O.66 - Cladosporium cladosporioides H39: A new antagonist for biological control of apple scab

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Abstract

Apple scab caused by *Venturia inaequalis* is a major disease in apple production. Scab epidemics during summer are driven by conidia produced only on apple leaves. In this situation, antagonists present in the phyllosphere may interfere with conidia of the pathogen during sporulation or infection. Within the EU-funded project REPCO, more than 100 fungi were isolated from sporulating scab colonies and tested on apple seedlings for their potential to reduce sporulation of the pathogen. Since the aim of the study was to contribute to the development of a biocontrol product, only candidates were selected which fulfilled a range of additional criteria considering the major constraints in the development of biocontrol products. The best antagonists were applied under orchard conditions during two growing seasons. The antagonist *Cladosporium cladosporioides* H39 significantly reduced sporulation of *V. inaequalis* after most applications. However, in a few cases no effect was found. Protocols for production, down streaming and formulation have been developed for the antagonist. A new formulation as a wettable powder is now available for further orchard testing.

Introduction

Apple scab caused by *Venturia inaequalis* is the major disease in European apple production. In organic farming, control of the disease currently depends on frequent application of copper fungicides. Biological control of apple scab may offer alternative options for disease control. Research on biological control of *V. inaequalis* until now mainly focused on the overwintering stage of the pathogen in fallen leaves. However, during the summer epidemic sporulating colonies of *V. inaequalis* may harbour antagonistic micro-organisms which may affect the sporulation capacity of the pathogen. The objectives of our study were (1) to build-up a collection of micro-organisms obtained from *V. inaequalis* colonies on apple leaves, (2) to select possible antagonists suppressing sporulation of the pathogen, (3) to develop prototype production protocols and formulations for selected antagonists, and (4) to test selected antagonists under field conditions.

Collection of candidate antagonists

Scab infected leaves were collected in the Netherlands, Belgium, northwest Germany and central Germany from old standard trees (without any further cropping management), e.g. planted along secondary roads, and in organically managed or abandoned orchards. Fungi were isolated from sporulating colonies of *V. inaequalis*. A surprisingly high number of very different fungi was found in sporulating lesions of *V. inaequalis*.

Pre-screening

A rapid throughput system was used for a first check of candidate antagonists regarding their potential risks and economical feasibility of the development of a biocontrol product. Main criteria were: sufficient production of spores during fermentation, cold tolerance and drought tolerance. Fungi belonging to the genera *Aspergillus*, *Penicillium* or *Fusarium* were discarded because of the potential of various species within these genera to produce mycotoxins. Isolates growing at 36°C were

discarded because such isolates may demand special risk studies during a registration procedure. In total, 160 isolates were tested. Less than 80 isolates fulfilled all pre-screening criteria.

Seedling assays

The potential of candidate antagonists to suppress conidia production of *V. inaequalis* on infected leaves was tested on young apple seedlings. Seedlings were pre-inoculated with *V. inaequalis.* After 2 days incubation the *V. inaequalis*-inoculated seedlings were sprayed with antagonist suspensions. The number of *V. inaequalis* conidia produced on leaves was quantified after additional incubation for 10 days. Fourteen experiments on seedlings were carried out. Most of the 80 candidate isolates tested on seedlings did not statistically significantly reduce conidiation of *V. inaequalis*. Only four isolates caused a significant reduction of *V. inaequalis* on the leaves in subsequent independent experiments. A few more isolates showed a strong statistically significant antagonistic effect in one experiment but such effects could not be repeated. Enhancement of conidiation of *V. inaequalis* after application of candidate isolates was never observed.

Orchard experiments

Experiments were carried out in summer 2006 and 2007 in an organically managed orchard at Applied Plant Research, Randwijk, the Netherlands. Apple trees var. Jonagold were pruned in spring and summer to obtain new shoots with young leaves highly susceptible to *V. inaequalis*. Eight experiments were carried out in 2006 on different sets of trees in the period between 22 June and 28 September. Seven treatments were carried out in a completely randomised block design with 6 blocks. Treatments consisted of spraying water as control, or suspensions of freshly produced spores of selected antagonists. The antagonist *Cladosporium cladosporioides* H39 was also applied using fermenter-produced spores which had been formulated as a dry powder and resuspended in water. A compressed air-driven knapsack sprayer was used for spray applications at 250 kPa until run-off. Experiments started with the first treatment 1 to 3 days after an infection period for *V. inaequalis* had been predicted according to the Mills table based on leaf wetness duration and temperature. During all experiments, subsequent treatments were carried out at 3 to 4 day intervals. Leaves were sampled 3 to 5 weeks after the first treatment. Sampled leaves were shaken in bottles in water containing 0.01% Tween 80 with a flask shaker and the concentration of conidia of *V. inaequalis* was determined for each of the obtained suspensions. The leaf surface of all leaves per sample was measured with an area meter.

After applications of a product containing formulated spores of the antagonist *Cladosporium cladosporioides* H39 significant reductions of *V. inaequalis* was found. Conidia production was reduced by 35 to 55% in the different experiments. Applications of the other selected antagonists had no consistent effects on *V. inaequalis.*

In 2007, a similar experiment was carried but the same trees were treated during a longer period of 8 weeks and leaves were repeatedly sampled from such trees. Applications of Cladosporium *cladosporioides* H39 significantly reduced *V. inaequalis* sporulation by 51 to 69%. However, at a third assessment date no difference between treated and untreated trees was found. This may be due to a reduced quality of the available antagonist product during the field experiment which had an insufficient shelf life.

Production and formulation

Selected antagonist candidates were assessed in small scale Solid-State Fermentation (SSF) for their suitability for large scaled biotechnological production processes. Different fermentation conditions regarding media and incubation conditions were evaluated. Only antagonists which passed this screening step were tested in subsequent experiments in the orchard. In final experiments protocols for mass production, down streaming and formulation were developed for the antagonist *Cladosporium cladosporioides* H39 in this new formulation. Fermentation was based on the Prophyta laboratory scaled SSF system and formulated pilot products were applied in the orchard experiments.

Unfortunately, samples of the produced pilot products of *Cladosporium cladosporioides* H39 used during the field experiments showed a limited shelf life. A new formulation as a wettable powder, not yet tested under orchard conditions, has been developed. Conidia of *Cladosporium cladosporioides*

H39 were viable and able to germinate on nutrient agar even after 8 months of storage at 4°C or -20°C. Conidia of *Cladosporium cladosporioides* H39 in this specific formulation also showed a lower susceptibility to exposure to UV light than spores produced and formulated under other conditions.

Perspectives

A first experiment will be carried out in 2008 in which the new formulation of conidia *Cladosporium cladosporioides* H39 will be tested under orchard conditions. Further research is now needed to investigate the effect of newly developed formulations of *Cladosporium cladosporioides* H39 on *V. inaequalis* under field conditions and to assess the effect of antagonist applications on the entire summer epidemics of apple scab. Since epidemics of apple scab are polycyclic, the levels of reduction of *V. inaequalis* sporulation during single reproduction cycles as found in our experiments may have a strong effect on the progression of the entire summer epidemic.

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