



Population growth of the stored product pest *Tyrophagus putrescentiae* (Acari: Acaridae) on environmentally and medically important fungi

Guilherme Liberato da Silva^{1,2} · Isadora Zanatta Esswein¹ · Daiane Heidrich³ · Fabíola Dresch¹ · Mônica Jachetti Maciel¹ · Danielle Machado Pagani² · Patrícia Valente² · Maria Lúcia Scroferneker² · Liana Johann¹ · Noeli Juarez Ferla¹ · Onilda Santos da Silva²

Received: 10 January 2019 / Accepted: 7 May 2019
© Springer Nature Switzerland AG 2019

Abstract

The stored food mite *Tyrophagus putrescentiae* (Schrank) (Acari: Acaridae) has been associated with the presence of several fungal species. The aims of this work were to evaluate *T. putrescentiae* population growth associated to environmental and medically important fungal species to determine on which fungal species populations of *T. putrescentiae* performs best, and to evaluate their ability to disperse each fungal species. First, 24 fungal species were inoculated separately in Petri dishes containing Sabouraud agar medium. One week after inoculation, 50 mites were added to each plate. On the 28th evaluation day, mites and eggs were counted in each plate, and 50 mites randomly collected from each replicate were transferred to new plates containing only Sabouraud agar medium. Then, mites, eggs, and fungal population were evaluated in each plate on day 28 again. The highest population increases were on *Trichophyton mentagrophytes*, *Alternaria* sp., *Microsporum gypseum*, and *Aspergillus chevalieri*. With *Fusarium guttiforme* and the medically important fungi *Microsporum canis*, *M. gypseum*, *T. mentagrophytes*, and *Sporothrix* sp., mites were observed to feed on whole mycelium. Only eight fungal species were dispersed by *T. putrescentiae* to the new Petri dishes: *Aspergillus clavatus*, *Candida tropicalis*, *Candida albicans*, *Fusarium guttiforme*, *Hyphopichia burtonii*, *Penicillium citrinum*, *Rhizopus azygosporus*, and *Trichophyton mentagrophytes*. The best performance of *T. putrescentiae* was found feeding on *F. guttiforme*, *P. citrinum*, and *T. mentagrophytes*. In conclusion, *T. putrescentiae* successfully used fungi as a food source, and it proved to be an important tool for disseminating both environmental and medically important fungi.

Keywords Dispersion · Mycophagy · *Fusarium guttiforme* · *Trichophyton mentagrophytes* · Stored food mite

✉ Guilherme Liberato da Silva
gibaliberato_148@hotmail.com

Extended author information available on the last page of the article

Introduction

The cosmopolitan mite *Tyrophagus putrescentiae* (Schrank) (Acari: Acaridae) is one of the most abundant and frequent species found in stored foods (wheat, soy, cheese etc.), and it is a species with economic and medical-veterinary importance (Duek et al. 2001; Mullen and O'Connor 2009). These mites can also colonize human-created habitats, such as households, dust in urban environments, medical and laboratory facilities, farms, and food factories (Franz et al. 1997; Solarz et al. 2007). High infestations by this mite cause degradation of food and feed products, speeding up their deterioration in storage facilities (Kucerova and Horak 2004).

Unsatisfactory sanitary conditions (e.g., if the food is inadequately stored) may provide conditions for growth of fungi (Osborne and Stein 2007), which can be concurrently infested with mites (Stejskal and Hubert 2008). Studies concerning mite-fungus interaction have shown: (1) fungal consumption by fungivorous mite species, such as *T. putrescentiae*, which can develop and reproduce exclusively on fungal diets (Nesvorná et al. 2012; Smrž et al. 2016); (2) selective transfer of fungal species by mites, with fungi attractive to mites being dispersed more (Hubert et al. 2003); and (3) grain contamination by fungi which are pathogenic to mites (Armitage and George 1986). The migration of *T. putrescentiae* populations in a wide range of ecosystems is known to contribute to the dispersal of viable spores of several fungal species, which can be transported on the vector's body surface or deposited with their feces, rapidly spreading the spores throughout the substrate on which they live (Nesvorná et al. 2012; Franzolin et al. 1999; Hubert et al. 2004). Fungi may benefit from the dispersal of spores attached to the body surface or of undigested spores in the excreta (Hubert et al. 2003; Griffiths et al. 1959). In addition, mite grazers are attracted by fungal volatiles (Vanhaelen et al. 1980). These interactions promote the dissemination of fungi throughout the stored grains, influencing both pests.

