+Chitosan) (provided by P2)
6. Capsule formulation **Typ III** (Alginate+Lignin) (provided by P2)
7. Capsule formulation **Typ IV** (Alginate+Gelantine) (provided by P2)
8. Capsule formulation **Typ V** (Combination of all) (provided by P2)

The screening of these treatments was done in a greenhouse trial (23 ± 1°C air temperature and 65% air humidity) with a similar experimental set up as previously used in greenhouse trial 1 (Fig. 2). The plants were grown in small plant pots (9 cm diameter) for about 2 weeks (plant growth stage BBCH 12) and then transferred to soil in larger plant pots (15 cm diameter). The BIPESCO 5 treatments were applied to the large plant pots by evenly mixing the treatment with soil prior to the transfer of the plants. This ensured a good distribution of the BIPESCO 5 treatments in the soil of the larger pots. The plants were carefully removed from the soil in the small plants pots, any loosely adhering soil removed from the roots and then inserted into the treated soil of the larger pots. 13 replicates (= 13 plant pots) were set up for each treatment.

The soil was treated with $3x10^8$ BIPESCO 5 spores / plant (= 1×10^{14} spores / ha) and was therefore higher than in the greenhouse trial 1 (= $2x10^7$ BIPESCO 5 spores / plant) to ensure better control efficacies. The spore concentration is still within the

advised concentration for an application in the field (= $1x10^{12}$ - $1x10^{14}$ spores / ha). Each capsule formulation contained $1x10^{8}$ Spores / g capsules and therefore 3g capsules of each formulation were applied per plant pot.

10 days after the application of the BIPESCO 5 treatments, 350 WCR eggs were inoculated into the soil at 7 cm depth. Such an egg density is higher than usual (~100 eggs/plant) but accounts for a lower egg viability previously measured in a viability check. The time of first hatch and the hatching rate was monitored in a Petri dish. The time of first larval hatch was observed 16 days after egg inoculation and 25 days after the BIPESCO 5 treatments were set up. After 21 days of larval development (most of the larvae were at the last larval stage), the soil and roots were removed from each plant pot and placed in a Kempson Extraction chamber. The larvae were extracted from the soil with heat (60°C) for 72 hours and the number of extracted WCR larvae/plant counted. With this approach the larval density per plant could be determined and the efficacy of the BIPESCO5 treatments calculated.

A quality control of each BIPESCO 5 treatment was set up to ensure that the spores were viable for fungal growth and infection. The growth of spores was monitored in Petri dishes by placing one capsule of each formulation on water agar or by evenly spreading unformulated spores on the water agar. Six Petri dishes were set up for each of the eight treatments. The infection was measured in small plastic boxes (10 x 7.5 x 4 cm) filled with the same soil as used in the greenhouse trial. The equivalent spore or capsule concentration as used in the plant pots was applied to the soil. Five mealworm larvae (*Tenebrio molitor)* were added to each plastic box (Fig. 2) and four plastic boxes (= replicates) were set up for each treatment. The mealworm larvae were checked for *M. brunneum* infection every week for 4 weeks (Fig. 2). Dead mealworm larvae with no signs of *M. brunneum* infection were placed in a moisture chamber (a Petri dish with moist filter paper) to check for *M. brunneum* infection at a later time.

Quality control of fungal growth showed good viability of all the tested BIPESCO 5 treatments and *M. brunneum* growth was observed in all treatments (Fig. 1). Infection of *M. brunneum* in mealworm larvae was also observed in all treatments and none in the control. The percentage of mycosed mealworm larvae ranged from 40% – 43% in the unformulated treatments with the spore suspensions (“S_E” and “S_O”) and from 36% (capsule formulation **Type II**) to more than 50% (capsule formulation **Type I, IV**

and V) with BIPESCO 5 capsule formulations. The production, formulation, transport and application procedure of the BIPESCO 5 spores therefore did not significantly affect viability of the fungus.

