Using honey bees to disseminate the biocontrol agent *Gliocladium catenulatum* J1446 to strawberries for grey mould control

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The problem

- The fungus ***Botrytis cinerea*** causes **grey mould** disease - a serious economic problem on a number of field crops, including strawberry.
- *B. cinerea* has the ability to quickly adapt to new chemistries and has become resistant to most chemical fungicides.
- Pesticide residues in strawberries (food) and the environment.
- Need for more environmentally friendly plant protection methods.

Chemical vs. Biological control

- Biological fungicide Prestop Mix contains spores and mycelium of the naturally occurring soil fungus ***Gliocladium catenulatum* J1446** (10^8 cfu/g).
- Approved for organic production.
- The powder formulation is not hygroscopic, thus it suits well for outdoor use and for spreading by honey bees.
- *G. catenulatum* remains viable on the flowers up to 5 weeks.

Modes of action:
- Competition
- Hyperparasitism
- Does not produce antibiotics.

Spraying vs. Bees

- Bees can transport significant amounts of biocontrol organisms attached to their body hair - microbes such as fungal and bacterial antagonists of plant diseases.
- Due to their foraging behaviour bees provide a continuos treatment of the flowers, new flowers are treated as soon as they open - no need for repeated spraying, which may damage the plants mechanically and pollute the environment.
- Searching for nectar and pollen bees deliver the biocontrol agents directly to the target location - the flower - reducing the cost of the biopesticide and leaving the rest of the environment clean.
- Added bonus: pollination - higher yield with better quality.
- Biocontrol method mostly against pathogens and diseases that infect the flowers.
- A bee leaving the hive carries about 1000-10 000 spores (cfu) of *G. catenulatum.*
- A mean of ≈ 1000 spores (cfu)/flower *G. catenulatum* has been measured.
- Only a few hundred spores (cfu)/flower are needed for control of *B. cinerea*.

Aim of the study

- **Field test** to study if using honey bees in dispersing the Prestop Mix preparation to control grey mould in strawberry would be efficient in Estonian conditions:
  - landscape more heterogeneous with many competing flowers
  - strawberry pollen and nectar are not very attractive for honey bees
- **Laboratory experiments** to test the effect of Prestop Mix to the respiration rate of bumble bees.

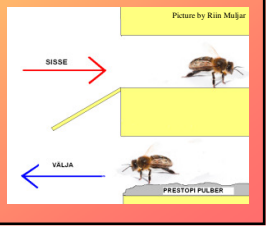
Materials and methods

FIELD TEST

- 2010 in 2 strawberry plantations in Tartu County: Nõo and Vasula, in 5 experimental areas:
  - Nõo 3 areas with the strawberry variety 'Sonata', each area 4 replicates
  - Vasula 1 area with variety `Sonata and 1 with 'Polka', both areas 4 replicates
- 2 treatments:
  - bee-excluded untreated check, covered with exclusion cages
  - bee-delivered Prestop Mix treatment
- Exclusion cages were removed after flowering

Materials and methods

- Honey bee hives (2 hives/ha) were placed at the edge of strawberry fields at first bloom, to each hive we attached a special dispenser containing the Prestop Mix preparation.
- Prestop Mix was added in the dispenser daily
- Exiting the hive the bees walk through the Prestop Mix powder, which sticks on their legs and hairy body.
- The dispener is a flat box with two openings, bees go in through one opening and out from the other, that way they won´t carry the powder into the hive.



Materials and methods

- Bee dissemination of Prestop Mix was started at the onset of strawberry flowering, and was continued until the end of flowering.
- No chemical treatment was used.
- Twice a week we counted the number of honey bees on the flowers.
- Ripe strawberries were picked every other day, the number of healthy and diseased berries were recorded.
- We compared:
  - The bee-disseminated treatment with the untreated check by counting the healthy and *Botrytis*-infected berries in both treatments.
  - *Botrytis* infection in strawberry varieties 'Sonata'and 'Polka'.

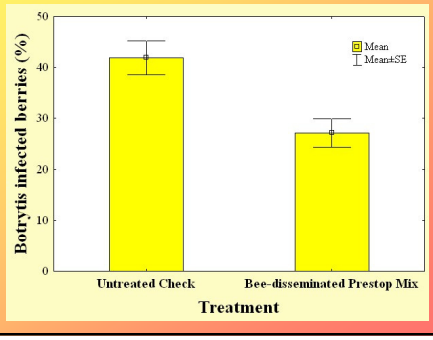
Materials and methods

LABORATORY TESTS

- **Feeding test** – commercially produced bumble bee colonies were fed for three weeks:
  - Test bumble bees with pollen and a mix of sugar solution and the Prestop Mix preparation
  - Control bumble bees with pollen and sugar solution only.
- **Contact test**
  - Test bumble bees dusted with the Prestop Mix powder
  - Control bumble bees untreated
- Infrared gas analyser was used to measure the respiration rate of the test and control bees by recording the amount of CO2 release (VCO2 ml h-1).

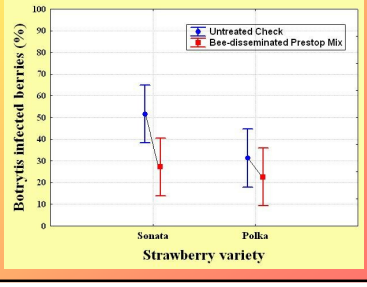
FIELD TEST Results

- Treating strawberry plants with the bee-dispersed Prestop Mix significantly reduced the proportion of infected berries - over 10% compared to the check ($p < 0{,}001$).



FIELD TEST Results

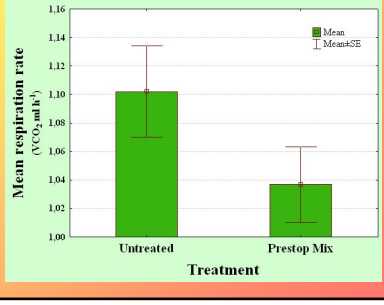
- Efficiency of the treatment depended somewhat on the strawberry variety - more efficient in 'Sonata' (p=0,067):
  - In `Polka` about 10% less diseased berries
  - In `Sonata` up to 25% less diseased berries
- 'Sonata' more attractive?



LABORATORY TEST Results

Contact test:

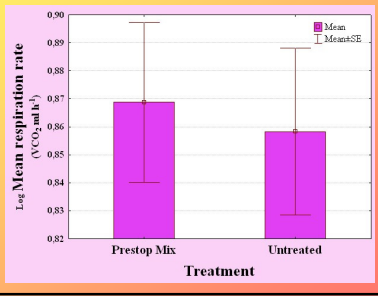
- Dusting the bumble bees with Prestop Mix powder lowered somewhat the respiration rate of the treated bumble bees, but no significant effect was found (p=0,1206).



LABORATORY TEST Results

Feeding test:

- Feeding the bees with Prestop Mix had no effect on the respiration rate of treated bumble bees compared with the control (p=0,8).
- Previous studies - no negative impact to bee reporoduction and foraging behaviour has been found.



Conclusions

- Using honey bees to disseminate the biocontrol agent *Gliocladium catenulatum* J1446 to strawberries for grey mould control is effective in Estonian conditions.
- Effiectiveness of grey mould control may depend on the strawberry variety and its attractiveness to honey bees.
- *Gliocladium catenulatum* J1446 doesn´t have a negative impact on the respiration rate of bumble bees.
- This is a pilot study and further research is needed.
- This biocontrol method is a promising alternative for estonian organic farmers and for use in integrated pest management to gain effective control of grey mould in an environmentally friendly manner.

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Thank you for your attention!