Molds and yeasts are widely disseminated by air, dust, fomites, plants and animal microbiota. Although there are few reports of human pathogenic fungi in stored food, these fungi are found in the environment (Mohapatra et al. 2017). Therefore, cross-contamination of stored food and feed by human pathogenic fungi, which are transported by mites from their primary environmental habitats, is feasible. Food contamination with human pathogenic yeasts has already been reported, and virulence factors of foodborne yeasts demonstrate a potential risk to human health (Rajkowska and Kunicka-Styczyńska 2018). Furthermore, the presence of mycotoxigenic fungi in stored grains is well known (Mediavilla et al. 1996), as well as the presence of fungal species whose spores may be important as allergen-producing agents for humans, which could be carried by mites (Hubert et al. 2004; Walsh and Dixon 1996). In addition, *T. putrescentiae* has received attention as one of the leading causes of allergic asthma, allergic diseases, and rhinitis in humans (Arlian et al. 1984; Nuñez et al. 2016). Anaphylaxis after eating *T. putrescentiae*-contaminated food has already been described (Matsumoto et al. 1996). Therefore, studies on interactions between mites and human pathogenic fungi are urgently required.

Our main objective was to expand the current knowledge concerning the interaction between mites (in particular *T. putrescentiae*) and fungi, elucidating whether (I) *T. putrescentiae* can disperse and sustain population growth on human pathogenic fungi as well as on fungi isolated from stored food; (II) *T. putrescentiae* fitness is influenced by fungal species or cell morphology (filamentous x yeast-like fungi); and (III) *T. putrescentiae* preferentially disperses fungal species that provide it with the best performance.

Materials and methods

Stock colonies of *Tyrophagus putrescentiae*

Individuals of *T. putrescentiae* were obtained from cornmeal provided by an animal-feed producer in Teutônia, Rio Grande do Sul State, Brazil. The stock colony was initiated approximately 8 months before the beginning of the study. The colonies were maintained in a plastic Petri dish (15 × 2.5 cm) placed on a layer of foam matting continuously moistened with distilled water inside the dish. The edges of the dish were covered with a layer of cotton wool to prevent mites from escaping. Each colony unit was filled up with dry yeast (*Saccharomyces cerevisiae*), which was used as food source for the mites, and environmental conditions were not controlled. Unsexed adults were used in the assays.

Fungal cultures

Two groups of fungi were used in the experiments: eight environmentally important species, isolated from samples of cornmeal and rice flour using the method set forth by Normative Instruction (IN) no. 62 of August 26, 2003 (Brazilian Ministry of Agriculture, Livestock and Food Supply—MAPA: Ministério da Agricultura, Pecuária e Abastecimento); and 16 isolates, each with a medically relevant fungal species or genus maintained in the Culture Collection of the Federal University of Rio Grande do Sul (UFRGS), Brazil. One week before the experiments, each fungus was spread in Petri dishes containing Sabouraud agar with chloramphenicol (0.24 g/L) and cultures were incubated in a climate chamber at 25 ± 1 °C. The list of fungal species is described in Table 1.

Identification of fungal species

The identification of the clinical isolates was maintained as they were received. Identification of stored food fungi at the genus level was performed through macro- and micromorphological observations of the isolates. The genomic DNA of the isolates was extracted using Power Soil DNA Isolation Kit® (MoBio, USA), following the manufacturer's guidelines. Polymerase Chain Reaction (PCR) conditions and primers for identifications of environmentally important fungi are in Table 2. The PCR was comprised of primers described in Table 2; 1 × buffer PCR (Invitrogen); 2 mM magnesium chloride for β -tubulin gene (Ben-A), elongation factor 1 α gene (EF-1 α); and 3 mM for Internal Transcribed Spacer (ITS) and part of calmodulin gene region (Calm); 0.12 mM dNTP mix for ITS and 0.4 mM for the other regions; 0.04 U Taq DNA polymerase for ITS and 0.03 U Taq platinum DNA polymerase (Invitrogen) for the other regions; and 5 μ L of DNA. The PCR products were purified using ExoSAP-IT (Affymetrix, USA) according to the manufacturer's instructions. Sequencing was performed in an ABIPRISM 3100 Genetic Analyzer (Applied Biosystems). In all environmental isolates, ITS rDNA region sequencing was compared with the type strains deposited in GenBank using the Basic Local Alignment Search Tool (BLAST) algorithm. To confirm *Penicillium* and *Aspergillus* species, calmodulin was also compared to the type strains (Samson et al. 2004, 2014). Phylogenetic trees were performed using sequencing of ITS, Ben-A, and EF-1 α to identify *Fusarium* species (Watanabe et al. 2011),

Table 1 Eight environmentally important fungal species isolated from food samples and their GenBank accession numbers and 16 medically important fungal species from the Culture Collection of Federal University of Rio Grande do Sul (UFRGS) used in the experiments

