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Short Communication

Paecilomyces farinosus destroys powdery mildew colonies in detached leaf cultures but not on whole plants

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Abstract

Since 2001, several isolates of *Blumeria graminis*, the causal agent of cereal powdery mildew, maintained on detached leaves at the John Innes Centre, Norwich, UK, have spontaneously become infected with an unknown filamentous fungus whose mycelia have quickly overgrown the powdery mildew colonies and destroyed them completely. A total of five isolates of the contaminant were obtained and identified as *Paecilomyces farinosus* based on morphological characteristics and rDNA ITS sequence data. To determine whether these *P. farinosus* isolates can be considered as biocontrol agents (BCAs) of powdery mildews, we studied the interactions between *P. farinosus* and the following four powdery mildew species: *B. graminis* f.sp. *hordei* infecting barley, *Oidium neolycopersici* infecting tomato, *Golovinomyces orontii* infecting tobacco and *Podosphaera fusca* infecting cucumber. The powdery mildew colonies of all these four powdery mildew species were quickly destroyed by *P. farinosus* in leaf cultures but neither conidial suspensions nor cell-free culture filtrates of *P. farinosus* isolates could suppress the spread of powdery mildew infections on diseased barley, tomato, tobacco or cucumber plants in the greenhouse. It is concluded that *P. farinosus* cannot be considered as a promising BCA of powdery mildew infections although it can destroy powdery mildew colonies in detached leaf cultures and can be a menace during the maintenance of such cultures of cereal, apple, cucurbit and tomato powdery mildew isolates.

Powdery mildew fungi are obligate biotrophic parasites of many plants and as such can survive in living host plant tissues only. For various research purposes, many laboratories maintain isolates of different powdery mildew fungi on detached leaves or leaf segments of cereals (Brown and Wolfe, 1990; Wyand and Brown, 2003), cucurbits (Bardin et al., 1997; Nicot et al., 2002; Shishkoff and McGrath, 2002; Romero et al., 2003), tomato (Kiss et al., 2001), tobacco (Szentiványi and Kiss, 2003), apple (Scheewe and Ketzel, 1994; Urbanietz and Dunemann, 2005), and other crops. In general, the leaves of the host plant are surface-sterilized, placed in small plates or tubes on artificial media, for example on benzimidazole agar or on mannitol sucrose agar (MSA), then the leaves are infected with conidia of a given powdery mildew isolate under near sterile conditions and kept for 2–6 weeks at 15–20 °C under artificial illumination. This method makes possible the maintenance of a large number of powdery mildew isolates each originating from a single colony or even a single conidium (Nicot et al., 2002). However, maintenance is laborious and the detached leaf cultures

sometimes become contaminated with other fungi that might destroy some of the powdery mildew isolates.

Isolates of the cereal powdery mildew pathogen, *Blumeria graminis*, have long been maintained on detached leaf segments of wheat, barley and other graminaceous host plants at the John Innes Centre, Norwich, UK (Brown and Wolfe, 1990; Wyand and Brown, 2003). Since 2001, many detached leaf cultures of *B. graminis* isolates have spontaneously become infected with an unknown filamentous fungus whose mycelia have covered the powdery mildew colonies and destroyed them completely. Thus, no more viable powdery mildew conidia could be obtained from the contaminated leaf cultures. Apparently, this contaminant interacted directly with powdery mildew colonies rather than being a saprophyte that simply overgrew them in culture. The objectives of the present work were (i) to identify the fungus responsible for the destruction of many isolates of *B. graminis* maintained on detached leaves, and (ii) to test its anti-powdery mildew capacity and its potential use as a biocontrol agent (BCA) of powdery mildew fungi.

To isolate the contaminant, small amounts of its mycelium were taken from detached leaf cultures of *B. graminis* f.sp. *tritici*, using sterile glass needles under a stereomicroscope. Mycelium was placed on Czapek-Dox agar supplemented with 2% malt extract (MCzA) and 0.5% chloramphenicol. Five isolates of the contaminant were obtained and maintained on MCzA and potato dextrose agar (PDA) for further studies. All colonies were of a white colour and extended at the rate of 0.3–0.4 mm radial growth day^{-1} at room temperature. The colonies produced conidia in abundance on both PDA and MCzA and also on the original contaminated fungal host. The ellipsoidal to fusiform conidia measured $2–3 \times 1–2$ μm and were produced on 110–300 μm long conidiophores bearing several groups of 7–14 μm long phialides (Figure 1). According to Samson (1974) and Domsch et al. (1980), these morphological patterns are characteristic of *Paecilomyces farinosus*, a ubiquitous entomopathogenic fungus. This species has previously been isolated from several sources worldwide, including many arthropods, soils (Samson, 1974; Domsch et al., 1980; Chandler et al., 2000) and also plants naturally infected with powdery mildew (Hijwegen and Buchenauer, 1984). Based on the morphological and cultural