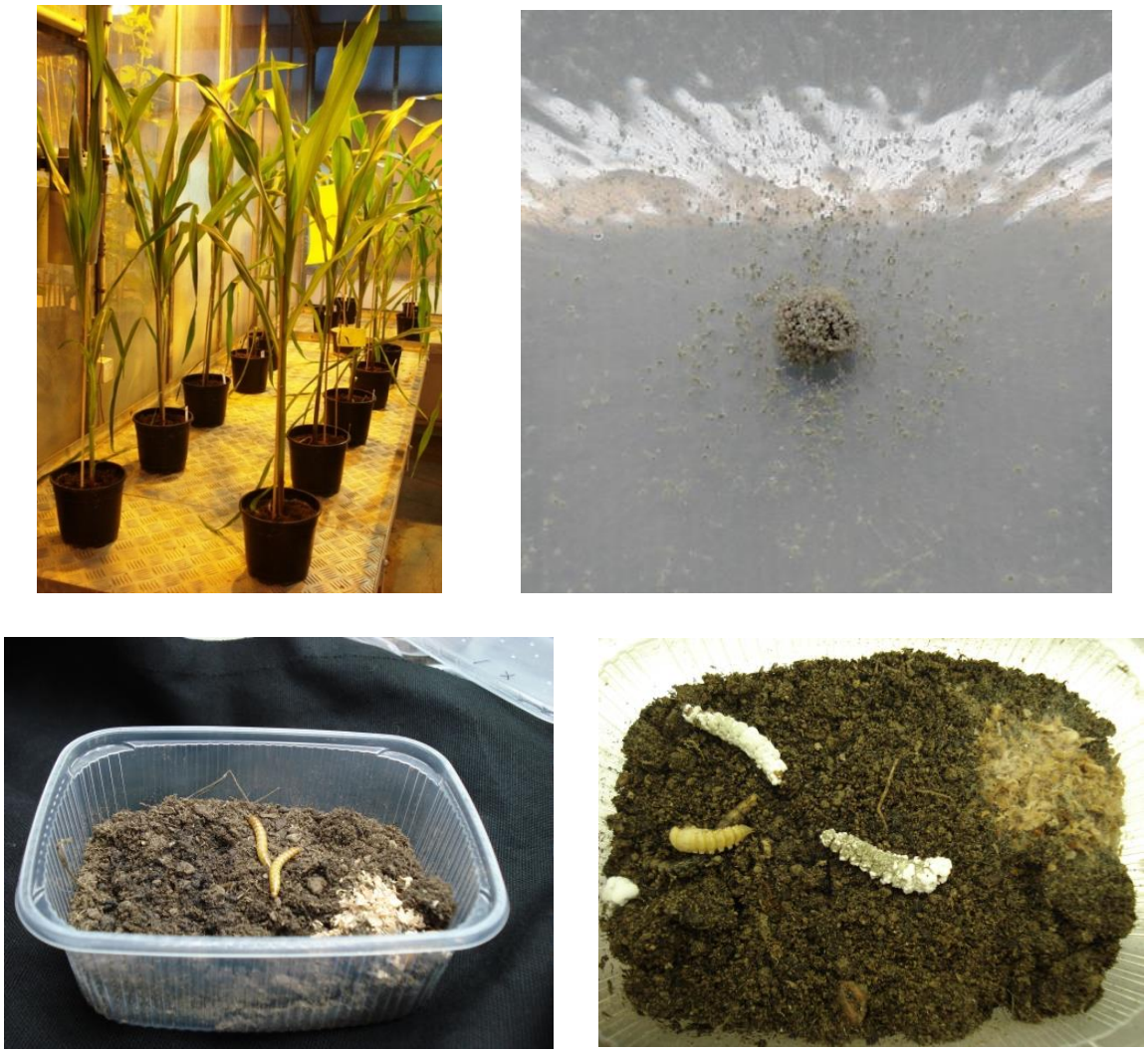

Fig. 2: Experimental set up of greenhouse trial to test different BIPESCO 5 formulations (TOP left picture); *Metarhizium brunneum* growth from a capsule (Formulation TYPE II) (TOP right picture); Quality control of *Metarhizium brunneum* infection with mealworm larvae (bottom pictures)

The larval density / plant after 21 days of larval development was significantly affected through the application of BIPESCO 5 treatments (One way ANOVA $F_{7, 96}$ = 3.19, P < 0,05 followed by Bonferroni test, Fig. 3). The unformulated BIPESCO 5 treatments (= spore suspension “S_O” and “S_E”) caused a low but non-significant reduction in larval density (6.77 larvae in each spore suspension treatment) compared to the untreated control (8.77 ± 1.1 larvae). Three BIPESCO 5 capsule

formulations (Type III – V) caused a higher reduction in larval density than the unformulated BIPESCO 5 treatments; the larval density in these treatments (4.84 – 6.64 larvae/plant), however, was also not significant compared to the untreated control. Two BIPESCO 5 capsule formulations (Type I and II) resulted in a significant reduction in larval densities (3.9 larvae/plant) compared to the untreated control. There was a lower but non-significant reduction in larval density with formulation Type I and II compared to the unformulated treatments ("S_O" and "S_E").