Environmentally important fungi	Date of isolation (all March 2016)	Sample	GenBank accession number of ITS rDNA region
<i>Aspergillus chevalieri</i>	10th	Corn meal	MH532415
<i>Aspergillus oryzae</i>	11th	Rice flour	MH569333
<i>Fusarium guttiforme</i>	11th	Corn meal	MH569332
<i>Hyphopichia burtonii</i>	10th	Rice flour	MH532416
<i>Penicillium aethiopicum</i>	13th	Corn meal	MH675468
<i>Penicillium citrinum</i>	11th	Corn meal	MH532414
<i>Rhizopus azygosporus</i>	10th	Corn meal	MH553362
<i>Trichoderma longibrachiatum</i>	11th	Corn meal	MH675467
Medically important fungi (clinical isolates; origin: culture collection)			
<i>Alternaria</i> sp.			
<i>Aspergillus clavatus</i> , <i>A. flavus</i> , <i>A. fumigatus</i> , <i>A. niger</i> , <i>A. terreus</i>			
<i>Candida albicans</i> , <i>C. parapsilosis</i> , <i>C. tropicalis</i>			
<i>Epidermophyton floccosum</i>			
<i>Microsporium canis</i> , <i>M. gypseum</i>			
<i>Sporothrix</i> sp.			
<i>Trichophyton mentagrophytes</i> , <i>T. rubrum</i>			
<i>Trichosporum</i> sp.			

whereas ITS and EF-1 α were required for the identification of *Trichoderma* species (Druzhinina et al. 2008).

Evaluation of *Tyrophagus putrescentiae* growth on fungal species

The isolates of 24 fungal species (eight environmental and 16 medically important species) were inoculated separately in the center of the Petri dishes (9×1.5 cm) containing Sabouraud agar with chloramphenicol, and incubated at 25±1 °C for 1 week, in triplicates. The study was divided in two phases. The first phase started 1 week after fungal inoculation, when 50 mites were added to each previously inoculated Petri dish. The dishes were covered with Parafilm M® (Bemis NA, Neenah, Wisconsin, USA) to prevent mites from escaping. The Petri dishes were incubated at 25 °C for 28 days and observed at weekly intervals using a stereoscopic microscope Leica EZ4 HD with a digital camera (Leica). After the 28th day, mites (live immature stages and adults) and eggs were counted in each Petri dish. Afterwards, the second phase of the study was initiated.

Tyrophagus putrescentiae spread of fungal species to new Petri dishes

After the end of the first phase, 50 mites or less (according to the maximum obtained) from each replicate of fungal species were collected using a sterile brush. Mites were collected outside visible fungal colonies and transferred into Petri dishes containing Sabouraud agar

Table 2 Identification methods of environmentally important fungal species by sequencing

Locus	Primers	Primer concentration (pmol/ μ L)	PCR Program	Reference
Internal transcribed spacer (ITS)	ITS1, ITS4	0.64	95 °C (5 min) 95 °C (1 min)/55 °C (1 min)/72 °C (2 min) [30 $\times$] 72 °C (10 min)	White et al. (1990)
β -tubulin (BenA)	Bt2a, Bt2b	0.64	95 °C (4 min) 95 °C (45 s)/66 °C (30 s)/72 °C (2 min) [35 $\times$] 72 °C (7 min)	Glass and Donaldson (1995)
Calmodulin (Calm)	CL1, CL2A	0.8	94 °C (4 min) 95 °C (30 s)/60 °C (1 min)/72 °C (1 min) [35 $\times$] 72 °C (7 min)	Otonelli et al. (2014)
Elongation factor 1- α (EF-1 α)	EF1, EF2	0.24	94 °C (1 min) 94 °C (30 s)/62 °C (45 s)/72 °C (1 min) [34 $\times$] 72 °C (5 min)	Watanabe et al. (2011)

with chloramphenicol (0.24 g/L). The Petri dishes were covered with Parafilm, incubated at 25 °C for 28 days, and observed at weekly intervals by taking photos. On the 28th day, mites (immature stages and adults) and eggs were counted in each Petri dish.

Control studies

Two types of control studies were performed: (1) one replicate containing each fungal species and no mites, and (2) a triplicate containing only mites (no fungus) to check whether *T. putrescentiae* was contaminated by a different microorganism than the one with which it was fed (dry yeast—*S. cerevisiae*). Both controls were performed in Petri dishes containing Sabouraud agar with chloramphenicol (0.24 g/L). Control plates (1) were monitored for 21 days to evaluate fungal growth in the absence of mites. The aim of control (1) was to observe normal fungal growth without interference from mites to evaluate if mites in the experiments were consuming the fungi. Control (2) was monitored for 7 days to evaluate mite contamination. Photos were taken to show that no contamination by other microorganisms occurred, and to show fungal growth without mites.

