Influence of antagonistic micro-organisms on the growth of strawberry plants in the presence of *Verticillium dahliae* and *Phytophthora cactorum*

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Abstract

Soil borne diseases are an increasing problem in organic strawberry production. In a national founded project, a strategy for biological control of Verticillium *spp. and* Phytophthora *spp. was investigated. After an in vitro selection, four antagonistic micro-organisms (*Trichoderma harzianum*,* T. atroviride*,* Metarhizium anisopliae and Bacillus subtilis*) were tested in greenhouse and field trials (2010-2011) in the presence of* V. dahliae *and* P. cactorum*.*

In greenhouse experiments the growth of strawberries in the presence of V. dahliae *could be positively influenced by the antagonist. But these results were not confirmed by first field trials. In case of* P. cactorum *inoculation, neither in greenhouse nor in field trials differences could be observed.*

Keywords: Strawberry, soilborne diseases, *Trichoderma*, *Bacillus, Metarhizium*

Introduction

Strawberry (*Fragaria* x *ananassa* Duchesne) is an important berry culture in Germany. In organic, but also in conventional production, soil-borne diseases (e.g. *Verticillium* spp., *Phytophthora* spp.) are an increasing problem because no efficient control systems are available.

Verticillium dahliae, distributed across the temperate and subtropical zones of the world, is a pathogen for a wide range of dicotyledonous plant species. (Barbara & Clewes, 2003; Fradin & Thomma, 2006). The pathogen invades the vascular system, with consequent wilting and eventually death of the plant (Barbara & Clewes, 2003; Dessimoz *et al*., 2011). In the soil, the fungus produces microsclerotia (MS) that can survive and infect plants up to 15 years (Fradin & Thomma, 2006; Dessimoz *et al*., 2011).

The pathogen *Phytophthora cactorum* has a wide host range and causes leather rot on fruits and crown rot in strawberry plants. The pathogen enters the plants through wounds (Maas, 1998); the plant wilts and browns at the base of the petiole and the upper part of the crown (Lilja *et al*. 2006).

Within an *in vitro* screening four micro-organism with antagonistic potential against the two pathogens were selected for further *ad planta* experiments. *Trichoderma* spp. and *Bacillus subtilis* are known to be antagonistic to different plant pathogens. *Metarhizium anisopliae*, an entomopathogenic fungus, is used for insect control. The aim of these experiments was to evaluate if/how the selected antagonists were able to influence the growth of the strawberry in the presence of soil-borne pathogens.

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Material and Methods

Pathogen production

V. dahliae was produced as conidia in liquid Czapek-Dox (grown 7 days at 25 °C on a rotary shaker) and as MS following the description of Neumann (personal communication). *P. cactorum* was grown on rye agar with the supplementation of ß-sitosterol (25 °C for two weeks, afterwards 15 °C until use). Spores and mycelium were scraped from the agar plate with sterile water.

For both pathogens the inoculation concentration was 10^5 conidia or spores mL^{-1}.

Antagonists production

T. harzianum T58 and *T. atroviride* P1 were grown for 14 days on boiled wheat at 25 °C in the dark. Afterwards the conidia were washed off with a 0.01% Tween® 80 solution. The concentration of the suspension used for the experiments was 10^5 conidia mL^{-1}.

M. anisopliae Ma43 was grown under the same conditions as *Trichoderma* but on a rice-oat mix (4:1) as substrate. Conidia were washed off with 0.1% Tween® 80 solution and also adjusted to a concentration of 10^5 conidia mL^{-1}.

B. subtilis FZB24® was provided as product and used as described on the package.

The Mixture was made using the four antagonist suspensions in the same proportion.

Greenhouse experiments

Tests were performed with Frigo strawberry plants. The cv. Honeoye was used in the trials with the pathogen *V. dahliae*, the cv. Sonata with the pathogen *P. cactorum*. For application of the antagonists the roots of the plants were dipped for 15 min in the antagonist suspension (or in water for the control) and then planted in pots containing 0-soil (Fruhstorfer Erde®). The pathogen inoculation was performed by pipetting 1 mL of conidial suspension directly on the roots before covering with soil. Afterwards, every two weeks (for four times) 250 mL of the antagonist suspension were added to the pot. The strawberries were kept at a soil temperature of 20 °C with a day/night photoperiod of 16/8 h. After four months the plants were harvested and the growth and yield parameters were determined. The trials were repeated three times with six plants each.

