Artificial hideouts for control of fruit moths: Persistence of the entomopathogenic fungi *Metarhizium anisopliae* and *Isaria fumosorosea* under semi-field condition

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Abstract

The usage of corrugated cardboard and mulches as artificial hideouts for cocooning and pupating of different fruit moths is under discussion. Therefore, under semi-field conditions the persistence of two entomopathogenic fungi formulated in oil or water and two different application strategies on mulches were compared. Additionally, for Isaria fumosorosea *the persistence of different produced spores was investigated. The results demonstrate that rape seed oil and* I. fumosorosea *persist on mulches for at least two months. But the persistence is influenced by environmental factors, especially rainfall. In contrast to* I. fumosorosea *the efficacy of* M. anisopliae *was not sufficient. Submerged spores of* I. fumosorosea *were as persistent as aerial conidia and its persistence was not influenced by the formulation, but by the application.*

Keywords: *Cydia funebrana*, biological control, entomopathogenic fungi, persistence

Introduction

Within a national funded project for biological control of the Plum Fruit moth (*Cydia funebrana*) we investigated the potential of artificial hideouts treated with entomopathogenic fungi. In Germany plum fruit moths generate two generations with overwintering larvae of the second one. Assuming that larvae of both generations pupate between the bark of the tree or closed to the stem one control strategy by treating these or artificial hideouts with entomopathogenic fungi is under discussion. One constrain of the application of living microorganisms is the environmental stability of the microorganism. Therefore, experiments on the persistence of the two selected entomopathogenic fungi *Metarhizium anisopliae* and *Isaria fumosorosea* on mulch was investigated over two months under semi-field conditions. Because the persistence and efficacy of biocontrol agents is influenced by the produced inoculum and the formulation two type of spores produced in liquid or solid state fermenter and oil or water based formulations were compared. Because the applicability in the field is often a constrain for implementation two different application strategies were compared.

Material and Methods

Because it was not possible to establish an artificial rearing of *Cydia funebrana* these experiments were carried out with larvae of *Cydia molesta* as a model species. For artificial rearing of *Cydia molesta* adults were kept in cylinder containing plastic foil for egg deposition. After three days the plastic foil with deposited eggs was transferred in boxes containing artificial diet (according to Ivaldi-Sender, 1974) and was kept at 25° C and 16 h light. Hatched larvae penetrated the artificial diet. Just before pupating the larvae (L_5) left the artificial medium and these larvae were used for all experiments.

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Production and formulation of the entomopathogenic fungi: Conidia of *Metarhizium anisopliae* (strain Ma43 = BIPESCO5 = F52) were produced in a Prophyta- laboratory solid state fermenter for 14 days at 25° C with a flexible aeration rate of 0.05-0.15 Nl/h and gram substrate (mixture of paraboiled rice and oat, 4:1). Conidia of *I. fumosorosea* (Strain Pfr4) were produced in 1-liter solid state fermenter for 14 days at 25° C with a fixed aeration rate of 0.15 Nl/h and gram substrate (mixture of paraboiled rice and oat, 4:1). For the production of submerged spores strain Pfr4 was grown in a liquid fermenter (Minifors, Infors, Switzerland) with 3.5 l medium (25g/l glucose, 20g/l Cornsteep, 4g/l NaCl) for 72h at 25° C. For separation of submerged spores the liquid medium was filtered over three layers of mull.

For preparing the oil based formulation the overgrown substrate of the solid state fermentation was suspended in rape seed oil and sieved with a mesh size of 150µm. For the water based formulation conidia were washed with deionised water containing 0.01% Tween 80. For comparison of formulations of Ma43 the formulations were adjusted to a concentration of $1x10^7$ conidia/ml (0.01 % Tween 80 in water) or $2x10^7$ conidia/ml (rape seed oil). For comparison of isolates (Ma43, Pfr4) and production system (aerial and submerged spores) concentrations of $1x10^6$ spores/ml were used. Per treatment 4000g of dried mulch (Plantop Dekormulch - natur, Ziegler, Germany) was mixed with 4000ml water based or 2000ml oil-based formulation. In 2011 spore suspensions were mixed or sprayed with a knapsack sprayer on the top of the substrate with an equivalent volume.