All experiments were conducted in a climate chamber (EletroLab EL 202) at 25 ± 1 °C and $80 \pm 5\%$ r.h. in the dark. All materials used during the study were sterilized using autoclave or UV ray.

Data analysis

Population growth response to diet was studied using Kruskal–Wallis test. Differences among fungal species were compared using Student–Newman–Keuls post hoc tests. Both tests were performed using the *BioEstat* 5.0 statistical program ($\alpha=0.05$) (Ayres et al. 2007).

Results

Identification of environmentally important fungi

Isolates obtained 99 or 100% of identity and $\geq 97\%$ of cover with type strains of each fungal species. Identification and ITS region GenBank accession numbers of the isolates are shown in Table 1.

Development of *Tyrophagus putrescentiae* population on fungi

Mite population increased when feeding on most fungal species, whereas it decreased or showed a non-significant increase in the presence of *Rhizophus azygosporus*, *Sporothrix* sp., *Aspergillus terreus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus oryzae*, and *Aspergillus flavus* (Table 3). The highest population increases were on *Trichophyton mentagrophytes*, *Alternaria* sp., *Microsporum gypseum*, and *Aspergillus chevalieri* (Kruskal–Wallis test: H ratio = 68.44, df = 23, $p < 0.05$) (Table 3). With *Fusarium guttiforme* and the medically important fungi *Microsporum canis*, *M. gypseum*, *T. mentagrophytes*, and *Sporothrix* sp., mites were observed to feed on whole mycelium on day

Table 3 Mean ($\pm$ SD) population growth (no. mites per replicate) of *Tyrophagus putrescentiae* on environmentally and medically important fungal species

Fungal species	Number of mites
<i>Trichophyton mentagrophytes</i>	1350 $\pm$ 298.16a
<i>Alternaria</i> sp.	1066.66 $\pm$ 208.26ab
<i>Microsporum gypseum</i>	988.33 $\pm$ 18.93ab
<i>Aspergillus chevalieri</i>	986.66 $\pm$ 412.04abc
<i>Candida tropicalis</i>	801.33 $\pm$ 153.13abcd
<i>Fusarium guttiforme</i>	793.66 $\pm$ 83.34abcd
<i>Candida parapsilosis</i>	631.66 $\pm$ 112.50abcd
<i>Microsporum canis</i>	586.66 $\pm$ 90.73abcd
<i>Trichosporum</i> sp.	508.33 $\pm$ 93.05abcde
<i>Epidermophyton floccosum</i>	410 $\pm$ 45.82abcdef
<i>Penicillium aethiopicum</i>	368.33 $\pm$ 48.83abcdefg
<i>Trichophyton rubrum</i>	333.33 $\pm$ 28.86bcdefgh
<i>Penicillium citrinum</i>	323 $\pm$ 110.01bcdefgh
<i>Aspergillus clavatus</i>	298.33 $\pm$ 36.17cdefghi
<i>Hyphopichia burtonii</i>	270 $\pm$ 58.94defghi
<i>Candida albicans</i>	246.66 $\pm$ 92.91defghi
<i>Trichoderma longibrachiatum</i>	218.33 $\pm$ 92.80defghi
<i>Rhizopus azygosporus</i>	53.33 $\pm$ 34.01efghi
<i>Sporothrix</i> sp.	53.33 $\pm$ 14.04efghi
<i>Aspergillus terreus</i>	50 $\pm$ 11.78efghi
<i>Aspergillus fumigatus</i>	27 $\pm$ 6.24fghi
<i>Aspergillus niger</i>	9 $\pm$ 7.54ghi
<i>Aspergillus oryzae</i>	2 $\pm$ 2.64hi
<i>Aspergillus flavus</i>	0i

Means followed by different letters are significantly different (Student–Newman–Keuls test: $p < 0.05$)

28, ‘clearing’ the plates (Fig. 1). Total mortality of individuals was observed only on *A. flavus*, as mite population did not survive until the end of the 28-day period.

From the 24 fungal species tested, only eight formed colonies in the new Petri dishes after transfer by *T. putrescentiae* (second phase). Therefore, mite population increase was calculated only for these eight species (Table 4). *Aspergillus clavatus*, *Candida tropicalis*, *Candida albicans*, *F. guttiforme*, *Hyphopichia burtonii*, *Penicillium citrinum*, *R. azygosporus*, and *T. mentagrophytes* were the fungal species that were dispersed, showing positive mite growth and used as food by mites. *Tyrophagus putrescentiae* populations showed better performances on *F. guttiforme*, *P. citrinum*, and *T. mentagrophytes* when compared with the others (Kruskal–Wallis test: H-ratio = 18.59, df = 7, $p < 0.05$) (Table 4; Fig. 2). Although it had negative growth rates in the first phase when feeding on *A. fumigatus* and *A. niger*, *T. putrescentiae* dispersed these fungi to new plates, but could not keep their colonies. *Tyrophagus putrescentiae* showed negative population growth on *R. azygosporus* in the second phase, reinforcing the poor growth obtained in the first phase on this fungal species. The controls confirmed that the mite stock was not contaminated by fungi other than *S. cerevisiae*, with which it was fed during storage, and experimental conditions were suitable for fungal development (data not shown).

