**University of Sopron**

**Faculty of Forestry**

**Thesis of PhD dissertation**

# THE POSSIBILITIES OF BIOLOGICAL CONTROL AGAINST *CETONIA AURATA, MELOLONTHA MELOLONTHA* AND *M. HIPPOCASTANI* GRUBS USING *BEAUVERIA* FUNGI

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## 1. Introduction and aims

Species of *Melolonthinae* have been causing severe harm for centuries under and over soil. The two most important species regarding forest protection are *Melolontha melolontha* and *M. hippocastani*. Climate change has a positive impact on their population thus increasing their importance. Besides these species *Cetonia aurata* can often be found, the grubs of which are hard to differentiate from *Melolontha* grubs. Pest control against cockchafer larvae is getting harder with the diminution of chemical control, as the use of numerous active ingredients are banned or restricted. That is why alternative control methods (biological, combined) need to be developed, examined and implemented. In Hungary, only *Beauveria bassiana* strains were used in forest protection practice with weak results. This thesis can therefore be considered the first attempt to use *B. brongniartii* (ART). It is usually not sufficient to apply insect pathogenic fungi alone, as other pests need to be controlled in nurseries, forests, and agricultural lands. However, the effects of the other used chemicals on the fungi are not known well enough.

First the data of harm caused by cockchafers of the last 10 years were analyzed. Then the growth of *Beauveria bassiana* and *B. brongniartii* was studied under laboratory conditions. The next step was to investigate the impact of chosen herbicides, pesticides and fungicides on the growth of these fungi. The last experiments were conducted to observe the interaction between fungal strains and the species *Melolontha melolontha*, *M. hippocastani*, and *Cetonia aurata*. The experiments were carried out under laboratory conditions and then semi-field conditions for *C. aurata*, using different soil types and temperatures.

### Aims

The following questions were to be answered:

1. Is the growth rate of *Beauveria bassiana* and *Beauveria brongniartii* the same under identical conditions?

2. How do the used herbicides, pesticides and fungicides change the growth rate and thus the efficacy of the fungal strains?

3. What is the pathogenity rate of *Beauveria bassiana* and *B. brongniartii* species on Ce*tonia aurata*, *Melolontha melolontha* and *M. hippocastani* species under identical conditions?

4. How is the impact of *Beauveria bassiana* and *B. brongniartii* species on *Cetonia aurata* grubs dependent on the soil type and the temperature?

## 2. Materials and methods

*Evaluation of the cockchafer-harm data*

For data evaluation, the 2013-2022 data provided by the Országos Erdőkár Nyilvántartó Rendszer (NFK EFO, SOE ERTI) were used.

*Growth rate examination of Beauveria species under laboratory conditions*

The experiment was carried out in the laboratory of the Faculty of Forest Protection in the University of Sopron, in 22±1°C constant temperature and with 10-11 hours per day natural light exposition.

For the experiment one strain of the entomopathogenic fungus used in biological control against cockchafer grubs; *Beauveria bassiana* (labelled as BORA), and four strains of another entomopathogenic fungus: *Beauveria brongniartii* (labelled as ART8, ART64, ART315 and ART2829) were used. The strains were put on sterile PDA (potato dextrose agar) in petri dishes, 4 replications each. The petri dishes were sealed and kept in the laboratory under the same conditions.

The first evaluation occurred after one month, and the second one occurred after another month. All petri dishes were photographed on millimeter paper during each evaluation, and the growth rates were determined based on this scale.

*The impact of four chosen chemicals used in practice on the growth rate of Beauveria strains under laboratory conditions*

The experiment was carried out in the laboratory of the Faculty of Forest Protection in the University of Sopron, in 22±1°C constant temperature and with 10-11 hours per day natural light exposition.

For the experiment one strain of *Beauveria bassiana* (labelled as BORA), and three strains of *Beauveria brongniartii* (labelled as ART8, ART64 and ART315) were used. The strains were put on sterile PDA (potato dextrose agar) in petri dishes, 15 replications each. After two weeks the four chosen chemicals were applied to the samples 3-3 replications each per strain, leaving 3 control samples untreated per strain. The four chemicals were chosen based on their usage in practice at the forestries (Taifun 360 – herbicide with active ingredient gliphosate; Basudin – insecticide and soil disinfectant with active ingredient diazinon; Vegesol eReS – fungicide with active ingredient sulphur; Topaz – fungicide with active ingredient penkonazol). Evaluation happened 7 days post application. All petri dishes were photographed on millimeter paper during each evaluation, and the growth rates were determined based on this scale.