The treated mulch was transferred in a grid of nine plots each of 80x80cm size. Before, the grass vegetation was cut and covered with a weed-fleece. The plots were exposed to full sunlight and rainfall. Mulch samples of 20x20 cm (250g mulch) were taken weekly or in two week intervals over a period of two months. Three times 50 g of each sample were put in separate boxes and 25 L_5-larvae of *C. molesta* were added, incubated at 25° C darkness and the number of hatched moths was monitored over four weeks. Additionally, in the field the ambient temperature, humidity and rainfall, and additionally in 2011 the substrate and soil temperature and the substrate moisture was measured over the time. Because the experiments were not repeated over time, no statistical analysis was conducted.

Results and Discussion

In 2010 and in 2011 experiments on the persistence of entomopathogenic fungi were conducted under semi field conditions. In both years mulch treated with rape seed oil alone resulted in a high reduction of number of hatched moths of *C. molesta* (fig. 1 and fig. 2). In contrast, the water based formulation did not show any clear effect. The effect of oil corresponds to results of laboratory experiments (Stephan and Herker, 2011). When conidia of *M. anisopliae*, strain Ma43, were suspended in water or oil especially the oil based formulation resulted in a reduction of hatched moths which can be explained mainly by the oil effect. But in the first three weeks (fig. 1B) a slight reduction of hatched moths compared to the blank formulation was visible. When suspended in water Ma43 showed a slight effect only in the first two weeks (fig. 1B). For both formulations the effect of Ma43 over the time was unsatisfying. When the effect of conidia of Ma43 and Pfr4 formulated in water were compared, for both isolates a high fluctuation of effects over time was measured (fig.1C). At the beginning of the experiment a clear effect was only visible for Pfr4. But after two weeks exposure in the field no effect was observed. On the other hand for Ma43 no effect was visible at the beginning of the experiment but after one week exposure, only less than 50% of moths hatched.

