Potential of the entomopathogenic fungus
*Beauveria bassiana* as an endophyte in grapevine *Vitis vinifera*

Yvonne Rondot and Annette Reineke
Hochschule GEISENHEIM University
Institute of Phytomedicine

IOBC-WRPS meeting "Integrated Protection and Production in Viticulture"
Ascona, 16th Oct. 2013
Beauveria bassiana

- entomopathogenic fungus
- worldwide distribution, naturally occurring in soil
- wide host range
- is able to colonize plants endophytically

- commercial products:
  - Naturalis® (contains strain ATCC 74040)
  - BotaniGard® 22WP (contains strain GHA)

- strains are registered on Annex 2, Regulation (EC) No. 1107/2009
Tritrophic interaction

- **endophyte**: Beauveria bassiana
- **plant**: *Vitis vinifera*
- **pathogens**: e.g. *Planococcus ficus*, *Otiorhynchus sulcatus*, *Plasmopara viticola*
Endophytic establishment of *B. bassiana*

Influence on insect pests

Potential against plant pathogens
Endophytic establishment of *B. bassiana*

- Is it possible to establish *B. bassiana* as an endophyte within *Vitis vinifera* plants?
- Is any effect of endophytic *B. bassiana* on plant growth of the plants evident?

Influence on insect pests

- Is any effect of endophytic *B. bassiana* on mortality and growth of pest insects evident?
- Are the insects influenced by endophytic *B. bassiana* in their host choice behavior?

Influence on plant pathogens

- Is any effect of endophytic *B. bassiana* on the disease severity of *Plasmopara viticola* evident?
Inoculation

Treatments:

• different strains (ATCC 74040 and GHA)
• different conidia concentrations
• conidia suspension vs. product Naturalis®
• inoculation conditions:
  wrapping the plants in plastic bags for 24 h after inoculation

→ all treatments were inoculated with handheld-sprayer on the upper and lower leaf surface
Re-isolation of *B. bassiana*

**surface sterilization**
2 min in 0.5 % NaOCl, 2 min in 70 % ETOH, rinsed with sterile dest. water

**leaf discs**
obtaining 6 leaf discs with a cork borer

**molecular detection**

**visual assessment**

incubation (24 °C, dark)
Endophytic establishment of *B. bassiana*

- Is it possible to establish *B. bassiana* as an endophyte within *Vitis vinifera* plants?
- Is any effect of endophytic *B. bassiana* on plant growth of the plants evident?

- Endophytic **colonization** of grapevine leaves by *B. bassiana* is possible
- Presence of *B. bassiana* in grapevine plants was confirmed up to **28 days** after inoculation
- Different **treatments** had no significant effect on the colonization rates
- Endophytic *B. bassiana* had no negative effect on **growth** of potted grapevine plants
Influence on insect pests

- Is any effect of endophytic *B. bassiana* on mortality and growth of pest insects evident?
- Are the insects influenced by endophytic *B. bassiana* in their host choice behavior?
Vine mealybug *Planococcus ficus*

**Symptoms/Damage**
- phloem feeding causes vine weakening, defoliation
- honeydew excretion covers berries and leaves, growth of sooty mold
- transferring Grapevine leafroll-associated virus-3 (GLRaV-3)!

**Occurrence**
- in viticulture mainly in South Europe and California

---

Fig.: Bovey et al. 1980  
Fig.: Scaglìusi et al. 2002
Detached leaf assay with *P. ficus*

- spray inoculation of plants with *B. bassiana* (Naturalis® 3%)
- assessment of numbers of alive mealybugs and size every week
Influence on insect pests

- Is any effect of endophytic *B. bassiana* on mortality and growth of pest insects evident?
- Are the insects influenced by endophytic *B. bassiana* in their host choice behavior?
Black vine weevil *Otiorhynchus sulcatus*

**Symptoms and damage:**
- maturation feeding of adults on leaves and flowers
  → characteristic crescent shaped notches
- root feeding of soil-borne larvae feed
  → results in wilting/stunting or could be lethal for host plants

**Host plants:**
- major pest in many horticultural crops
- partly in forestry
- in viticulture mainly in grapevine nurseries
Choice assay with *Otiorhynchus sulcatus*

- spray inoculation of plants with
  - *B. bassiana* (Naturalis® 3%)
  - formulation of Naturalis® without conidia
  - water as control
- one adult *O. sulcatus* per tube
- each test lasted 1 h
- pre-test for validation of the olfactometer test system
Choice assay – validation of the test system

→ the weevils are able to select a tube with a plant over an empty tube
Influence on insect pests

- Is any effect of endophytic *B. bassiana* on mortality and growth of pest insects evident?
- Are the insects influenced by endophytic *B. bassiana* in their host choice behavior?

- a significant effect of endophytic *B. bassiana* on growth and on mortality one week after the initial settlement of vine mealybugs was evident

- adult *O. sulcatus* chose significantly more often the control plants as a host plant compared to grapevine plants with endophytic *B. bassiana*
Influence on insect pests
• Is any effect of endophytic *B. bassiana* on mortality and growth of pest insects evident?
• Are the insects influenced by endophytic *B. bassiana* in their host choice behavior?

Endophytic establishment of *B. bassiana*
• Is it possible to establish *B. bassiana* as an endophyte within *Vitis vinifera* plants?
• Is any effect of endophytic *B. bassiana* on plant growth of the plants evident?

Influence on plant pathogens
• Is a protective effect of endophytic *B. bassiana* on the disease severity of *Plasmopara viticola* evident?
Inoculation tests with *P. viticola*

- **Day 0**: Treatment with *B. bassiana*
- **Day 7**: Inoculation with *P. viticola*
- **Day 14**: Assessment of disease severity

Leaf level:
1. 0
2. 5
3. 10
4. 15
5. 20
6. 25
7. 30
8. 35
9. 40
10. 45

Affected area (%) on the abaxial leaf surface.
Influence on plant pathogens

- Is a protective effect of endophytic *B. bassiana* on the disease severity of *Plasmopara viticola* evident?

- A significant protective effect of endophytic *B. bassiana* on disease severity of *Plasmopara viticola* is evident.

- A systemic protection of the newly formed leaves could not be observed.
Tritrophic interaction

endophyte
Beauveria bassiana

plant
Vitis vinifera

pathogens
e.g. Planococcus ficus,
Otiorhynchus sulcatus,
Plasmopara viticola
Thanks to:

Deutsche Bundesstiftung Umwelt (DBU)
Prof. Dr. A. Reineke
Prof. Dr. H.-M. Poehling
Roman Peiter (M. Sc. Thesis)
Gordan Behaderovic (B. Sc. Thesis)

Thanks for your attention!