*The interaction between Beauveria strains and Melolontha and Cetonia grubs under laboratory conditions.*

The experiment was carried out in the basement of the Faculty of Forest Protection in the University of Sopron, in 18±1°C constant temperature and natural light exposition.
For the experiment, the four strains of the two enthomopathogenic fungi were used, as in the previous experiment. The treated grubs were labelled additionally (*Melolontha melolontha* - M.m., *Melolontha hippocastani* - M.h., *Cetonia aurata* - C.a.).
The experiment lasted for five months. Soil with high humus content was put in 0,4l plactic cups and one L3 grub was put in each of them. The cups were treated with 5000µ of a solution of 1400 ml distilled water and 10 ml fungus suspension. Additional 2000 µ distilled water was added to the samples for moisturizing. The treatments were as follows: *Melolontha melolontha* – 17 pcs/strain, *Melolontha hippocastani* – 17 pcs/strain and *Cetonia aurata* – 34 pcs/strain. Additionally 17 grubless cups/strain were treated and 17 control cups/strain were left without treatment. The cups were placed in plastic boxes, 17 in each.

*The interaction between Beauveria strains and Cetonia aurata grubs in different media and temperature, under semi-field conditions.*

The experiment was carried out using L2 grubs of *Cetonia aurata* and for the treatment the fungal suspension of a strain of *Beauveria brongniartii* labelled ART64, which appeared to be the most effective based on the previous experiments. 60 plastic cups of 0,5l volume were filled with high humus-content soil, and 60 with sand. One *Cetonia aurata* gub was placed in each of them, and 30 of each soil type was treated with 5000µ of a solution of 1400 ml distilled water and 10 ml fungus suspension. Additional 2000 µ distilled water was added to the samples for moisturizing. A slice of potato was put into the sandy samples as nutrient supply. The samples were placed in sealable plastic boxes, with 10 samples of soil and 10 samples of sand in each. This resulted in 3 treated boxes and 3 control boxes, which were arranged as follows:

- 1 treated and 1 control box in the orchard sank to the gound, covered with soil; temperature is altering, same as (< 10 °C)
- 1 treated and 1 control box in 16-18 °C (~ 17 °C)
- 1 treated and 1 control box in 23-25 °C (~ 24 °C)

The experiment lasted for 6 months, the samples were checked every one and a half months (4 times).

*Statistical data processing*

The effect of the treatments were evaluated using ANOVA (=Analysis Of Variance) in the Statistica programme with default settings. Where it was needed Dunnet post hoc and Tukey post hoc tests were applied. For further evaluation Microsoft Excel programme was used and for processing the data of harm Spearman correlation was applied.

## 3. Results

*Evaluation of the cockchafer-harm data*

Between 2013 and 2022 cockchafer harm data were mainly reported from sandy plots, with varying – 10-100 vigor. This coincides with previous data that states that *Melolontha* species mainly occur in sandy lands.

In all ten years the majority of the incoming data was from the Belső-Somogyi-homokvidék, the other regions were much less infested. The grubs were the main problem everywhere over the bugs. Their cyclicness shows a noticeable pattern; a peak in 2014 an one in 2018 that fits the life cycle of the species.

In all years *Quercus robur* was the most infested tree species, followed by *Quercus cerris*, and mostly *Pinus sylvestris*, except for 2013, when *Pinus nigra* and in 2015, when *Populus canescens* was on the third place.

The occurence of harm caused by grubs and the harm caused by bugs depends differently on the production site and stand features. As the health of the stand declines, both harm forms are getting stronger, but while with altitude rising grub-harm is less frequent but more severe, bug-harm is less frequent and less severe as well. Besides this the amount of foliage shows inverse effect on them too; range of grub-harm decreases with the decrese of the foliage closure, but range of bug-harm increases significantly at the same time.

*Growth rate examination of Beauveria species under laboratory conditions*

*Beauveria bassiana* (labelled as BORA) started to grow significantly at the beginning of the examination. Their colonies were three times larger than the other strains on average, and by the time of the second measurement they almost or actually filled up the whole petri dish. Later the growth rate decreased a bit. This sample's deviation was the only one that decreased between the two measurements, partly due to reaching the maximum size.

The other four strains' deviation shows the double at the second measurement as at the first one. This shows that BORA samples are more stable to produce the measured growth rate, the average can be evaluated in its case. The difference between deviation values comes from the significant size differences between the colonies of the different strains. The difference between averages also decreased, average BORA colony sizes were only 1,9 times the size of the average size of the second biggest; ART2829.

Growth of the *Beauveria brongniartii* strains' colonies was approximately constant between the two measurements, the doubling of the samples confirms it.