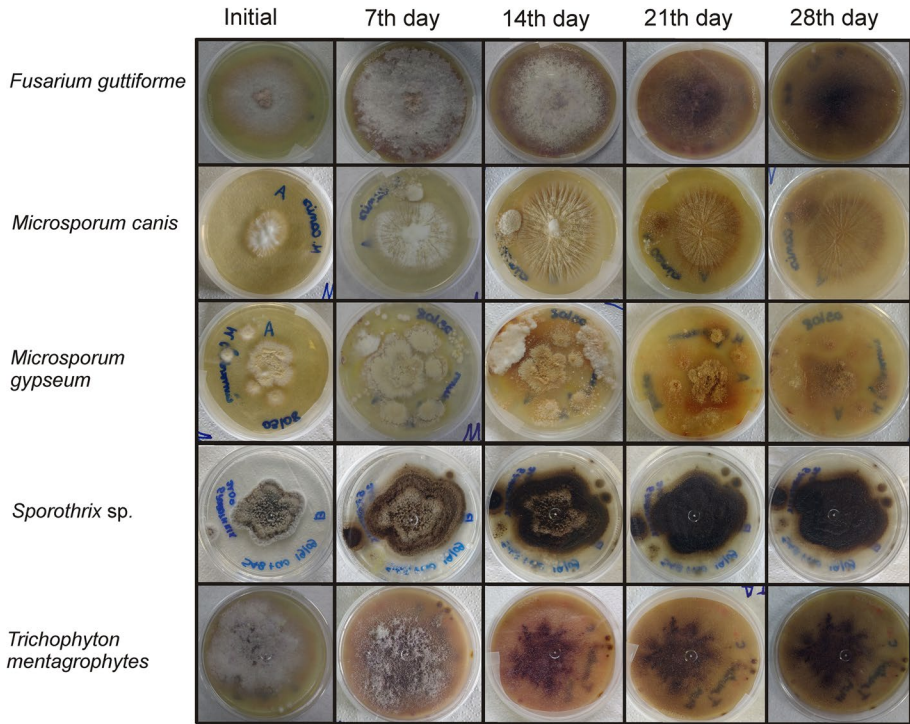


Fig. 1 Fungal species utilized in the first phase of experiment. Initially (after 1 week of fungal inoculation, 50 *Tyrophagus putrescentiae* were added), and after 7, 14, 21 and 28 days with mites

Table 4 Mean ($\pm$ SD) population growth (no. mites per replicate) of *Tyrophagus putrescentiae* on dispersed fungal species

Fungal species	Number of mites
<i>Fusarium guttiforme</i>	770 $\pm$ 114.56a
<i>Penicillium citrinum</i>	522.33 $\pm$ 109ab
<i>Trichophyton mentagrophytes</i>	370.33 $\pm$ 248.25abc
<i>Candida tropicalis</i>	241.33 $\pm$ 116bcd
<i>Hyphopichia burtonii</i>	239 $\pm$ 21.51bcd
<i>Candida albicans</i>	174 $\pm$ 62.98bcd
<i>Aspergillus clavatus</i>	98.67 $\pm$ 25.42cd
<i>Rhizopus azygosporus</i>	46.33 $\pm$ 20.64d

Means followed by different letters are significantly different (Student–Newman–Keuls test: $p < 0.05$)

Discussion

This is the first study on the specific population growth of *T. putrescentiae* on several fungi, particularly some medically important species. *Tyrophagus putrescentiae* population increased when feeding on several fungi species, dispersed and kept feeding on the environmental fungi *F. guttiforme*, *H. burtonii*, *P. citrinum*, and *R. azygosporus* (isolated

