Screening *Trichoderma asperellum* as a Mycoparasite on *Phytophthora ramorum*[1]

Timothy L. Widmer[2] and Gary J. Samuels[3]

Abstract

Despite efforts of eradication and sanitation, *Phytophthora ramorum* persists in the United States and abroad. Fungicides have limited effectiveness, but there are concerns that they may only inhibit pathogen growth and resistance may develop. Biological control is an active control measure that can work continuously as long as the agent is alive and active. The goal of this study was to examine whether *Trichoderma asperellum* isolates are mycoparasitic on *P. ramorum*. Sixteen isolates of *T. asperellum* and other *Trichoderma* spp. that have demonstrated antagonism towards other *Phytophthora* spp., or were suspected to have mycoparasitic activity, were selected. The rate of mycoparasitism was determined by overlaying a strip of *Trichoderma*-colonized agar on a V8 agar plate colonized by *P. ramorum* (A2 mating type). Every 7 days for 4 weeks agar plugs were removed and transferred to V8 agar amended with benomyl (V8+B) or a wounded leaf disk of *Rhododendron* 'Cunningham's White.' Control plugs of *P. ramorum*, without exposure to the *Trichoderma* spp., always showed growth on V8+B and produced necrosis on the leaf disks. The different *Trichoderma* spp. isolates demonstrated variable mycoparasitic activities. Some isolates showed no inhibition of *P. ramorum* growth on V8+B or reduction in necrosis even from plugs removed directly below the *Trichoderma* strip. Other isolates showed a reduction in growth and necrosis over time, but did not completely eliminate the pathogen after 4 weeks. Six isolates of *T. asperellum* were consistent among replicated trials in eliminating growth of *P. ramorum* from the agar plugs and preventing leaf disk necrosis within 2 weeks exposure. Further testing of four of these six *T. asperellum* isolates against two different *P. ramorum* isolates (A1 and A2 mating types) resulted in the same high level of mycoparasitic activity. We believe the results demonstrate that specific *T. asperellum* isolates have the potential to remediate *P. ramorum*-infested soil. Tests are ongoing to determine whether *P. ramorum* soil populations can be eliminated when treated with these isolates.

Introduction

Studies have shown that *Phytophthora ramorum* can survive in potting medium around containers of infected plants in a nursery (Jeffers 2005, Tjosvold and others 2009). Aerated steam and chemical fumigants are known methods to eliminate soilborne pathogens. Linderman and Davis (2008) found that *P. ramorum* populations in potting media were killed by aerated steam heat treatments of 50 °C or higher or treatment with metam sodium concentrations of 0.25 ml per l of medium. However, using these techniques increases the chance of destroying beneficial microorganisms and of working in a hazardous environment.

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[2] USDA/ARS, Foreign Disease and Weed Science Research Unit, 1301 Ditto Avenue, Fort Detrick, MD 21702.

[3] USDA/ARS, Systematic Botany and Mycology Laboratory, Beltsville, MD 20705.

Corresponding author: tim.widmer@ars.usda.gov.

Some of the most studied and promising fungi used in a biocontrol system are *Trichoderma* spp. (Harman and others 2004). Populations of *Trichoderma* spp., which often are abundant in composts and compost-amended media, typically suppress *Pythium* and *Phytophthora* root rots within days after their formulation (De Ceuster and Hoitink 1999). *Trichoderma* spp. are reported to suppress soilborne diseases caused by *Phytophthora* spp. in containerized systems (da S. Costa and others 2000, Sharifi Tehrani and Nazari 2004). Currently, *T. asperellum* is being studied as a biological control agent to manage black pod disease of cacao in Cameroon. Recent results show that disease incidence was lower when *T. asperellum* was applied on infected cacao trees (Tondje and others 2007). It was the purpose of this study to screen selected *Trichoderma* spp. for antagonism towards *P. ramorum*.

