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Temperature-water and competitive interactions *in vitro* of two toxic black molds, *Fusarium oxysporum* and *Rhizopus stolonifer* isolated from a shower setting

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Abstract

We report differences in secondary resource capture and upper lethal temperature as survival attributes for *Fusarium oxysporum* (FOSC) and *Rhizopus stolonifer*, two medically important fungi that were co-isolated from a table shower caddy. Isolates of these two shower-associated fungi have been deposited at the University of Alberta Microfungus Collection and Herbarium, Centre for Global Microfungal Biodiversity (UAMH, Toronto, CAN) as *R. stolonifer* UAMH 11965 and *F. oxysporum* (FOSC) UAMH 11966; this paper provides growth characteristics for these two strains that can be used for further studies on black molds in bathrooms given the relevance to public health. Both *R. stolonifer* UAMH 11965 and *F. oxysporum* (FOSC) UAMH 11966 require contact with a wet surface substrate with water activity ≥ 0.95 a_w for growth and sporulation. In contrast to *F. oxysporum*, *R. stolonifer* has a 4-6x, faster radial growth rate, superior colonizing ability, and capacity for overgrowth (exploitative competition). This *F. oxysporum* (FOSC) strain, however, is thermotolerant, as demonstrated by broader thermal growth range and higher optimum temperature, which puts fewer limitations on its growth compared to *R. stolonifer*. This is a point of public awareness for mold-sensitive and immunocompromised people that wet, wood substrates in a shower can permit colonization and competitive interactions that can concentrate *R. stolonifer* and *F. oxysporum* spores.

Key words – Bathroom – fungal ecology – public health – radial growth – resource capture

Introduction

High relative humidity, moisture, warm temperature, soap and shampoo surfactants create an ideal habitat for a variety of pathogenic and allergenic molds to people (Hamada & Abe 2009, 2010a,b, Short et al. 2011). *Acremonium*, *Alternaria*, *Aureobasidium*, *Cladosporium*, *Cyphellophora*, *Exophiala*, *Fusarium*, *Paecilomyces*, *Phoma*, *Ramichloridium*, *Rhodotorula*, and *Scolecobasidium* (ascomycetes and basidiomycetes) are some of the medically important molds that have been recovered from bathroom settings (Hamada & Abe 2009, 2010a,b). Of these, the most noteworthy and frequent among the bathroom molds is *Fusarium*, a genus that consists of numerous potentially harmful species (Short et al. 2011, Levetin et al. 2016, Egbuta et al. 2017). There are few reports of bathroom contamination with zygomycetes. However, Garner & Machin (2008) reported *Rhizopus pusillus* with a high frequency of isolation from a patient's shower in connection with water damage, air contamination, and in association with wooden substrates.

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Under adequate moisture and temperature conditions, any of these fungal bathroom contaminants are capable of producing copious amounts of spores, conidia and sporangiospores (Amari et al. 2011). Children, the elderly, mold-sensitive, and immunocompromised people, in particular, are at an elevated risk of infection via spores. The spores gain access to the inside of the body by handling, inhalation, introduction into broken skin, or by way of wounds (Egbuta et al. 2017). Although seldom reported in clinical literature, dual infections in skin and respiratory system can take place, including harmful *Rhizopus-Fusarium* combinations (de Almeida Júnior et al. 2016). A *Rhizopus-Fusarium* combination also occurs in decomposing potatoes (Ibrahim et al. 2014).

Here, we report a *Rhizopus-Fusarium* growth that was isolated from a wood table caddy that was being used in a bathroom shower. The two fungi were identified as *Rhizopus stolonifer* UAMH 11965 and *Fusarium oxysporum* species complex (FOSC) UAMH 11966. We have chosen to keep the company of the wood shower table caddy confidential. This specific source information is not pertinent to this investigation, only that a wood table shower caddy can be a substrate for *Rhizopus* and *Fusarium* fungal growth in a shower. Because this *R. stolonifer* strain, and similarly this *F. oxysporum* strain, were isolated from a shower setting make these strains of particular public health value in relation to bathroom hygiene.

This study compares the growth characteristics of *R. stolonifer* UAMH 11965 and *F. oxysporum* (FOSC) UAMH 11966, with these goals: 1. to determine how each fungus grows in the presence of each other (i.e., combative strategy); and 2. to define the factors that are limiting to fungus growth and sporulation (water availability and temperature requirements). We are testing the hypothesis that these *F. oxysporum* and *R. stolonifer* strains may differ in their water and temperature requirements.