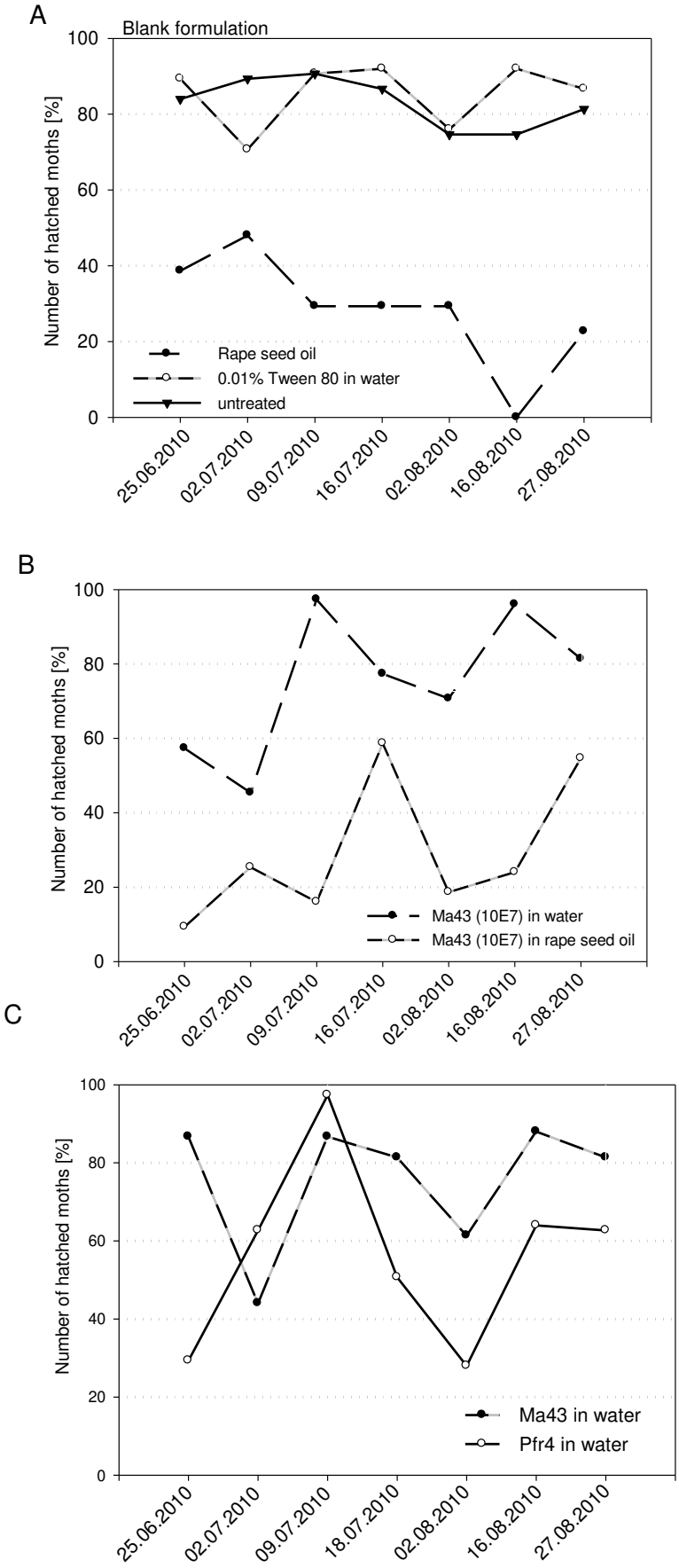

Figure 1: Persistence of oil or water based formulations of two entomopathogenic fungi mixed with mulches in 2010