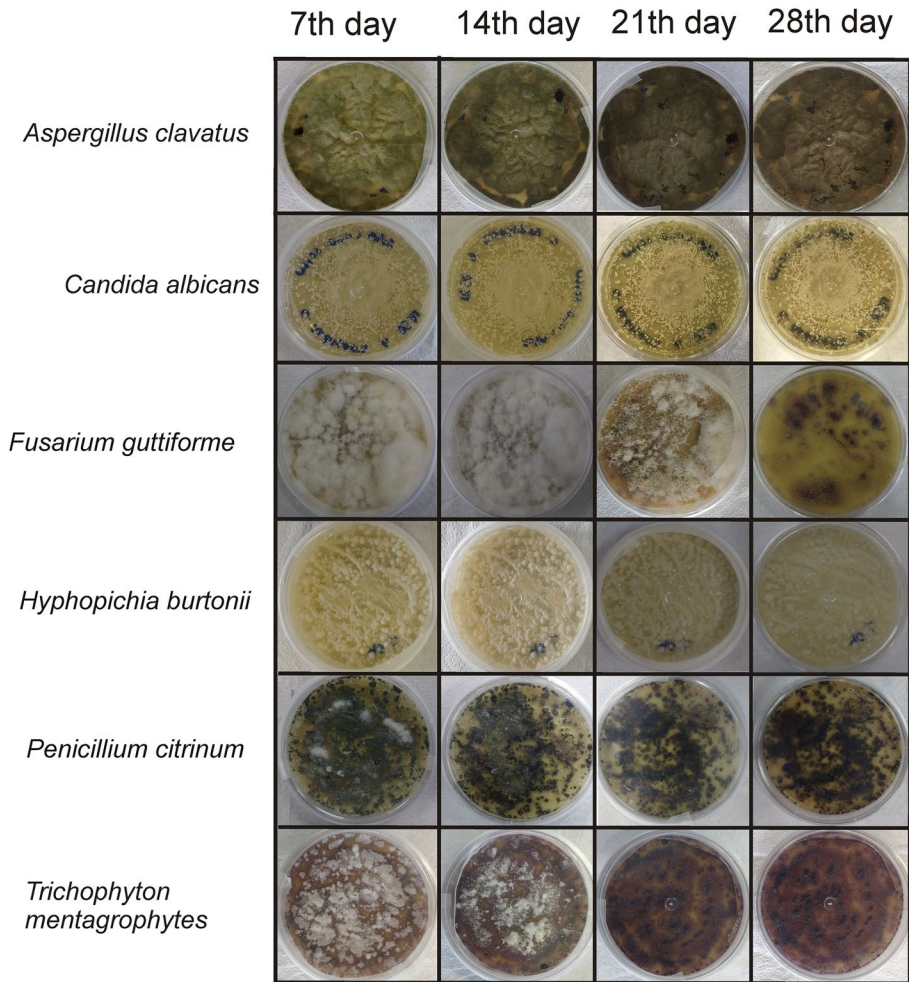


Fig. 2 Fungal species utilized in the second phase of experiment, showing the effects of *Tyrophagus putrescentiae* grazing on fungal species after 7, 14, 21 and 28 days

from food samples) and the medically important fungi *A. clavatus*, *C. tropicalis*, *C. albicans*, and *T. mentagrophytes*. The consumption of these fungal species resulted in a mite population explosion. The growth on other tested fungal species was not as significant, but mite populations increased on most fungi. Fungi are known to stimulate mite population growth, especially the fungivorous ones (Nesvorná et al. 2012; Hubert et al. 2004).

Although mite growth on the medically important fungi maintained in Culture Collections is known, and *T. putrescentiae* has already been demonstrated to feed on the spores and hyphae of all the dermatophytes, other moulds, and yeasts (Duek et al. 2001), it was quite surprising to observe the sharp population growth of mites on several human pathogenic fungi.

The yeast-like *H. burtonii* is known as ‘chalky mould’, and can spoil foods through powdery and filamentous colonies due to the fragmentation of hyphae into short lengths (Deschuyffeleer et al. 2011). This is the first study that reports the interaction between

mites and *H. burtonii*. The latter is known to produce powerful amylases (Takeuchi et al. 2006), and could provide support to mite digestion on stored products such as cereals and grains. It is a clearly beneficial interaction for the mite while it supports fungal dispersion, and this kind of interaction may occur with other fungal species, as well. The experiments in the present study were carried out in Sabouraud agar, which has glucose as its main carbon source. It would be interesting to evaluate the interaction between mites and yeast in a medium containing starch as carbon source to see whether stored-product mites could actually benefit from amylase production by the yeast. Yeasts and arthropods are known to play an important role on each other's development. For instance, yeast spores can survive digestion by *Drosophila melanogaster* (Diptera), which suggests that these flies can serve as effective vectors of yeasts under natural conditions (Reuter et al. 2007). On the other hand, larvae of these insects show preference for yeast species that lead to faster development and increased adult body weight (Anagnostou et al. 2010).

Duck et al. (2001) reported an association between *T. putrescentiae* and species belonging to the yeast genus *Candida*. Our study demonstrated that *T. putrescentiae* was able to grow and disperse on some human opportunistic yeast pathogens (*C. albicans* and *C. tropicalis*) while others were not dispersed although mites were able to develop on them (*C. parapsilosis* and *Trichosporum* sp., Tables 3 and 4). As the four species form pseudohyphae and/or true hyphae, cell morphology does not seem to explain the lack of dispersal of *C. parapsilosis* and *Trichosporum* sp. in the present study.