Materials & Methods

Fungus isolation and identification

Random block design was used to select three different sites from a patch of mold that had developed on the undersurface of the wood table shower caddy. Each site was swabbed with three, individual cotton tipped applicators (Puritan Medical Products, Guilford, ME). The sample was streaked for isolation onto Potato Dextrose Agar (PDA) in a 100 x 15 mm Petri dish (Fisher Scientific, St. Louis, MO). A total of nine Petri dishes were inoculated from the test mycelium. Incubation was in darkness, 25 ± 0.5ºC (Percival incubator, Perry, IA). Isolations were made by subculturing hyphal tips from the advancing edge of isolated colonies (40-45x light microscopy). Each hyphal tip was considered an isolate. Isolates were identified at the University of Alberta Microfungus Collection and Herbarium, Centre for Global Microfungal Biodiversity (UAMH, Toronto, CAN).

All experiments were conducted on PDA, which is the medium of choice for maintaining stable spore size/shape, hyphal branching pattern, hyphae diameter, and colony pigmentation in *Rhizopus* (Hernádez-Lauzardo et al. 2005) and *Fusarium* (Rafique et al. 2015). Design of temperature-water activity, growth, and competition experiments follows standard methods (Lockwood 1992, Wicklow 1992, Baldrian & Gabriel 2002).

Determination of optimum temperature and water activity for growth

Computer-programmable incubators (SD < ± 0.5ºC; Percival) were used to control temperature. Glycerol (> 99.5% pure; Sigma Chemicals, St. Louis, MO)-supplemented PDA was used to adjust the water activity (a_w) of the agar media (Rousseau & Donèche 2001). Each water activity was measured using a hygrometer (SD ± 0.005% a_w; Thomas Scientific, Philadelphia, PA). Based on interpretations according to Christian (1980), the water activity of pure water = 1.00 a_w, the moisture content of a surface with a water activity ≥ 0.90 a_w is “wet”; and the moisture content of a surface with a water activity 0.65 - 0.85 a_w is “damp”.

All experiments were conducted on solidified media in 100 x 15 mm Petri dishes (Fisher) that had been scored into quadrants by drawing two lines that crossed each other on the bottom of the dish. Two-week old cultures of each test fungus were used, using a 0.5 cm^2 agar plug, made with the

end of a sterile pipette (Fisher) from the edge of the mycelium. The plug was placed on top solidified PDA at the center of the dish, overtop the intersection of the two lines that had been drawn on the bottom. Dishes were incubated and examined daily by 40-45x light microscopy. Five measurements were taken on each line (n = 4) as the mycelium spread over the agar surface; n = 20 growth measurements/dish, set up in triplicate; thus, each growth rate is the mean (± SE) of 60 individual measurements. Plain agar plugs containing no fungus served as controls.

Radial growth rate (K_r) was calculated: $K_r = (R_1 - R_0)/(t_1 - t_0)$, where R_1 and R_0 are colony radii at the beginning of the linear (t_0) and stationary (t_1) phases of growth. Experimental growth period was ten days or until five measurements had been taken. Radial growth rates were compared using an analysis of covariance (ANCOVA; $p < 0.05$) and response surface methodology for examination of optimization effects of combined temperature and water activity (SPSS 14.0 Microsoft Excel and Minitab, Chicago, IL).

Determination of competitive ability by primary resource capture

Two 0.5 cm^2 agar plugs of fungus (from two-week old culture) were placed opposite each other in a 100 x 15 mm Petri dish on solidified PDA. Each plug was placed 5 mm from the edge of the dish. Four lines for measuring the spread of the mycelium over the agar surface were drawn on the bottom of the dish such that the lines (separated by 36º from each other) radiated outward from the center of the plug. The two fungi were growing at the same time, opposite each other, in the dish. Growth measurements were stopped before the fungal hyphae made contact with each other, as determined by 40/45x light microscopy. Dishes were incubated at 25ºC. The radial growth rate (K_r) was calculated as described above for both fungi in the pairing as the mycelium of each approached the other.

