Laboratory experiments with entomopathogenic fungi on artificial hideouts for biocontrol of *Cydia pomonella* and *Cydia funebrana*

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Abstract

Cydia funebrana *(Plum fruit moth) is a serious pest of plum fruits. Therefore, we investigated whether the moth can be controlled by artificial hideouts treated with conidia of entomopathogenic fungi under lab conditions. Because we were not able to establish a mass rearing of* C. funebrana *we did additional experiments with the related species* Cydia pomonella*. We tested corrugated cardboards and different mulch substrates on acceptance for pupation. The results indicate that the moths accepted corrugated cardboard and especially bark mulch for pupation. When we compared oils and tensides, both insect species were sensitive to the formulation. In case of* C. pomonella *a mortality of 46% and 92% was determined for Tween 80 and sunflower oil, respectively. The addition of conidia of* Beauveria bassiana *did not enhance the mortality but even when low concentrations of conidia (10^4conidia/mL) were applied in oil, 90% mycosis was achieved. We also tested the effect of Naturalis-L, which is a* B. bassiana *containing product, and its blank formulation and received comparable results. Because we did not achieve high mortality rates with* B. bassiana *we tested other entomopathogenic fungi like* Lecanicillium lecanii*,* Paecillomycis fumosoroseus *and* Metarhizium anisopliae*.* M. anisopliae *and* P. fumosoroseus *showed the highest mortality rate on both moth species. Furthermore,* C. pomonella *seems to be more sensitive to entomopathogenic fungi than* C. funebrana*. The presented data demonstrate that vegetable oils have a dramatic effect on larvae and oil-based formulations can improve the efficacy of entomopathogenic fungi. Field trials should demonstrate whether artificial hideouts in combination with oil-based formulated entomopathogenic fungi can be integrated in a control strategy of codling and/or plum fruit moth.*

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Keywords: *Cydia funebrana*, Naturalis-L, entomopathogenic fungi, biological control, formulation

Introduction

Codling moth (*Cydia pomonella*) and Plum fruit moth (*Cydia funebrana*) are important pest insects of organic fruit production. Control options for organic growers have been limited to methods such as oils, trapping, matting disruption, manual removal of infested fruits or, in the case of codling moths, the application of commercial formulations of granulovirus to control newly hatched larvae. In addition, entomopathogenic fungi targeted for overwintering stages offer a control strategy. It is known that Codling moth and plum fruit moth hide in bark and below trees for overwintering. The idea is to spread out contaminated material so that the larvae could be infected by fungi while overwintering and accordingly pupation. Therefore, we investigated the potential of entomopathogenic fungi incorporated in artificial hideouts. For the experiments corrugated cardboard and commercial bark mulch were treated with Naturalis-L or entomopathogenic fungi which were re-isolated from commercialized products. Additionally, the effect of the formulation was investigated. For

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that the conidia had to be formulated into oil or tenside solutions because conidia are highly hydrophobic. Additionally, vegetable oil is well known in biological pest control. But it is not specific to insect species.