The high development and dispersal ability of *T. putrescentiae* on *T. mentagrophytes*, which is an etiological agent of dermatophytosis, is unsettling. *Trichophyton mentagrophytes* is considered a zoonotic species, and can be transmitted from animals to humans through direct contact. Therefore, the relationship between animal-mite-human in household environments may maximize clinical cases of dermatomycosis caused by this fungus. The association of *T. mentagrophytes* and mites belonging to the genus *Cheyletiella* (Acari: Cheyletidae) was shown to cause intensely itchy papules with necrotic areas on the skin areas in contact with animals (Dobrosavljevic et al. 2007). Thus, *T. putrescentiae* bites could transmit dermatophytes to human hosts, and this study highlights the great potential of *T. putrescentiae* to act as a host to human pathogenic fungi.

Although *T. putrescentiae* was able to develop on *E. floccosum*, *M. canis*, *M. gypsum*, and *T. rubrum* in the first phase, these fungi were not dispersed. The contrast between the dispersal of *T. mentagrophytes* and the lack of dispersal of the other dermatophytes in the present study reinforces that fungal cell morphology does not play a role in dispersal by mites.

The positive growth of *T. putrescentiae* feeding on *A. clavatus* in the two study phases represents a potential risk as both pests develop on cereals that can be used in animal feed (Varga et al. 2003). *Aspergillus clavatus* produces a neuromycotoxin that affects ruminants after ingestion of contaminated feedstuff (Botha et al. 2018) and, as *T. putrescentiae* is the most abundant and predominant mite species found in cereal-based food products, the association with *A. clavatus* is disturbing. The interaction between mite and fungus could occur in stored products (Kheradmand et al. 2007), processing and packaging areas of feed factories (Rybanska et al. 2016).

Even pathogenic or mycotoxigenic fungal species that resulted in negative growth or no growth of mite populations could be disseminated to the second phase. This is the case of two *Aspergillus* species (*A. fumigatus* and *A. niger*) tested in this study. Sinha (1966) showed that *A. niger* was rejected by astigmatid mites, which corroborates our results, since *T. putrescentiae* is also classified as belonging to the Astigmatina cohort. A possible explanation is that *T. putrescentiae* cannot feed on these species or their nutritional value is

very low to sustain mite population. The dissemination of fungi which are not used as food by mites points to a fortuitous dispersal of fungal spores attached to mites' bodies, regardless of any interaction between mites and fungi.

The fact that *T. putrescentiae* grew well on *A. chevalieri* but could not disperse it is interesting, as the latter is a species reported to cause opportunistic cutaneous aspergillosis (Naidu and Singh 1994) (Tables 3 and 4). Although no study on *Aspergillus* has been found, Hubert et al. (2014) investigated the effect of different *T. putrescentiae* densities on *Fusarium poae* transmission on non-sterile barley grains, and observed that high densities of fungus were overgrazed, while lower mite population density supported the transfer of *F. poae*. The present study did not address the effect of mite density, but it could be a likely explanation for *T. putrescentiae* behavior towards *A. chevalieri*. Alternatively, studies on *A. chevalieri* survival through mite digestive tract could help to elucidate this issue.

Tyrophagus putrescentiae could not survive in the presence of *A. flavus* in the experimental conditions of the present study. Franzolin et al. (1999) observed the growth of *T. putrescentiae* in the presence of *A. flavus* and using maize grains as food source, and observed an effective dispersal of viable fungal spores from the inoculated compartment to the uncontaminated one. The same authors reported an increase in aflatoxin concentrations whenever the mite was present. Notwithstanding, according to Rodrigues et al. (1984), aflatoxins can generally prevent or reduce mite oviposition but do not affect *T. putrescentiae*. Instead, *A. flavus* and *A. clavatus*, which could also produce mycotoxin, caused an astonishing increase in mite population. Therefore, the mycotoxin production of these fungal isolates could be evaluated to check the correlation between mycotoxin and mite survival. The opposite growth behavior of *T. putrescentiae* population on the various *Aspergillus* species evaluated does not seem to be related to fungus phylogeny, as both *A. clavatus* and *A. fumigatus* belong to *Aspergillus* section *Fumigati*, and mites hardly grew in presence of the latter.

Although this study demonstrated that it is difficult for *T. putrescentiae* populations to develop on *Sporothrix*, this fungus has been recently reported as partner in a new fungus-mite mutualism mediated by birds (Theron-De Bruin et al. 2018). The mite species *Glycyphagus* sp. and *Proctolaelaps vanderbergi* were abundant in inflorescences of *Protea* trees and on cape sugarbirds, carried *Sporothrix* fungal spores regularly, and were capable of using the fungus as food source. These findings emphasize that fungus-mite associations may be specific, and therefore, generalizations should be postulated with caution.