The experiment was conducted in triplicate; i.e., each radial growth rate is the mean (± SE) of 20 measurements (five per growth line), replicated three times, to total n = 60 individual measurements. Data were compared with ANCOVA. In all cases, microscopic examination of the mycelium margin, and hyphal, spore, and colony characteristics were used to track and distinguish each fungus.

Determination of competitive ability by secondary resource capture

A 0.5 cm^2 agar plug of fungus (inoculum), taken from a two-week old culture, was placed on top, at the center, of an established mycelium that had been growing in a Petri dish (100 x 15 mm) on solidified PDA for one week. The Petri dish had been scored with two lines such that the dish was divided into quadrants. Measurements were taken, as described above, along the lines for the fungus inoculum as it spread over the existing mycelium. Measurements were used to calculate the radial growth rate (K_r) of the inoculum as described above. Fungi were differentiated based on spore and colony characteristics, mycelium margin, and pure culture comparison by 40/45x microscopy. No fungal growth, K_r = 0.00 mm/day, was confirmed by 40/45x microscopy, as an absence of visible hyphae and subsequent spores. Each experiment was replicated three times, for a total n = 60 individual measurements for the growth rate of each fungus. ANCOVA ($p < 0.05$) was used to compare the radial growth rates (the mean ± SE; $p < 0.05$).

Determination of competitive ability by differential competition

Twenty 0.5 cm^2 plugs of fungus were placed 1 cm apart from each other in a 100 x 15 mm Petri dish on solidified PDA. The twenty plugs of fungus represented different proportions of the two fungus strains in each Petri dish. One strain was designated as fungus A and the other strain as fungus B. The plugs of different fungi were placed into the dish by random block design. The perimeter of the mycelium around each plug was measured daily with a digital planimeter (Professional Equipment Inc., Hauppauge, NY). Of 20 plugs of fungus in a Petri dish, test proportions were: 1.0 = 20 plugs fungus A; 0.8 = 16 plugs fungus A and 4 plugs fungus B; 0.6 = 12 plugs fungus A and 8 plugs fungus B; 0.4 = 8 plugs fungus A and 12 plugs fungus B; 0.2 = 4 plugs fungus A and 16 plugs fungus B; and 0.0 = 20 plugs fungus B.

A 0.5 proportion (10 plugs fungus A and 10 plugs fungus B) was used to calculate the relative crowding coefficient, RCC = [(area occupied by fungus A at 0.5:0.5)/(area occupied by fungus B at 0.5:0.5)]/ [(area occupied by fungus A at 1:0)/(area occupied by fungus B at 1:0)]. If fungus A has an advantage over fungus B, then the RCC > 1. Conversely, an RCC < 1 means that fungus B is a better competitor than fungus A. Results were compared using ANCOVA ($p < 0.05$), based on three replicates of each proportion of fungal species tested.

Results

Fungus isolation and identification

At the sampling site where mold was growing, 14 colonies were found at the first area swabbed, six at the second area swabbed, and 19 from the third area swabbed ($n = 3$ replicates at each area swabbed; $n = 9$) for a total of 39 isolations. The percentage breakdown of isolates was 62 ± 1.1% *Rhizopus*, 33 ± 0.4% *Fusarium*, and 5 ± 0.5% *Penicillium* (the mean ± SE; $P < 0.05$). One isolate was identified as Mycelia sterilia. The fungi were identified as *Rhizopus stolonifer* UAMH 114965 and *Fusarium oxysporum* UAMH 11966. These isolates have been deposited at the University of Alberta Microfungus Collection and Herbarium (UAMH), Toronto, CAN.

Optimum temperature and water activity

Rhizopus stolonifer grows at a faster rate than *F. oxysporum* (at each pairwise comparison at a particular temperature and water activity; $p < 0.05$; Fig. 1). For *R. stolonifer*, the thermal growth range is 15-30ºC, and growth peaks at 20ºC. There is no growth at 35ºC, 40ºC and 45ºC. In contrast, *F. oxysporum* has broader thermal growth range (15-35ºC), higher optimum temperature (25ºC), and there is growth at 35ºC.

Both *R. stolonifer* and *F. oxysporum* can grow down to 0.95 a_w, and they exhibit peak growth at 0.997 a_w. There is no growth at 0.90, 0.85, 0.80, 0.75, 0.70, 0.65, 0.60, 0.55, 0.50, and 0.40 a_w's. Sporulation for *R. stolonifer* and *F. oxysporum* took place at any temperature and water activity where there was measurable growth. The exception was for *R. stolonifer*, where at 0.95 a_w and 15ºC *R. stolonifer* grew without producing aerial hyphae and subsequently no spores. At 15ºC, the minimum water activity for producing spores by *R. stolonifer* is 0.96 a_w, and 0.95 a_w at higher temperatures like in *F. oxysporum*.