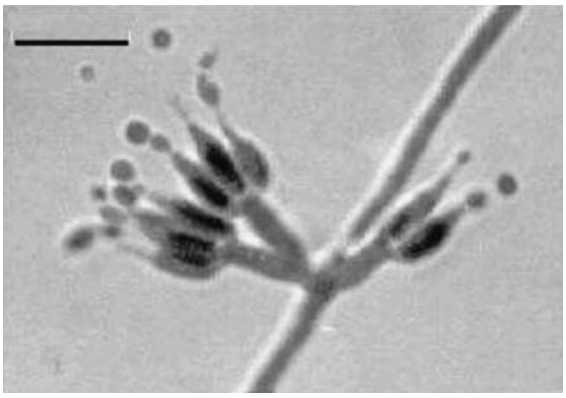

Figure 1. Phialides of the *Paecilomyces farinosus* isolate MYA-3388. Bar equal 10 μm.

data, the contaminant found in our cereal powdery mildew cultures maintained *in vitro* was identified as *P. farinosus* and an isolate was deposited in the American Type Culture Collection (Manassas, VA, USA) under the accession number MYA-3388.

To confirm the identity of our isolates, the ribosomal DNA (rDNA) internal transcribed spacer (ITS) sequence was determined in isolate MYA-3388 and compared with those available from DNA databases. DNA was extracted from mycelium using a Qiagen DNeasy Plant Mini Kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's instructions. The ITS region was amplified using the universal primer pair ITS1/ITS4 (White et al., 1990). PCR products were purified using a Qiagen QIAquick Gel Extraction Kit and then sent for direct sequencing in both directions using the same primer pair. The 500 bp long ITS sequence obtained (GenBank accession number: DQ374646) was identical with that of *P. farinosus* strain IFO7866 reported by Yokoyama et al. (2004) (GenBank accession number AB083033) and also with some other ITS sequences of various *P. farinosus* strains available in DNA databases, thus confirming the identity of our isolate MYA-3388.

More than 40 fungal species are known to suppress the growth and sporulation of powdery mildews in different ways and some of them have already been commercialized as BCAs of these plant pathogens (Bélanger and Labbé, 2002; Kiss, 2003). Curiously, some fungal antagonists of powdery mildews, such as *P. fumosoroseus* (Kavkova and Curn, 2005) *Lecanicillium lecanii* (syn.

Verticillium lecanii) (Romero et al., 2003; Miller et al., 2004) and *Meira geulakonigii* (Sztejnberg et al., 2004), are arthropod pathogens, as well. The anti-powdery mildew effect of *P. farinosus*, also mostly known as an arthropod pathogen (Samson, 1974; Domsch et al., 1980; Chandler et al., 2000), has not been studied in detail. In previous studies, a *P. farinosus* strain was included in a screening of the effects of a total of 17 fungal species against cucumber powdery mildew (Hijwegen, 1988), but did not show any specific anti-powdery mildew effect, nor did its cell-free culture filtrate (Hijwegen, 1989). Therefore, it was not selected for further studies on biocontrol of powdery mildews.

In the present work, we investigated the interactions between our *P. farinosus* isolates and four powdery mildew species: *B. graminis* f.sp. *hordei* maintained on barley, *Golovinomyces orontii* maintained on tobacco, *Oidium neolycopersici* maintained on tomato and *Podosphaera xanthii* (formerly known as *Sphaerotheca fuliginea*) maintained on cucumber. For all experiments outlined below, conidial suspensions of *P. farinosus* were produced by washing off conidia from 2 week-old colonies produced in 9 mm diam MCzA plates, using 4 ml sterile distilled water per plate. Concentrations were adjusted before use to 10^6 conidia ml^{-1} using a Bürker haemocytometer. Four experiments were carried out to examine the relationship between *P. farinosus* and the powdery mildew species included in this study. Each experiment was repeated 2–4 times.