Field experiments

In 2010 field experiments were set up on fields at the JKI, Institute for Biological Control. For these trials the same cv. as described before were used. Again, the strawberry roots were dipped for 15 min in the antagonistic suspension and planted in the fields. Afterwards, every two weeks (for four times) the plants were watered with the antagonist suspension (5 L of 10^5 conidia mL^{-1} per row, 30 plants per row) In case of field 1 no pathogens were added (from analysis and history of the field it was know that both diseases were present.); for field 2 pathogens were added in form of conidial suspension for *P. cactorum* and of a MS/sand mix for *V. dahliae*. The visual rating followed after one year (field 1) and nine months (field 2) after planting (1. vital plant – 5. dead plant).

Results and discussion

In greenhouse experiments two strawberry cultivars were inoculated with the soil-borne diseases and were treated with the four antagonists and a mixture of them. For both pathogens no clear infection symptoms were monitored. In the experiment with *V. dahliae* all antagonists show an increase of all growth parameters measured (tab. 1). When the four antagonists were compared no clear tendencies and no additive effects for the

mixture were observed. For *P. cactorum* no evident effects of the antagonists on the investigated growth parameters were seen (tab. 2).

Table 1: Results of greenhouse experiments cv. Honeoye inoculated with *V. dahliae*.

Treatments	n*	% biomass increase	Leaves			Roots	
			Mean of plant height (cm)	Sum	DW (g)	Mean of length (cm)	DW (g)
Control	15	54 (± 15)	15.5 (± 4.6)	144	59.62	22.0 (± 4.9)	9.27
B. subtilis FZB24	17	64 (± 10)	18.3 (± 1.9)	209	101.33	26.3 (± 2.8)	19.07
T. atroviride P1	16	63 (± 10)	16.8 (± 2.8)	170	78.56	24.5 (± 7.5)	18.55
T. harzianum T58	18	70 (± 7)	19.1 (± 2.8)	164	105.12	30.0 (± 4.3)	16.68
M. anisopliae Ma43	17	66 (± 9)	19.3 (± 2.1)	185	110.94	27.5 (± 6.6)	14.79
Mixture	17	63 (± 6)	18.8 (± 2.5)	176	95.01	25.6 (± 4.7)	10.87

DW=dry weight. *number of plants alive at the scoring day.

Table 2: Results of greenhouse experiments cv. Sonata inoculated with *P. cactorum*.

Treatments	n*	% biomass increase	Leaves			Roots	
			Mean of plant height (cm)	Sum	DW (g)	Mean of length (cm)	DW (g)
Control	18	68 (± 8)	23.5 (± 2.7)	158	104.46	27.9 (± 4.6)	13.90
B. subtilis FZB24	17	67 (± 10)	23.8 (± 4.3)	155	107.88	27.5 (± 3.5)	14.17
T. atroviride P1	17	66 (± 8)	22.5 (± 3.3)	168	108.34	27.4 (± 4.9)	12.80
T. harzianum T58	18	67 (± 9)	23.1 (± 2.9)	158	101.95	26.1 (± 4.1)	13.48
M. anisopliae Ma43	17	61 (± 12)	19.9 (± 5.4)	178	109.91	26.5 (± 5.7)	21.97
Mixture	18	62 (± 9)	22.5 (± 3.0)	143	95.49	28.3 (± 4.3)	13.84

DW=dry weight. *number of plants alive at the scoring day.