Primary resource capture

Radial growth rate of *R. stolonifer* was not reduced prior to hyphal contact by the addition of *F. oxysporum* within the same Petri dish (Table 1). Similarly, no decrease in radial growth rate by *F. oxysporum* occurred by the presence of *R. stolonifer*. No decrease in growth rate was observed in conspecific controls, as the result of when *R. stolonifer* and *R. stolonifer*, or *F. oxysporum* and *F. oxysporum*, were pitted against each other.

Secondary resource capture

Radial growth rate of *R. stolonifer* was reduced by 43% when it was placed on an existing, established mycelium of *F. oxysporum* (Table 2). However, *F. oxysporum* when added to the mycelium surface of an established *R. stolonifer* colony failed to initiate growth. Adding the same fungus to an existing mycelium of the same strain (conspecific controls) resulted in no measurable growth of the fungus that was introduced.

Differential competition

Rhizopus stolonifer spread over a greater surface area than *F. oxysporum* when they were placed in mixed proportions in a Petri dish (in each pairwise comparison, $p < 0.05$; Fig. 2). The relative crowding coefficient (RCC) for the *R. stolonifer-F. oxysporum* pair is 3.04. Increasing area of mycelium spread did not occur with increasing inoculum in a linear dose response: $y = 125x$, $R = 0.39$ for *R. stolonifer* and $y = 47x$, $R = 0.52$ for *F. oxysporum*.

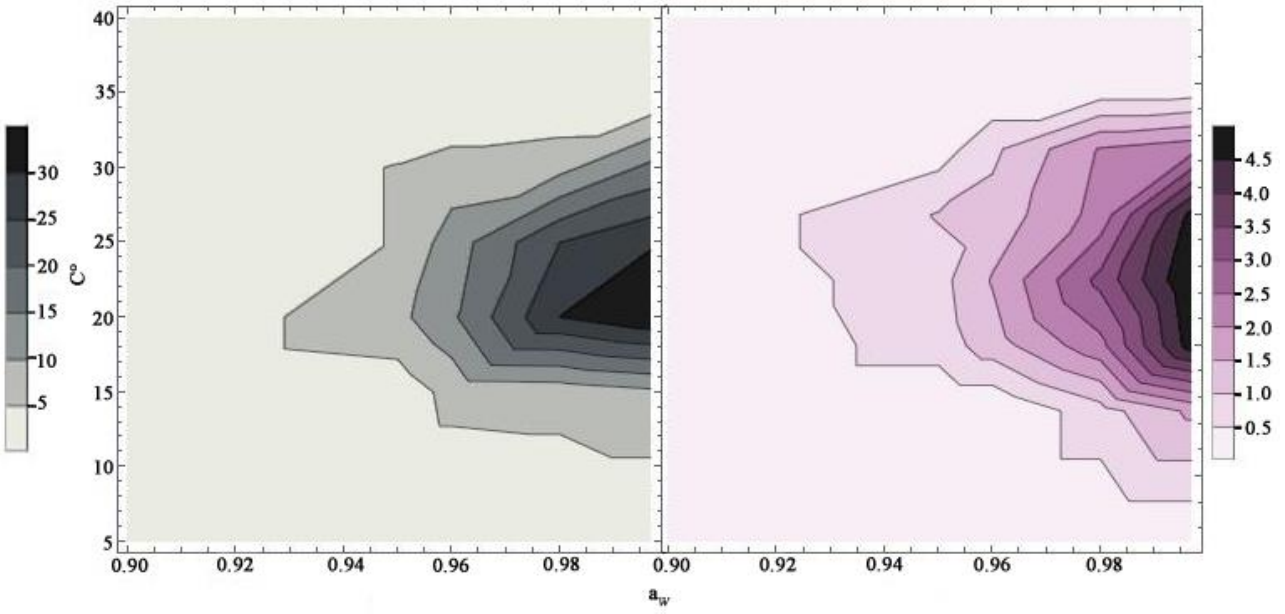

Fig. 1 – Contour plot of response surface showing optimization effects of temperature and water activity (a_w) on growth of *Rhizopus stolonifer* UAMH 11965 (gray plot) and *Fusarium oxysporum* UAMH 11966 (purple plot). Radial growth rates are the mean $K_r \pm SE \leq 0.34$.