In Experiment 1, conidia from each powdery mildew species studied were transferred to five plates containing 1.5% water agar (WA) medium covered by one layer of sterile cellophane. Forty microlitre conidial suspension of *P. farinosus* was pipetted onto powdery mildew conidia and the plates were incubated for 24 h at room temperature. Five plates not inoculated with *P. farinosus* served as controls. After incubation, pieces of the cellophane bearing fungal materials were cut out, placed on a slide, stained with cotton blue in lactophenol, and examined under a light microscope. This revealed that conidia of *P. farinosus* germinated on WA in less than 24 h and the emerging hyphae penetrated conidia of all the four powdery mildew species (Figure 2). However, no other effect of *P. farinosus* was observed during these interactions. The germination rate of powdery mildew conidia on WA was similar in the presence and in the absence of *P. farinosus* (data not shown) suggesting that the metabolites which might have been secreted by conidia and hyphae of *P. farinosus* in these cultures did not have any detectable anti-powdery mildew effect.

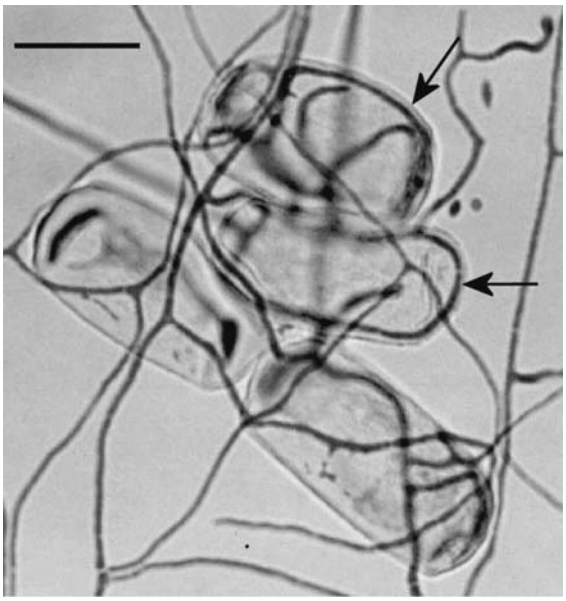

Figure 2. Growth of hyphae of *P. farinosus* in conidia of *Oidium neolycopersici* (arrows) on water agar medium. Bar equal 15 μm.

In Experiment 2, plates containing surface-sterilized barley, tobacco, tomato and cucumber leaves on MSA (mannitol, 20 g l^{-1}, sucrose, 10 g l^{-1}, agar, 7 g l^{-1}, tetracycline hydrochloride, 25 mg l^{-1}) were prepared as described previously (Bardin et al., 1997; Szentiványi and Kiss, 2003). Leaves in five plates for each host plant species were infected with the corresponding powdery mildew species in a horizontal air flow hood. Five other plates for each plant species were used without being infected with powdery mildew. Each plate was inoculated with 50 μl conidial suspension of *P. farinosus*. Non-inoculated plates with leaves with or without powdery mildew infection were used as controls. After incubation for 4–8 days, leaves with or without powdery mildew mycelia were taken out of the plates and examined under a light microscope. This study showed that *P. farinosus* readily germinated and hyphae quickly overgrew and destroyed all the powdery mildew colonies in the detached leaf cultures. Four to 6 days after inoculation, there were no more viable powdery mildew hyphae or conidiophores

on any of the leaves. Thus, the damage observed at the beginning of this study in the detached cereal powdery mildew leaf cultures was reproduced for all the four powdery mildew species by inoculation with *P. farinosus*. These experiments showed that *P. farinosus* is clearly a fungal antagonist of powdery mildews, at least in detached leaves. Light microscopy revealed that conidia of *P. farinosus* also germinated on the leaves maintained in plates without powdery mildew infection, but no mycelial mass of *P. farinosus* developed on these leaf surfaces. Thus, the development of mycelium of *P. farinosus* in these plates was possible only in the presence of powdery mildew colonies. This suggests that powdery mildew colonies were used as substrates by *P. farinosus* under these conditions.