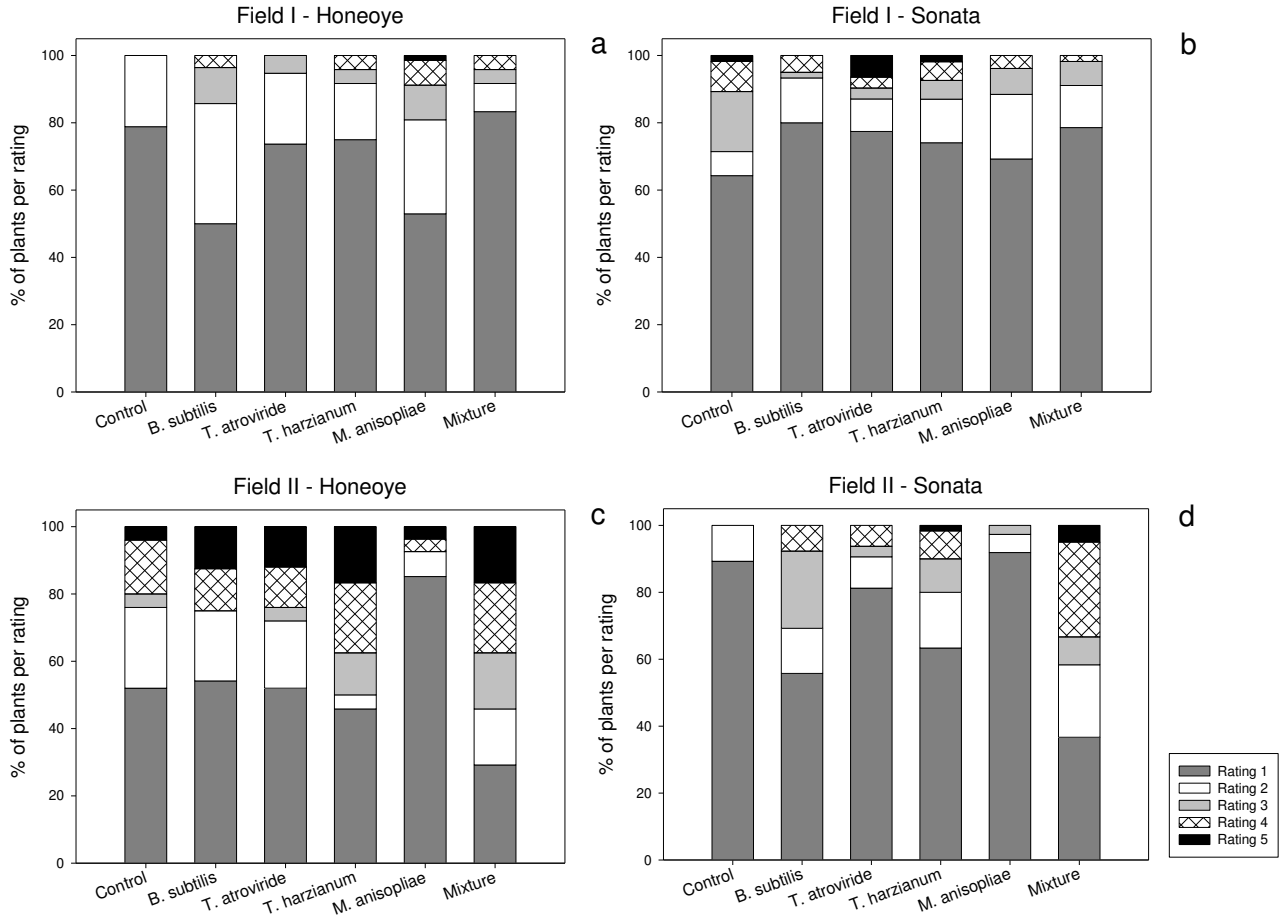

Figure 1: Visual rating of the two fields: Field 1 naturally infected; Field 2 artificially inoculated.

In the field 1 trials some differences between antagonists could be seen for the cv. Honeoye (figure 1a). For *B. subtilis* and *M. anisopliae* only ca. 50% of the plants were rated as vital (rating 1) whereas all other treatments, including control, show more than 70% of rating 1. After artificial inoculation (field 2, figure 1c) the number of plants with rating 1 was reduced for all treatments, except for *M. anisopliae*. The number of dead plants (rating 5) was increased in comparison to field 1.

For the cv. Sonata in field 1 (figure 1b) the percentage of plants with rating 1 was nearly similar. None of the treatments showed a positive effect. The artificial inoculation (field 2, figure 1d) did not show an increase of diseased plants in the control. It can be supposed that the weather conditions in 2010-2011 did not support the disease.

In field 2 (figure 1c and d) the negative effect of the Mixture and, on the other side, the positive effect of *M. anisopliae* can possibly be caused by other factors e.g. white grubs or different water capacity of the soil.

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