Material and Methods

Adults of *Cydia pomonella* (strain BW-FI-03) were kept in cylinder containing strips of parafilm (American National Can Company) for egg deposition. Parafilm strips were changed every three days and transferred in Petri dishes. Hatched larvae were transferred into 50-well plates containing 45 ml semi-artificial diet (according to Ivaldi-Sender, 1974 20.5 mM Benzoic acid, 16.4 mM Nipagin (Methyl-4-hydroxybenzoate), 42.6 mM L(+)-Ascorbic acid, 2.5% Agar-Agar, 6.25 % polenta, 6.25 % yeast, 6.25 % weatgerm) and kept at 25°C and 16 h light. After five weeks larvae reached L_5 stage and were set on corrugated cardboard for pupation. Within three weeks moths are hatching. Afterwards, the corrugated cardboard was transferred into cylinders for mating. *Cydia funebrana* larvae were collected in untreated field plots at the experimental station Dossenheim and were than used for the experiments immediately. The following fungi were used: *Lecanicillium lecanii* (re-isolate of the product: Mycotal), *Metarhizium anisopliae* isolate 43 (strain of the product BIO1020), *Beauveria bassiana* (re-isolate of the product: Naturalis-L) and *Paecilomyces fumosoroseus* isolate 4 and 8 of the JKI-strain collection. All fungi were incubated on MPA-medium (3 % malt extract broth, 0.5 % peptone from soy meal, 1.5 % agar-agar) for two weeks and 25°C in darkness. The conidia suspensions were obtained by washing the conidia with oil or 0.01 % Tween 80 containing solution from Petri dishes. Suspensions were filtrated through mull and concentration was determined by using counting cell chamber. Different conidia concentrations were adjusted and 75 µL pipetted in each flute of the corrugated cardboard. Then 4 stripes of corrugated cardboard (each 3 cm, 10 flutes) were placed in 1 L boxes with 25 larvae per experimental approach. After 14 days of incubation the numbers of dead larvae, pupae or moths were counted. Additionally, cadavers from each treatment were kept in Petri dishes containing wet filter paper. The Petri dishes were incubated for at least two weeks at 25° C and the mycosis was observed. Bark mulch was autoclaved for 10 min at 121°C before use and put into conditioning cabinet at 60°C for 48 h. 50 g of bark mulch were mixed with 20 mL of conidia suspension and transferred to 1 L boxes. Per approach 25 larvae were used.

Results

Acceptance of corrugated cardboard and different mulches

From *C. pomonella* we did know that corrugated cardboard is accepted for pupation but no data were available for *C. funebrana*. Our results have shown that almost 100 % of the larvae had chosen the flutes as place for pupation (data not shown). As an alternative we also tested different pupation materials (fig.1). As can be seen in figure 1 the larvae accepted all materials in particular commercial bark mulch. The number of cocooned or pupated larvae was here higher then with other materials. Also the mortality was less than with chipped wood or corrugated cardboard. We decided to use commercial bark mulch as substrate to test entomopathogenic fungi. On the one hand commercial bark mulch can easily be applied and spread out otherwise it is less sensitive to changing climate conditions like rain.

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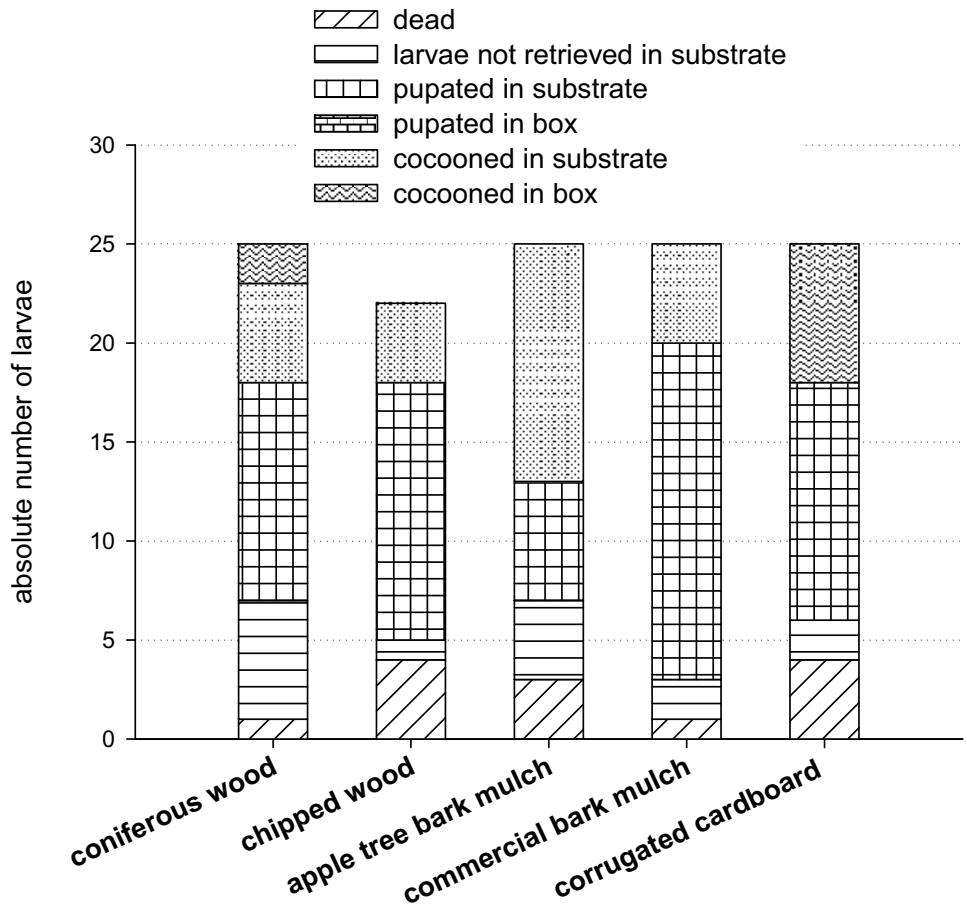