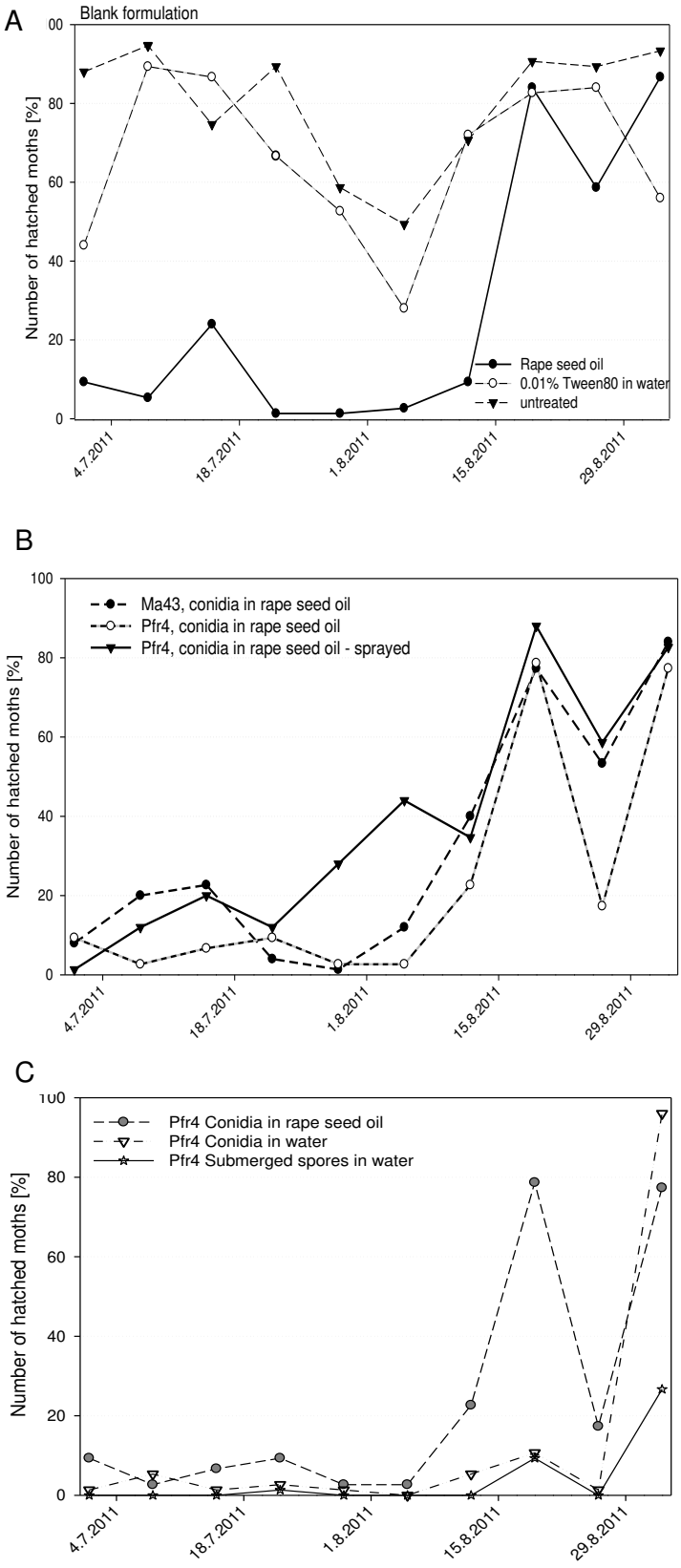

Figure 2: Influence of the fungal isolate, formulation, application and type of spores on the persistence on mulches in 2011

These results indicate that possibly, environmental factors like temperature and humidity influenced the persistence in field. The results of 2010 let assume that the environmental optima of the two isolate seem to be different (fig.1C). In 2011 again a clear oil effect was observed. The reduction of efficacy in mid of August can be explained by heavy rainfall (fig. 2A). In 2011 again the effect of oil formulated conidia of Ma43 was mainly caused by the oil formulation which was also the case for conidia of Pfr4 formulated in oil (fig. 2B). When the two application strategies - mixing or spraying - were compared a slight better effect was achieved with mixed material. This can be explained by a better distribution on the mulch. When conidia and submerged spores of Pfr4 were formulated in water nearly over the whole experimental time a dramatic reduction of hatched moths was achieved. Independently of using solid state produced conidia or liquid fermented submerged spores not more than 20% of the larvae developed to moths over the whole experimental time (except the last sample). Because water with 0.01% Tween 80 did not show any clear effect in laboratory experiments (data not shown) and in the persistence experiments of 2010 and 2011 the effect is caused by the entomopathogenic fungus. When the tests were analysed, *I. fumosorosea* grown out of the cocoons were found over the whole monitored period. Although in the field the mulch-temperature reached temperatures higher than 45° C the fungus was still effective. Additionally, submerged spores were as persistent as conidia. This is important, because this strain can be easily produced in liquid culture and an industrial production of this strain seems to be possible.

Conclusion

In laboratory experiments mulches and corrugated cardboard are accepted as artificial hideouts for cocooning and pupation of various moth species (Herker et al. 2010). Bioassays have shown that rape seed oil and the entompathogenic fungi *M. anisopliae* and *I. fumosorosea* were effecting the development of Cydia *funebrana* (Stephan and Herker, 2011) and other fruit moths (unpublished data). The presented results on the persistence of rape seed oil and entomopathogenic fungi demonstrate that both, oil and *I. fumosoroseus*, have a long persistence under semi-field conditions over the cocooning period of the first and second generation of the plum fruit moth. For both treatments, the consistency of results over the time was better when mixed with the substrate instead of spraying on the substrate. Submerged spores were as good as conidia but have the important advantage that they can easily be produced in large scale. Further field experiments have to be carried out to proof whether oil and *I. fumosorosea* can be integrated in a control strategy for control of e.g. *C. pomonella*, *C, funebrana*, *C. molesta* or *Eupoecilia ambiguella*.

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