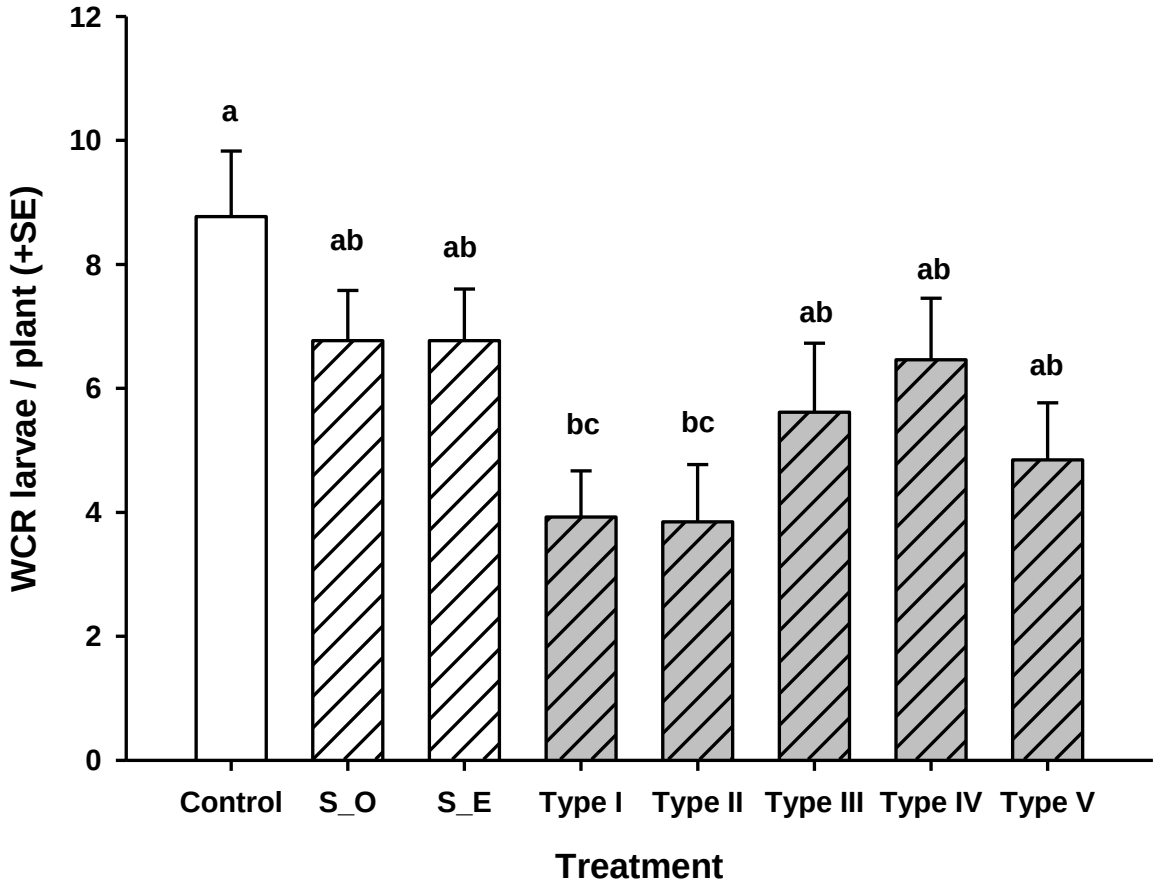

Fig. 3: Number of WCR larvae extracted from the root system of each plant 3 weeks after larval hatch; White hatched columns show unformulated spores applied as a spore suspension with spores from culture (S_O) and spores from spore powder (S_E); Grey hatched columns show formulated spores: Type I: Alginate; Type II: Alginate + Chitosan; Type III: Alginate + Lignin; Type IV: Alginate + Gelatine; Type V: Combination of all

The unformulated BIPESCO5 treatments “S_O” and “S_E” in greenhouse trial 2 did not result in a significant reduction of larval density. The significant control efficacy with unformulated BIPESCO5 evaluated greenhouse trial 1 can therefore not be replicated, despite the higher spore density applied in greenhouse trial 2. The main factor which might have caused such a discrepancy could be related back to the lower larval density of the control in greenhouse trial 2 (8.77 ± 1.06 larvae vs 17. 07 ± 2.97 larvae in greenhouse trial 1). A lower larval density may reduce the fungal dispersal and lower the chance of infection with a WCR larva. Infected larvae may also serve as an inoculum and create “spore hot spots” for further infections with other WCR larvae. This should subsequently increase spore density in the soil. A lower larval density could subsequently reduce the increase in spore density in the soil. We therefore hypothesize that the pest density in the soil has a significant effect on the efficacy of EPF.