Fig.1: C*. pomonella* (L_5-stage) larvae set on different substrates for pupation. Dead, pupated and cocooned larvae were rated.

Effect of formulation

We treated corrugated cardboard with different conidia concentrations of *B. bassiana* formulated in sunflower oil (SfO) or in water containing 0.01 % Tween80 (fig.2). Sunflower oil kills almost all larvae even in control (SfO). There is no visible dosage effect of increasing conidia concentrations from. Mycosis is always at high level. In contrast corrugated cardboard treated with 0.01 % Tween80 showed different effect. The mortality rate did not reach the level of that from sunflower oil (fig.2.). But with increasing conidia concentration also increased mycosis rate. Besides we did the same experimental approach with commercial bark mulch and obtained similar results. Almost al larvae died when commercial bark mulch was treated with sunflower oil. We also did comparative experiment between sunflower and rape oil. These experiments showed that the mycosis efficacy of conidia formulated in rape oil is even better (data not shown).

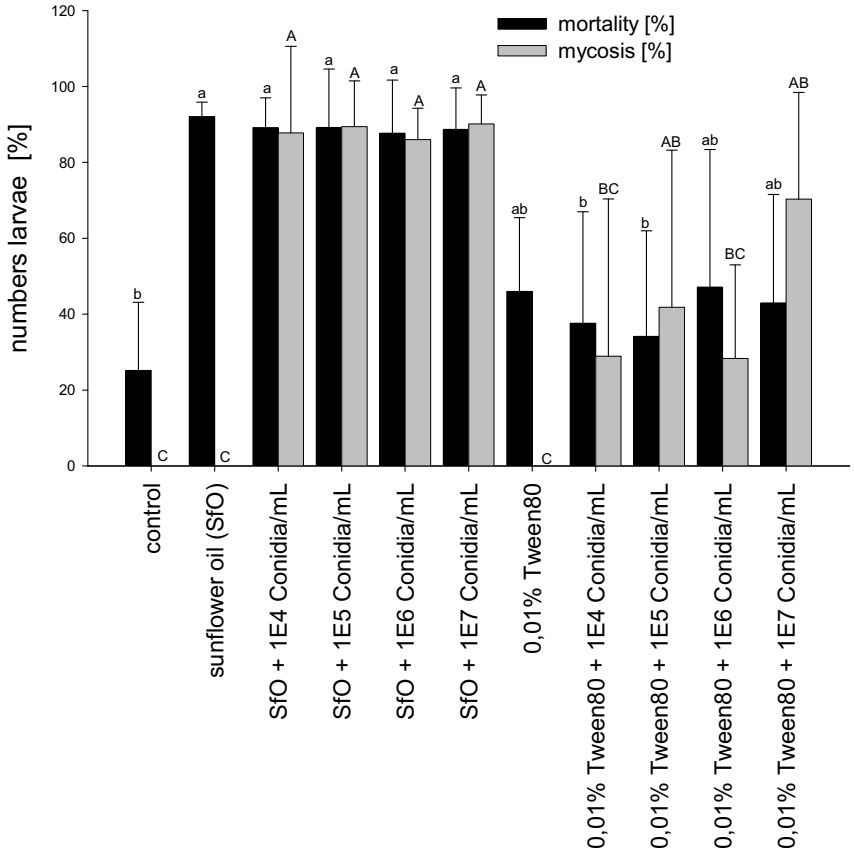

Fig.2: *B. bassiana* infection and mortality rate on *C. pomonella* (L_5-stage) in corrugated cardboards. Conidia from *B. bassiana* were formulated in sunflower oil (SfO) or 0.01% Tween80. Bar = Standard deviation, means of the arcsine transformed data with the same letter are not significantly different following the Student-Newman-Keuls Test ($P<0.05$)