Based on the measured values, BORA had the most vigorous growth and significantly differs from the others as well, it is true for the first and the second measurement too. ART2829 showed the second most vigorous growth, and it also differed from the others significantly. The other three strains showed growth rates very alike.

*The impact of four chosen chemicals used in practice on the growth rate of Beauveria strains under laboratory conditions*

The control samples showed that ART8 had the smallest - 117,88% and ART315 had the greatest – 211,78% growth rate. So even the slowest growing strain did more than double its size in two weeks on average, while the one that grew the most aggressively reached more than three times its original size in laboratory conditions. In this experiment, not BORA, like in the previous one, but ART315 showed the strongest growth without treatment.
Treatment with Taifun 360 herbicide showed slight growth inhibition in the case of all four strains. Treatment with Basudin had positive effect on the growth of the samples except for ART64. Growth enhancement was the biggest in the case of BORA (49,67%) and ART8 (38,03%)
Treatment with Vegesol eReS indicated slight growth inhibition except for ART8, while treatment with the other fungicide Topaz, resulted in growth inhibition in all cases. The smallest inhibition happened in the case of ART64 – 38,58 % inhibition, but the highest value shown by ART315 was 100,08%, In this case, the colony got even smaller than the original size at spraying. Growth rate inhibition was significant statistically in the case of Topaz treatment except for ART64 samples. This fungicide turned out to be much stronger than Vegesol eReS, which can be explained with their different active ingredients or with their different modes of action; Topaz is systemic, seems like it can move across the colony, while Vegesol is contact, and has a local effect.
The effect of the four used chemicals was different regarding the growth rate of the fungi. The herbicide and the insecticide and soil disinfectant had positive or neutral effects, the two fungicides had negative effect on them.
In all cases visual changes could be detected, even when there was a neutral effect in growth rate affection. This can be explained with the hidrophobic feature of the colonies, as the contact chemicals could only take effect where the droplets contacted the colonies. In many cases, pinkish decolorization occurred.
Using the recommended amount of each chemicals did not significantly inhibit fungal growth, allowing for simultaneous application if necessary. Control agents containing *Beauveria* species are more effective if not applied simultaneously with fungicides because even the slightest inhibition can result in loss of efficacy against *Melolontha* grubs.

*The interaction between Beauveria strains and Melolontha and Cetonia grubs under laboratory conditions.*

In the case of *Cetonia aurata* grubs, no direct lethal effect was detected. The death of the grubs is likely caused by the indirect effect of the fungi. It seems like these grubs don't even react to ART315, as there were no dead grubs among these samples.

In the case of the other two insect species, there were more grubs weakened by the fungi and killed by some other factor than experiencing a direct lethal effect. The latter is confirmed by the white fluffy coverage.

*Melolontha hippocastani* reacted the most sensitively to every strain. This species showed the highest rate of direct lethal effect, where ART2829 had the leading role with 24%, while indirect deaths were mainly caused by ART64 (58%).

In the case of *M. melolontha* grubs the highest rate of direct lethal effect was shown by ART64 (18%), while the indirect effect was the highest (29%) at ART2829.

Among the four strains ART64 was the most effective regarding both direct and indirect effects, followed by ART2829, ART315, and BORA, which performed similarly.

By the end of the fifth month the white fungal strains almost completely disappeared. The control samples and the ones that were treated but did not contain grubs did not show the presence of the fungi, that implies that in lack of the target organism (food) the fungi stay in 'sleeping' mode – aka spore and don't develop.

While in the first two experiments, BORA and ART315 appeared to be the fastest growing, in this third experiment, as practical use, a third strain; ART64 turned out to be the most effective.

*The interaction between Beauveria strains and Cetonia aurata grubs in different media and temperature, under semi-field conditions.*

In the semi-field experiment, the ART64 strain was selected based on the results of the previous experiment. *Cetonia aurata* larvae showed no direct infection earlier, so we wanted to know if different soil types or temperatures would help reach infection. There were four checks throughout the six months:

The first check revealed almost no difference, one specimen was missing from the 24°C samples.

The second check showed at least 70% healthy, living samples in every box. Higher temperature favored the grubs' development, the samples at 24 °C had 30% cocoons. The cocoons were in sandy samples with one exception.

The third check showed the help of the higher temperature; in the 24 °C samples, more cocoons and even bugs were found. The number of deaths also increased in almost all boxes.

According to the data from the last check, the rate of deceasing does not depend on the treatment. Two out of the three boxes showing the highest rate of dead specimen were control samples.

The least dead grubs (0) were found in the treated sandy samples of the outside box. The development of the specimen that remained alive was undoubtedly influenced by temperature.