Discussion

It is clear that *R. stolonifer* UAMH 11965 is the more aggressive, faster growing fungus (primary colonizer). As such, *R. stolonifer* UAMH 11965 is also an effective secondary colonizer, and *F. oxysporum* UAMH 11966 is the weaker, slower growing fungus of the pair; i.e., the RCC (relative crowding coefficient) is 3.0. The disproportionate growth favoring *R. stolonifer* results in large area of mycelium coverage at low inoculums (result of pairing a fast with a slow grower in the same dish); the response would otherwise be linear if growth rates of these fungi were similar, but this was not observed. Few, if any studies, report results for the direct fungus-fungus interaction *in vitro* for *Rhizopus* and *Fusarium*. We found that neither strain inhibited incoming hyphae when they were pitted against each other in the same Petri dish.

An established mycelium of *F. oxysporum* allowed for *R. stolonifer* to initiate growth, develop, establish, and produce spores, although at 43% the regular rate. At no time was the *F. oxysporum* replaced by *R. stolonifer*. Simply, *R. stolonifer* grew directly over *F. oxysporum*. Once past the edge of the *F. oxysporum* mycelium, where there is no inhibitory effect, the growth of *R. stolonifer* returned to its regular, fast rate (data not shown). Applying Lockwood's (1992) and Wicklow's (1992) interpretations, we conclude that *F. oxysporum* UAMH 11966 competes by interfering with *R. stolonifer* UAMH 11965 growth in order to secure limiting resources, whereas *R. stolonifer* UAMH 11965 is an exploitative competitor in addition to an interference competitor.

Contact with a wet (not damp) surface substrate is required for growth and sporulation by both *R. stolonifer* UAMH 11965 and *F. oxysporum* UAMH 11966. The minimum water activity ≥ 0.95 a_w for both of these shower-associated strains which is above the "wet", 0.90 a_w threshold as defined by Christian (1980). The growth of *R. stolonifer* and *F. oxysporum* peaks on agar that is close to water saturation. The optimum water activity for both species = 0.997 a_w and water = 1.00 a_w. We found that "damp" conditions of 0.65-0.85 a_w will stop growth of *R. stolonifer* and *F. oxysporum*. Indeed, both *Rhizopus* and *Fusarium* are known for their habitat preference and suitability for moisture-rich substrates and surfaces (Amari et al. 2011). We now extend this trend to include *Rhizopus* and *Fusarium* isolates from a wet, shower substrate. A water-saturated wood substrate makes *F. oxysporum* and *R. stolonifer* successful, especially in a regularly used shower that would keep the moisture supply relatively continuous on a frequent basis. The growth of *R. stolonifer* UAMH 11965 and *F. oxysporum* UAMH 11966 is likely influenced by frequency and duration of showering that

would contribute to the wood substrate holding more water for longer periods of time and less prone to evaporation and "dampness" which is unfavorable for fungus growth.

For the strains investigated here, *F. oxysporum* UAMH 11966 is high temperature adapted by having a higher upper lethal temperature of 35-40ºC and higher optimum temperature of 25ºC when compared to *R. stolonifer* UAMH 11965. Thus, *F. oxysporum* can initiate growth, sporulate, and establish over a broader temperature range than *R. stolonifer*. Under 22-24ºC, indoor, standard comfort conditions for people (Ferng & Lee 2002), the growth and sporulation of these shower-associated strains of *R. stolonifer* and *F. oxysporum* occur readily as long as surface substrates are "wet" to satisfy their water requirement. The mean 41ºC preferred water temperature of a typical shower (Buijze et al. 2016) would be too high for *R. stolonifer* and *F. oxysporum* growth; however, adequate growth conditions would resume once the shower has ended. This strain of *F. oxysporum* UAMH 11966 has a competitive advantage over *R. stolonifer* UAMH 11965 under hotter conditions, at the upper end of temperature range for a typical mesophile (Amari et al. 2011).