Naturalis-L was in the focus of our investigations, because this product works with an entomopathogenic fungus and is listed for the German market. Naturalis-L is an oil based formulation. 1 L boxes were prepared with commercial bark mulch and different conidia concentrations of Naturalis-L were adjusted. We also centrifuged a sample to remove conidia to test blank formulation. In water control almost 60 % of larvae emerge to moths (fig.3). High concentrations of conidia resulted in almost no adults, as expected. That suggests a dose-response relationship. Blank Naturalis-L formulation had huge effect on larvae mortality. Only a few moths were obtained.

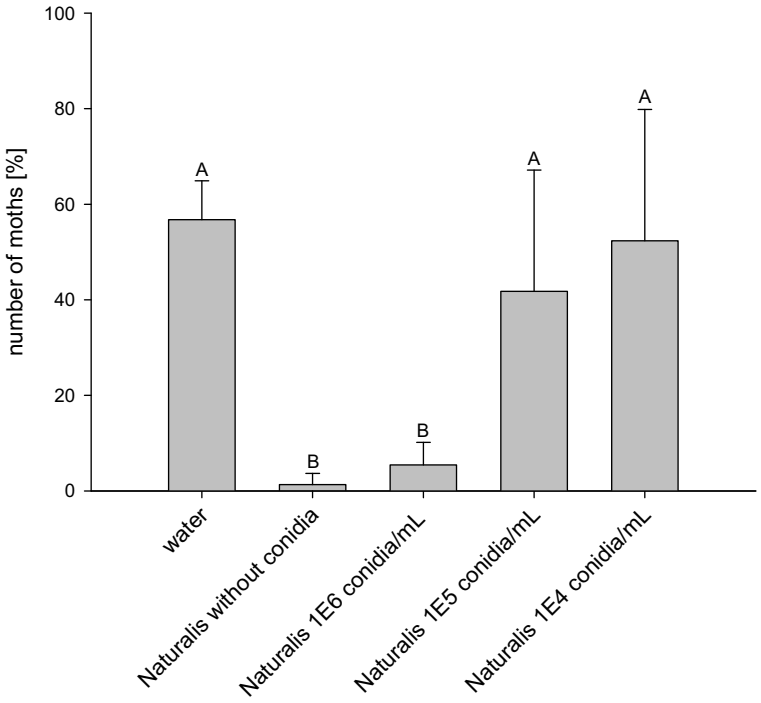

Fig.3: Survival of *C. pomonella* (L_5-stage) after infection with Naturalis-L on commercial bark mulch. The numbers of hatched moths were counted after four weeks of infection. Bar = Standard deviation, means of the arcsine transformed data with the same letter are not significantly different following the Student-Newman-Keuls Test ($P<0.05$)

Comparison of different entomopathogenic fungi

Oil formulations like sunflower or rape oil and blank Naturalis-L covered the efficacy of mycosis so we decided to test fungi isolates instead of commercial products. Because *B. bassiana* showed little effect on *C. pomonella* we also tested *Lecanicillium lecanii*, *Metarhizium anisopliae* and two isolates of *Paecilomyces fumosoroseus* on corrugated cardboard (fig.4) and commercial bark mulch (data not shown). All these fungi are known to infect insects (D.M.Glen et. al, 2008). The experiments showed that larvae from *C. funebrana* that were collected outdoor are less sensitive to the tested fungi than *C. pomonella* (fig. 4). Nevertheless, *Metarhizium anisopliae* and *Paecilomyces fumosoroseus* isolate 4 showed the highest mortality rate on both lepidopterean species. *B. bassiana* that is the infective micro-organism of Naturalis-L had neither impact on *C. pomonella* nor *C. funebrana*.