The encapsulation of the spore powder (used in treatment “S_E”) into different capsule formulations resulted in a varied efficacy depending on the type of capsule tested. The formulations Type III, IV and V did not result in significant control efficacies. The capsule types I + II resulted in a control efficacy of 55% and 56%, respectively, and could subsequently be suitable to WCR control. Capsule type I offers a cheaper and easier production possibility as only alginate needs to be used for formulation. Capsule type II would need a higher production input compared to capsule Type I. The anti-microbial property of the biopolymer chitosan as a capsule coating, however, offers protection from capsule contamination from other soil microorganisms. Capsule type II can therefore be regarded as technically more advanced over capsule type I.

The spore concentration in the capsules was higher (= $1x10^8$ *M. brunneum* Spores/ g capsules) than in previous experiments with other *M. brunneum* capsules (= $2.5x10^7$ *Metarhizium* Spores/ g capsules with strain ART 2825 for the evaluation of a “lure and kill” strategy against wireworm in field trials as part of deliverable 4.2). This resulted in a 10 % instead of 1 % EPF biomass. A lower EPF biomass/g capsules results in a better quality of the capsule formulation and may also promote better EPF growth and establishment. Future studies will therefore try and aim at a low EPF biomass in the capsule (1- 3% EPF biomass) to reach a range ~ $1x10^7$ *Metarhizium*-Spores/ g capsules.

The screening of five different BIEPSCO 5 formulations identified a positive effect of a spore formulation on the control efficacy of WCR larvae. Two formulations (capsule formulations Type I and II) can be regarded as suitable for further investigations. These formulations will be screened more thoroughly to identify the more suitable out of the two formulation types for future use. Type II is considered as technically more advanced and will therefore be used in an evaluation of a lure and kill strategy against WCR larvae in the semi field trials.

Greenhouse trial 3: Semi field trial on the "lure and kill" approach with a BIPESCO 5 formulation

The screening of the BIPESCO 5 formulation caused a delay in the evaluation of a "lure and kill" strategy against WCR larvae in a semi field trial. The semi field trial is currently ongoing and only the experimental set up and procedure will be described for this deliverable. The first results are expected in March 2014 and will help to develop application rates and procedures to test the lure and kill approach under field conditions in 2014 as part of deliverable 4.5.

The semi field plots (50cm x 80cm) were set up in 500 L containers in the greenhouse (Fig. 4). Maize plants are grown in seedling trays and transferred to the container at growth stage BBCH 12. Six maize plants are planted in each semi field plot (13 cm within and 60 cm between maize row distances) and grown until the maize plants have reached growth stage BBCH 14-15.

Fig. 4: Set up of the semi field trial to test a "lure & kill" strategy against WCR larvae in the greenhouse

The following treatments will be set up in the semi field trial:

1. Untreated control
2. BIPESCO5 capsules (Type II formulation) and CO_2 capsules applied between the maize rows (= “Lure and Kill” treatment)
3. BIPESCO5 capsules (Type II formulation) applied between the maize rows
4. BIPESCO5 capsules (Type II formulation) applied in the maize rows

Based on results from the previous greenhouse trials, BIPESCO5 capsules (Type II) will be used as the “Kill” component. CO_2 capsules were evaluated as attractants in WP2 and will be used as the “Attract” component. A co-formulation of these two capsules systems is under development at the moment and not available for the semi field trial. The lure and kill approach (treatment no. 3) will therefore be tested with two capsule systems (BIPESCO 5 and CO_2 capsules) which will be mixed prior to soil application. The capsule will be applied in a 5-7 cm deep furrow.

One week after capsule application, 100 WCR eggs will be applied to each plant. Hatching time and rate will be monitored as done in previous experiments. After 21 days of the first larval hatch, larval density/plant will be determined to calculate the efficacy of the different treatments. Quality control of BIPESCO 5 products and CO_2 capsules will be set up as done in previous experiments.

General discussion D 4.3

The screening of the *Metarhizium brunneum* strain BIPESCO 5 at different stages of the formulation process (i.e. mass production by P11 and formulation by P2) allowed excluding any negative effect on EPF viability for WCR larval control. The screening of different capsule formulations identified BIPESCO 5 capsules for WCR control. The results from these screenings helped to select the most suitable formulated “kill” component as part of the efficacy trial for a “lure and kill” approach. The semi field and field trials in WP4 will show whether this approach will be a suitable option to manage WCR larvae.