Taken together, *R. stolonifer* and *F. oxysporum* (FOSC) are an antagonistic mycoflora, which consists of numerous non-pathogenic and pathogenic strains that can cause severe allergies and secondary infections in people (O'Donnell et al. 2009, Levetin et al. 2016, Egbuta et al. 2017). The importance of this study is that it examines, for the first time, the interaction *in vitro* between these two medically important molds that are specifically bathroom-associated isolates. This study identifies differences in mycelium growth, upper lethal temperature, and secondary resource capturing as the key survival elements that promote the natural coexistence and increase abundance of *F. oxysporum* (FOSC) UAMH 11966 and *R. stolonifer* UAMH 11965 in a shower setting.

Table 1 Primary resource capture experiments showing the radial growth of *Rhizopus stolonifer* UAMH 11965 and *Fusarium oxysporum* UAMH 11966 in paired competition experiments, 25ºC. The mean radial growth rate (K_r, the mean ± SE) was determined before hyphae of the two fungi made contact. Data followed by the same letter with a column are not significantly different ($p > 0.05$).

Competitor	Vs.	Test Fungus	K_r (mm/d) of Test Fungus
None		*R. stolonifer*	28.7 ± 0.3^a
R. stolonifer		*R. stolonifer*	31.4 ± 0.4^a
F. oxysporum		*R. stolonifer*	31.2 ± 0.4^a
None		*F. oxysporum*	5.2 ± 0.3^b
F. oxysporum		*F. oxysporum*	4.7 ± 0.1^b
R. stolonifer		*F. oxysporum*	5.3 ± 0.2^b

Table 2 Secondary resource capture experiments showing the radial growth of *Rhizopus stolonifer* UAMH 11965 and *Fusarium oxysporum* UAMH 11966 when placed onto an existing mycelium of another fungus, 25ºC. NG, no growth. Data (the mean K_r ± SE) followed by the same letter with a column do not differ significantly ($p > 0.05$).

Existing Mycelium	Fungus Introduced	K_r (mm/d) of Fungus Introduced
None	*R. stolonifer*	29.2 ± 0.5^a
F. oxysporum	*R. stolonifer*	16.6 ± 0.4^b
R. stolonifer	*R. stolonifer*	NG (0.0 mm/d^c)
None	*F. oxysporum*	4.9 ± 0.3^d
R. stolonifer	*F. oxysporum*	NG (0.0 mm/d^c)
F. oxysporum	*F. oxysporum*	NG (0.0 mm/d^c)

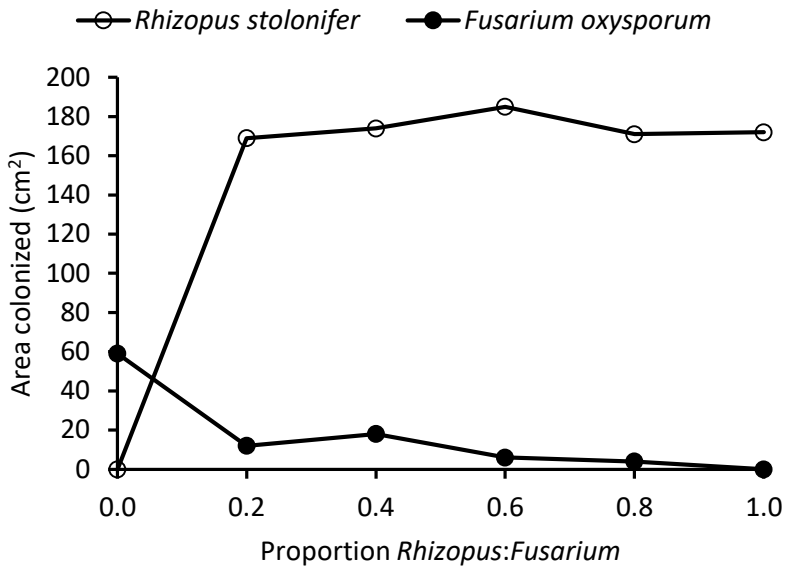

Fig. 2 – Differential competition colonizing ability *Rhizopus stolonifer* UAMH 11965 and *Fusarium oxysporum* UAMH 11966, 25ºC, in mixed proportions in the same Petri dish. Each point is the mean ± SE ≤ 3.7. The relative crowding coefficient (RCC) for this *R. stolonifer-F. oxysporum* pairing is 3.04.