Experiment 3 was designed to examine the anti-powdery mildew effect of *P. farinosus* on powdery mildew-infected plants. For this purpose, barley, tobacco, tomato and cucumber plants were grown in pots in the greenhouse until 2–8 fully expanded leaves developed. Eighteen pots for each plant species were inoculated with the respective powdery mildew species while 18 other pots were kept separately in another greenhouse and remained free of powdery mildew during the experiments. One set of experiments was carried out in spring and another one in autumn. In both cases, the natural daily illumination was supplemented with 2 h artificial light/day and temperature was kept between 18–24 °C and relative humidity between 70–80% in the two greenhouses. When sporulating powdery mildew colonies became visible on the leaves, nine pots for the infected and nine pots for the non-infected plants were sprayed until run-off with conidial suspensions of *P. farinosus*. The same number of pots of mildew-infected and non-infected plants were used for each species as controls. These were sprayed with water only. After spraying, 1/3 of the pots of each plant species were left uncovered in the greenhouse, 1/3 of them were covered with a transparent plastic bag for 24 h, and the remaining pots were covered for 48 h. The development and spread of *P. farinosus* on the treated plants was monitored daily using light microscopy. However, no significant differences were found between the powdery mildew severity of the *Paecilomyces*-treated and control plants in any of these tests (data not shown). Mycelia of *P. farinosus* did not develop at all on mildew-infected plants which were left uncovered or were covered with plastic bags for only 24 h after their treatment. Similarly, *P. farinosus* did not produce mycelia on any of the uncovered or covered healthy potted plants although light microscopy showed that its conidia germinated on the treated leaf surfaces. On mildew-infected plants kept covered 48 h after treatment, mycelia of *P. farinosus* appeared and started to sporulate on the older powdery mildew colonies in 4–7 days, but young powdery mildew colonies also appeared during this period and these were generally free of *P. farinosus* hyphae. Thus, the fungus could not suppress the spread of the disease in these tests. In addition, it required a long time period, 48 h, of incubation at 100% relative humidity to be able to colonize powdery mildew colonies. These results suggested that *P. farinosus* cannot be considered as a promising BCA of powdery mildew infections although it completely destroyed powdery mildew colonies on detached leaves during the previous experiment.

In Experiment 4, cell-free culture filtrates were prepared from liquid cultures of *P. farinosus* to examine any possible anti-powdery mildew effect of its metabolites produced in culture. Liquid cultures were obtained by inoculating 100 ml Czapek-Dox broth supplemented with 2% malt extract (MCzB), with 10–12 mg mycelia of *P. farinosus* (scraped off from 2-week old colonies grown in plates on MCzA). To maximize the amounts of mycelia obtained, inoculated flasks were shaken for 24 h, then left to stand without shaking for 48 h and then shaken again for a further 24 h. This procedure resulted in growth and sporulation of the mycelia in MCzB. Cell-free culture filtrates were obtained from 4, 14 and 30-day old liquid cultures using filters with pore size of 0.22 μm (Millipore Corporation, Bedford, MA). Before filtration, most of the mycelia were removed from the flasks and the remaining liquid phase was centrifuged at 18,000 rpm for 5 min. Potted barley, tobacco, tomato and cucumber plants infected with powdery mildew were sprayed with the freshly obtained culture filtrates until run-off and the disease progress was assessed daily. Five pots were treated for each plant species. The same number of pots were sprayed with water to serve as controls. None of these treatments lead to any promising results in terms of powdery mildew control. The disease severity was similar on treated and control plants in all our experiments (data not shown) which suggested that none of the metabolites which

might have been excreted by *P. farinosus* in liquid cultures had any detectable anti-powdery mildew effect.

In conclusion, our results showed that *P. farinosus* was not effective against powdery mildew infections of four different crops. This fungus could only spread on mildew colonies existing on detached leaves and on potted and mildew-infected plants kept covered with plastic bags for at least 48 h. In these cases mildew-infected leaves were maintained in high relative humidity for at least 2 days which seems to be a prerequisite for the successful spread of *P. farinosus* on powdery mildew colonies. Cell-free culture filtrates of *P. farinosus* were not effective against powdery mildew infections.

Thus, a promising anti-powdery mildew effect, reported recently for an isolate of the closely related *P. fumosoroseus* (Kavkova and Curn, 2005), was not observed in our studies. However, *P. farinosus* might exist in the field as a powdery mildew-associated fungus, in addition to being an entomopathogen and a saprophyte, as it was isolated from powdery mildew colonies infecting *Lupinus polyphyllus* in the field (Hijwegen and Buchenauer, 1984). However, this appears to be the only report on isolation of *P. farinosus* associated with powdery mildew. This suggests that *P. farinosus*, known as a ubiquitous entomopathogen worldwide, can be associated with powdery mildew colonies in the field, as well, and in certain conditions, such as on detached leaves, can become a damaging antagonist of these plant pathogens and a menace during the maintenance of detached leaf cultures of cereal, tobacco, cucurbit and tomato powdery mildew isolates.

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References

Bardin M, Nicot PC, Normand P and Lemaire JM (1997) Virulence variation and DNA polymorphism in *Sphaerotheca fuliginea*, casual agent of powdery mildew of cucurbits. European Journal of Plant Pathology 103: 545–554.

Bélanger RR and Labbé C (2002) Control of powdery mildews without chemicals: Prophylactic and biological alternatives for horticultural crops. In: Bélanger RR, Bushnell WR, Dik AJ and Carver TLW (eds) The Powdery Mildews (pp. 256–267) A Comprehensive Treatise. American Phytopathological Society Press, St. Paul, MN, USA.