Correlation between temperature and living grubs showed a significant negative, while between temperature and cocoons and bugs showed a significant positive correlation. Almost all grubs kept in higher (24 °C) temperature turned to cocoons or in many cases, developed into beetles. Some of the specimen kept at a lower temperature (17 °C) formed cocoons, while those exposed to less than 10 °C, sometimes even below 0, all remained in larval stage. The survival rate is the highest here too; 100%. This can be explained by the fact that the fungi don't have a significant effect on *C. aurata* grubs – and the lower temperature does not favor the fungi either so even an indirect effect could not occur.
In some cases, the fungi could be detected on the deceased specimen, and even an indirect effect could be suspected.
As a result of this semi-field experiment, it was confirmed that *Cetonia aurata* is not a target species for these fungi.

**Thesises**

**T1:** Strains of *Beauveria bassiana* and *B. brongniartii* do not show similar growth rates under similar, constant and ideal conditions. *B. bassiana* is capable of faster growth.
Among the studied one *B. bassiana* (BORA) and four *B. brongniartii* (ART8; ART64; ART315; ART2829) strains the *B. bassiana* strain developed the fastest under laboratory conditions. The four other strains also showed difference in growth rates. Growth rates compared to the maximum area (petri dish – 100%) were as follows: ART8 – 14% < ART64 – 14,43% < ART315 – 18,40% < ART2829 – 48,32% < BORA – 91,04%. On the whole we can say that the development of *B. bassiana* is maximal under optimal conditions and among the *B. brongniartii* strains studied the fastest developing one only shows half as good results as BORA, the other three following way behind. As sterility cannot be an issue, and the other given condition is temperature it is likely that for *B. brongniartii* a different temperature is optimal.

**T2:** Fungicides applied simultaneously with *Beauveria* species mostly inhibit fungal growth.
The two fungicides used in the experiment (Topaz and Vegesol eReS) both inhibited the growth of the studied fungal strains, except for one case, that can be explained with incorporative or covering defect. The rate of inhibition varied between 3,36% and 100,08%. The difference is derived from the different modes of action in case of the two fungicides; contact and systemic. Topaz was better in inhibiting growth so if it is inevitable to apply *Beauveria* and a fungicide simultaneously, of the two, Vegesol is to be preferred, otherwise *Beauveria* can be ineffectual.

**T3:** Studied strains of *Beauveria bassiana* and *B. brongniartii* species are more likely to be effective against *Melolontha* species among *Cetonia aurata*, *Melolontha melolontha* and *M. hippocastani* grubs under similar, and constant conditions.
The experiments showed no obvious effect on *C. aurata* grubs, their development was undisturbed in most cases. It is not the same regarding *Melolontha* species, as in many cases direct or indirect effect was detected between fungal treatment and the death of *Melolontha* specimen. However, under ideal, laboratory conditions the 30% effectiveness is very likely to be lower in case of semi-field and field application.

**T4:** The effect of *Beauveria bassiana* and *B. brongniartii* strains on *Cetonia aurata* grubs is not affected by the change of soil type or temperature.
There was no obvious or significant infection neither in different media (soil with high humus content or sand), nor at different temperatures (< 10°C; ~17°C, ~24°C) in treated and control samples either. Derived from this it can be stated thet *C. aurata* is not a target species for the used fungi. However, by the alteration of temperature, the development rate of the insect changed. In low temperature (<10°C) no development occurred, all samples remained in larval stage. In middle temperature (~17°C) 40% of the samples remained in larval stage, but no bugs were detected. In higher temperature (~24°C) only 3% of the samples remained in larval stage, 21% became cocoon, and by the end of the experiment 26% developed into a bug.

## 4. List of publications


1. **HORVÁTH ESZTER**, TUBA KATALIN, LAKATOS FERENC (2016): The effect of *Beauveria* species on cockchafer and rose chafer grubs. 5th Interdisciplinary Doctoral Conference, Pécs, 27-29. May, 2016.

2. TUBA KATALIN, MOLNÁR MIKLÓS, **HORVÁTH ESZTER**, MERŐ NÁNDOR (2017): Pajorok elleni védekezési kísérletek *Beauveria* törzsekkel. Biokultúra 28 1 pp. 22-25.

3. **HORVÁTH ESZTER** (2017): A cserebogarak (*Melolonthidae*) elleni gombaszuszpenziós (*Beauveria*) biológiai védekezés hatékonysága. Soproni Egyetem, Diplomamunka, 34 oldal.

4. **HORVÁTH ESZTER**, LAKATOS FERENC, GISELHER GRABENWEGER, TUBA KATALIN: Examination of the effect of four pesticides used in practice on *Beauveria* strains under laboratory conditions. Acta Silvatica – befogadói nyilatkozat 2024.