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References

Amari A, Chai W, Schnabel G. 2011 – Effect of nutrient status, pH, temperature and water potential on germination and growth of *Rhizopus stolonifer* and *Gilbertella persicaria*. Journal of Plant Pathology 93, 603–612.

Baldrian P, Gabriel J. 2002 – Intraspecific variability in growth response to cadmium of the wood rotting fungus *Piptoporus betulinus*. Mycologia 94, 428–436.

Buijze GA, Sierevelt IN, van der Heijden BCJM, Dijkgraaf MG, Frings-Dresen MHW. 2016 – The effect of cold showering on health and work: A randomized controlled trial. PLoS– One. 11:e0161749.

Christian JHB. 1980 – Reduced water activity. In: Silliker JH, Elliott RP, Baird-Parker AC, Bryan FL, Christian JHB, Clark DS, Olson JC Jr, Roberts TA (eds), Microbial Ecology of Foods. Academic Press, New York, pp. 70–91.

de Almeida Júnior J, Ibrahim KY, Del Negro GM, Bezerra ED et al. 2016 – *Rhizopus arrhizus* and *Fusarium solani* concomitant infection in an immunocompromised host. Mycopathologia 181, 125–129.

Egbuta MA, Mwanza M, Babalola OO. 2017 – Health risks associated with exposure to filamentous fungi. International Journal of Environmental Research and Public Health 14, 719–735.

Ferng SF, Lee LW. 2002 – Indoor air quality assessment of daycare facilities with carbon dioxide, temperature, and humidity as indicators. Journal of Environmental Health 65, 14–18.

Garner D, Machin K. 2008 – Investigation and management of an outbreak of mucormycosis in a paediatric oncology unit. Journal of Hospital Infection 70, 53–59.

Hamada N, Abe N. 2009 – Physiological characteristics of 13 common fungal species in bathrooms. Mycoscience 50, 421–429.

Hamada N, Abe N. 2010a – Comparison of fungi found in bathrooms and sinks. Biocontrol Science 15, 51–56.
Hamada N, Abe N. 2010b – Growth characteristics of four fungal species in bathrooms. Biocontrol Science 15, 111–115.
Hernádez-Lauzardo A, Bautista-Baños S, Velázquez del Valle M, Trejo-Espino J. 2005 – Identification of *Rhizopus stolonifer* (Ehrenb.: Fr.) vuill., causal agent of *Rhizopus* rot disease of fruits and vegetables. Revista mexicana de fitopatología 24, 65–69.
Ibrahim M, Shehu K, Tafinta IY, Iman UA, Hassana YI. 2014 – Efficacy of some plant extracts on growth and germination of *Rhizopus stolonifer* and *Fusarium oxysporum* isolated from rotten Irish potato tubers. Annals of Biological Science 2, s63–67.
Levetin E, Horner WE, Scott JA. 2016 – Taxonomy of allergenic fungi. Journal of Allergy and Clinical Immunology in Practice 4, 375–385.
Lockwood JL. 1992 – Exploitation competition. In: Carroll GC, Wicklow DT (eds), The Fungal Community – Its Organization and Role in the Ecosystem, 2nd ed. Marcel Dekker, New York, pp. 243–263.
O'Donnell K, Gueidan C, Sink S, Johnston PR et al. 2009 – A two-locus DNA sequence database for typing plant and human pathogens within the *Fusarium oxysporum* species complex. Fungal Genetics and Biology 46, 936–948.
Rafique K, Rauf CA, Naz F, Shabbir G. 2015 – DNA sequence analysis, morphology and pathogenicity of *Fusarium oxysporum* f. sp. *lentis* isolates inciting lentil wilt in Pakistan. International Journal of Biosciences 7, 74–91.
Rousseau S, Donèche B. 2001 – Effect of water activity (a_w) on the growth of some epiphytic micro-organisms isolated from grape berry. Vitis 40, 75–78.
Short DP, O'Donnell K, Zhang N, Juba JH, Geiser DM. 2011 – Widespread occurrence of diverse human pathogenic types of the fungus *Fusarium* detected in plumbing drains. Journal of Clinical Microbiology 49, 4246–4272.
Wicklow DT. 1992 – Interference competition. In: Carroll GC, Wicklow DT (eds), The Fungal Community – Its Organization and Role in the Ecosystem, 2nd ed. Marcel Dekker, New York, pp. 265–274.