

CONSISTENT CONTROL OF VERTICILLIUM WILT BY COMBINATION OF *PSEUDOMONAS FLUORESCENS* P60 AND *TALAROMYCES FLAVUS*.

Termorshuizen, A. J.¹, Soesanto, L.¹, and Raaijmakers, J. M.²

1: Biological Farming Systems,

2: Laboratory of Phytopathology, Wageningen University and Research Centre, The Netherlands

Introduction

Verticillium dahliae is a soil-borne fungal pathogen that causes yield losses in a wide variety of economically important crops, including potato, several vegetables, fruits and fruit trees, fibre and oil seed crops, ornamentals and shade trees. Control of verticillium wilt has largely been based on soil fumigation with broad-spectrum pesticides, most of which will soon be banned from the market given their adverse effects on the environment. Except for solarization in mediterranean countries, there are currently no effective control methods available. In the past decades, a renewed interest in biological control of plant diseases in agriculture and horticulture evolved, partly as a response to public concern about the use of hazardous chemicals. Several attempts have been made to control *Verticillium* wilt. Application of mycoparasites has been attempted with *Trichoderma* spp. and *Gliocladium* spp. Recently, *Talaromyces flavus* has been registered for control of verticillium wilt in oil-seed rape and other horticultural crops. In addition, several rhizobacteria, including *Stenotrophomonas maltophilia*, *Bacillus subtilis* and *Pseudomonas fluorescens* have shown activity against *V. dahliae*. To date, however, the efficacy of most of these antagonists has been inconsistent.

Results and Discussion

In a series of six different experiments with *Arabidopsis thaliana* and eggplant, *P. fluorescens* P60 consistently reduced the number of newly formed microsclerotia 4 to 10-fold, slowed down the senescence of *A. thaliana* to a level identical to treatments without *V. dahliae*, and significantly reduced infection of the stembases of eggplant from 27% to 2%. Combination of *P. fluorescens* P60 with *Talaromyces flavus* isolate R1 further improved control of *V. dahliae*: on *A. thaliana*, the number of newly formed microsclerotia was reduced by 26 to 44-fold. Moreover, with increasing densities of *V. dahliae* up to 100 microsclerotia per gram of soil, the combination of P60 and *T. flavus* still led to disease reduction. Thus, the combination resulted in almost complete control of the disease and, even more interesting, prevented the build-up of microsclerotia, the survival structures and primary inoculum of *V. dahliae*. Subsequent hybridization and PCR with primers and probes specific for biosynthetic genes involved in the production of 2,4-diacetylphloroglucinol (Phl) demonstrated that *P. fluorescens* strain P60 harbors genes for Phl. Furthermore, HPLC-analysis showed that P60 produces Phl in vitro. The antibiotic Phl has activity against various pathogenic micro-organisms including *V. dahliae*. Mycelial growth of nineteen isolates of *V. dahliae* was completely inhibited at a Phl concentration of 100 µg/ml PDA. Interestingly, the formation of microsclerotia in vitro was significantly reduced at sublethal Phl concentrations. Collectively, these results suggest that Phl is a key determinant in the suppression of *Verticillium* wilt by strain P60. Bioassays with mutants of P60 defective in Phl production will be required to support this hypothesis.