Brown JKM and Wolfe MS (1990) Structure and evolution of a population of *Erysiphe graminis* f.sp. *hordei*. Plant Pathology 39: 376–390.

Chandler D, Davidson G, Pell JK, Ball BV, Shaw K and Sunderland KD (2000) Fungal biocontrol of Acari. Biocontrol Science and Technology 10: 357–384.

Domsch KH, Gams W and Anderson T-H (1980) Compendium of Soil Fungi, Academic Press, London, UK.

Hijwegen T (1988) Effect of seventeen fungicolous fungi on sporulation of cucumber powdery mildew. Netherlands Journal of Plant Pathology 94: 185–190.

Hijwegen T (1989) Effect of culture filtrates of seventeen fungicolous fungi on sporulation of cucumber powdery mildew. Netherlands Journal of Plant Pathology 95(Suppl 1): 95–98.

Hijwegen T and Buchenauer H (1984) Isolation and identification of hyperparasitic fungi associated with Erysiphaceae. Netherlands Journal of Plant Pathology 90: 79–84.

Kavkova M and Curn V (2005) *Paecilomyces fumosoroseus* (*Deuteromycotina*: *Hyphomycetes*) as a potential mycoparasite on *Sphaerotheca fuliginea* (*Ascomycotina*: *Erysiphales*). Mycopathologia 159: 53–63.

Kiss L (2003) A review of fungal antagonists of powdery mildews and their potential as biocontrol agents. Pest Management Science 59: 475–483.

Kiss L, Cook RTA, Saenz GS, Cunnington JH, Pascoe I, Bardin M, Nicot PC, Takamatsu S, Sato Y and Rossman AY (2001) Identification of two powdery mildew fungi, *Oidium neolycopersici* sp. nov. and *O. lycopersici*, infecting tomato in different parts of the world. Mycological Research 105: 684–697.

Miller TC, Gubler WD, Laemmlen FF, Geng S and Rizzo DM (2004) Potential for using *Lecanicillium lecanii* for suppression of strawberry powdery mildew. Biocontrol Science and Technology 14: 215–220.

Nicot PC, Bardin M and Dik AJ (2002) Basic methods for epidemiological studies of powdery mildews: Culture and preservation of isolates, production and delivery of inoculum, and disease assesssment. In: Bélanger RR, Bushnell WR, Dik AJ and Carver TLW (eds) The Powdery Mildews (pp. 83–99) A Comprehensive Treatise American Phytopathological Society Press, St. Paul, MN, USA.

Romero D, Rivera ME, Gazorla FM, DeVincente A and Pérez-Garcia A (2003) Effect of mycoparasitic fungi on the development of *Sphaerotheca fusca* in melon leaves. Mycological Research 107: 64–71.

Samson RA (1974) *Paecilomyces* and some allied *Hyphomycetes*. Studies in Mycology 6: 1–119.

Scheewe P and Ketzel A (1994) In vitro screening for resistance against powdery mildew (*Podosphaera leucotricha* [Ell. et Ev.] Salm.) in apple. Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz 101: 368–377.

Shishkoff N and McGrath MT (2002) AQ10 Biofungicide combined with chemical fungicides or AddQ spray adjuvant for control of cucumber powdery mildew in detached leaf culture. Plant Disease 86: 915–918.

Szentiványi O and Kiss L (2003) Overwintering of *Ampelomyces* mycoparasites on apple trees and other plants infected with powdery mildews. Plant Pathology 52: 737–746.

Sztejnberg A, Paz Z, Boekhout T, Gafni A and Gerson U (2004) A new fungus with dual biocontrol capabilities: Reducing the numbers of phytophagous mites and powdery mildew disease damage. Crop Protection 23: 1125–1129.

Urbanietz A and Dunemann F (2005) Isolation, identification and molecular characterization of physiological races of apple powdery mildew (*Podosphaera leucotricha*). Plant Pathology 54: 125–133.

White TJ, Bruns T, Lee S and Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ and White TJ (eds) PCR Protocols: A Guide to Methods and Applications (pp. 315–322) Academic Press, San Diego.

Wyand RA and Brown JKM (2003) Genetic and forma specialis diversity in *Blumeria graminis* of cereals and its implications for host-pathogen co-evolution. Molecular Plant Pathology 4: 187–198.

Yokoyama E, Yamagishi K and Hara A (2004) Development of a PCR-based mating-type assay for *Clavicipitaceae*. FEMS Microbiology Letters 237: 205–212.