Materials and Methods

Sixteen different *Trichoderma* spp. isolates were cultured on half-strength PDA (1/2PDA). This included 12 isolates of *T. asperellum*, two isolates of *T. virens*, one isolate of *T. koningiopsis*, and one undescribed isolate *Trichoderma* sp. nov. Three different *P. ramorum* isolates, WSDA-1772, 5-C, both A2 mating type and clonal lineage NA1, and PRN-1 (CBS 101327), mating type A1 and clonal lineage EU1, were cultured on 20 percent clarified V8 agar.

An agar plate bioassay was conducted as described by Krauss and others (1998). The *Trichoderma* spp. were grown on 1/2PDA in 90 mm diameter Petri plates until they completely colonized the plate. A 4 X 1 cm strip of *Trichoderma*-colonized 1/2PDA was removed and transferred to a *P. ramorum*-colonized V8 agar plate. A non-colonized 1/2PDA strip was used in the same way as a control. Every week for 4 weeks a 1 cm X 4.5 cm strip perpendicular to the original *Trichoderma* strip was removed. The strip was cut lengthwise in half and divided into 0.5 cm cubes to give two sets of nine cubes. From one of the sets the cubes were placed individually on the abaxial side of nine wounded *Rhododendron* 'Cunningham's White' leaf disks (6 mm diameter). The corresponding set was placed on a 20 percent V8 agar plate supplemented with 50 mg/l of benomyl. After 1 week at 20°C, observations were made on the leaf disks for necrosis and mycelial growth originating from the cubes on the V8 agar plate. The experiment was conducted twice for each *Trichoderma* isolate on the three different *P. ramorum* isolates.

Results

There was an observed correlation between the lack of necrosis on the leaf disks and no mycelial growth from the corresponding plug on the agar plate. Nine *Trichoderma* spp. isolates showed some reduction in necrosis after 1 week and complete reduction in necrosis and *P. ramorum* growth within 2 weeks after exposure. Eight of these isolates were *T. asperellum* and the other one was *T. koningiopsis*. There was no difference among the *P. ramorum* isolates tested.

Microscopic examination of the interaction between the antagonistic *Trichoderma* spp. and *P. ramorum* revealed mycoparasitism of *P. ramorum* chlamydospores and sporangia (fig. 1). This confirms the observation by Watanabe and others (2007), who first reported mycoparasitism as the mode of action of *T. asperellum*.

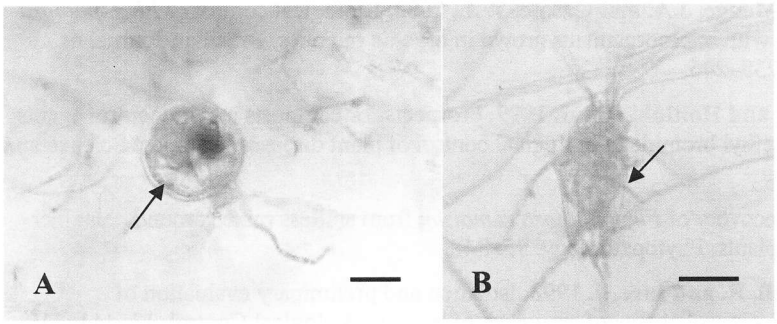

Figure 1—*Trichoderma asperellum* (arrows) demonstrating mycoparasitism of a *P. ramorum* **A)** chlamydospore and **B)** sporangium. Bar = 20 µm.

Discussion

Specific *T. asperellum* and *T. koningiopsis* isolates have potential as biocontrol agents against *P. ramorum*. Further tests have been started to determine if selected isolates can reduce *P. ramorum* soil populations to nondetectable levels over time. In addition, tests are ongoing to determine if selected isolates can parasitize and eliminate viability of *P. ramorum* propagules in infested leaf litter. If the *Trichoderma* spp. are found to be effective in eliminating the population of *P. ramorum* in the soil and infested leaf litter, then the potential exists to use this as a biologically based method to remediate infested nursery beds.

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