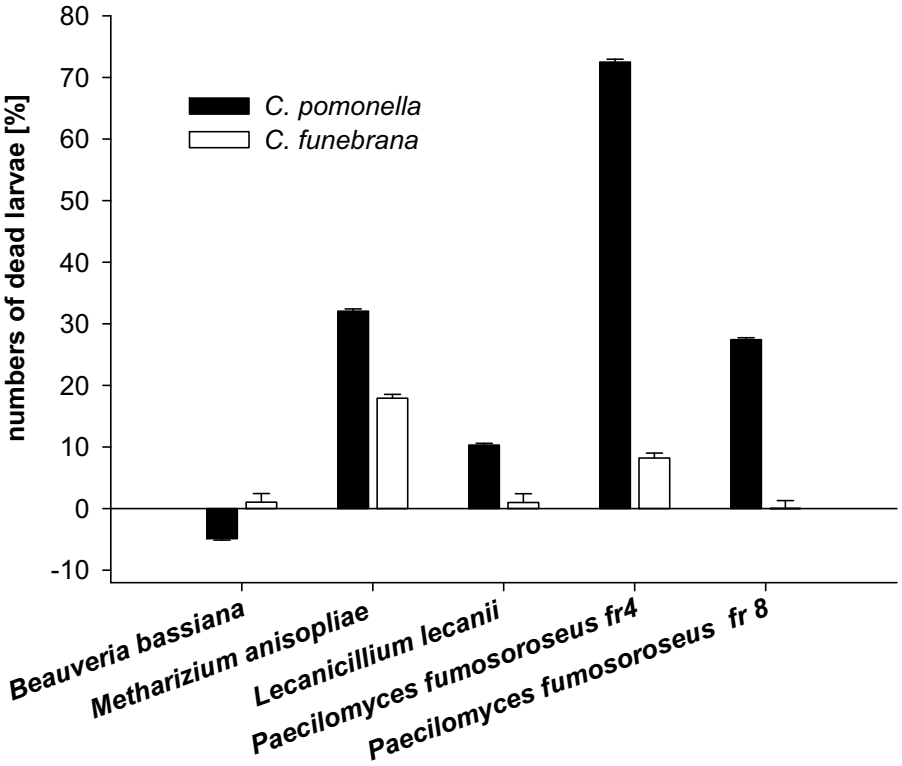

Fig.4: Comparison of entomopathogenic fungi (10^5conidia/mL) against *C. pomonella* and *C. funebrana* L_5-larvae. Numbers of dead larvae were counted (corrected according to Abbott, Bar = Standard deviation).

Discussion

We showed that corrugated cardboard and commercial bark mulch will be accepted as artificial hideouts by *C. funebrana*. Additionally, larvae can be infected with entomopathogenic fungi by inoculating cardboard or commercial bark mulch with the fungi. First results indicate that the environmental conditions like humidity seem to be important for the infection process. Commercial bark mulch keeps the humidity better than for example cardboard and therefore it offers stable climatic conditions for larvae and fungi which is important for application in the field. Oils like sunflower or rape oil have an unspecific insecticidal effect. It is known that oil based formulations enhance the infection efficacy of entomopathogenic fungi. Therefore, it was anticipated that the mycosis was increased by using oils for suspending the conidia independently of the used substrates. In the case of the commercial product Naturalis-L we also showed within our experimental design that the product formulation itself has a high impact on the insects. But *B. bassiana* showed only little effect on *C. funebrana* and is, possibly improper for population control (fig.4). But we found good evidence that *M. anisopliae* and *P. fumosoroseus* could be used as pathogens against Plum fruit moth. The fungi showed up to 20 % mortality with a low conidia concentration of 10^5 conidia per mL for *C. funebrana* in corrugated cardboard (fig.4). Possibly, reared insects (*C. pomonella*) are more sensitive to entomopathogenic fungi than insects from outdoor (*C. funebrana*). One explanation could be that the immune system of reared animals is delicately than that of outdoor animals. However it also could be because *C. funebrana* and *C. pomonella* are two different species.

Finally, we have good evidence to test *M. anisopliae* and *P. fumosoroseus* in field approaches. We are also preparing the same experimental approaches for other moths like *Cydia molesta* (Oriental peach moth) and *Eupoecilia ambiguella* (Vine Moth).

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