We demonstrated that *T. putrescentiae* was able to use *F. guttiforme* as food source, confirming the suitability of *Fusarium* species to support mite growth (Nesvorná et al. 2012; Hubert et al. 2012). Hubert et al. (2012) demonstrated changes in bacterial community associated with *T. putrescentiae* after shifting to *Fusarium* diets, suggesting a preference for Bacillaceae species known as chitinase producers, which might participate in fungal mycelium hydrolysis. *F. guttiforme* is a well-known plant pathogen which causes pineapple fusariosis in Brazil (Ploetz 2006) and can contaminate stored grains with mycotoxins (Botalico and Perrone 2002); however, it is still hard to determine whether such mite-fungus interaction would lead to the suppression of *F. guttiforme* growth in fruits. Based on our findings, we suggest that infestations of *T. putrescentiae* could disseminate the fungus on different types of food, reaching a high economic damage level.

Penicillium citrinum and *Penicillium aethiopicum* produce mycotoxins responsible for intoxication in humans and animals (Frisvad et al. 2004; Rosa 1986). Although *T. putrescentiae* was able to grow on both fungi in the first experiment phase, only *P. citrinum* was dispersed and allowed for mite development in the second phase. Differential influence of *Penicillium* species has already been demonstrated for mites *Glycyphagus domesticus* and

Histiostoma sp.; some species are suitable for mite multiplication, while others were toxic for mites (Racovitza 1969). *Tyrophagus putrescentiae* could support the dissemination of *P. citrinum*, as well as other mycotoxigenic fungi, in storehouses, mainly when kept under unsatisfactory sanitary conditions.

In this study, the fungus *R. azygosporus*, which has been reported associated with zygomycosis (Ribes et al. 2000), was isolated from the food sample. The weak population growth rate of *T. putrescentiae* in the first phase and its negative growth in the second phase could be explained by the high density of filaments of *R. azygosporus*, possibly preventing mite motility when grazing on fungal mycelium. In spite of that, *T. putrescentiae* was capable of successfully dispersing *R. azygosporus*. Therefore, *T. putrescentiae* demonstrated potential for *R. azygosporus* transmission and could infect immunosuppressed hosts either in household environments or in hospitals under unsatisfactory sanitary conditions.

Two factors are determining for a successful interaction: the mite's ability to use the fungus and the digestibility of a particular fungal species (Maraun et al. 2003; Pankiewicz-Nowicka et al. 1984). The reason why *T. putrescentiae* did not develop its populations on several fungi might be associated with fungal digestibility. To understand fungal digestibility, it is important to know that ancestors of storage mites were mycophagous (O'Connor 1982), and they have been found to host bacterial communities in the gut, parenchymal tissues, and reproductive tract (Kopecky et al. 2014). Also, *T. putrescentiae* is known to acquire bacteria and fungi (*Trichoderma* spp.) with chitinolytic activity to digest chitin from fungal cell walls (Smrž et al. 1991; Smrž and Čatská 2010), and its feeding habits change due to the bacterial community associated with the gut and parenchymal tissues (Smrž 2003). Mites produce bacteriolytic enzymes that hydrolyze the cell walls of gram-positive bacteria, showing that microorganisms can either enable or hamper both mite fitness and population growth (Erban and Hubert 2008; Erban et al. 2016). Several fungal species differ in the chemical structures of their cell walls, with a diversity of chitins and many types of chitinolytic enzymes that are specific to such substrates (Gow et al. 2017). Smrž et al. (2016), using histological methods and fluorescence microscopy, proved that there is a nearly symbiotic relationship between chitinolytic bacteria and digested fungi (spores and mycelium) associated to *T. putrescentiae*. It is possible that the many cases where mites did not feed on fungi could be related both to the associated chitinolytic bacteria efficiency and/or to the resistance of fungal spores to hydrolysis. Therefore, one possible inference is that these factors could affect the digestibility of fungi by mites.

Another possible explanation for the inability of *T. putrescentiae* to grow on some fungal species could be the size of fungal spores, which could impair fungal passage through the buccal apparatus of mites. However, as the average range of fungal spores is 2–15 µm and the pharynx of mites can be distended from 2 to 3 µm up to 20 µm to accommodate solid structures (Colloff 2009), we do not believe that spore size was a limiting factor for the use of fungi as food by mites. Even when fungi presented cellular structures larger than the upper limit for distension of the mouth apparatuses of mites, as is the case of *P. citrinum*, which has giant cells (500–1000 µm) (Katoh et al. 1978), *T. putrescentiae* was capable of multiplying on it, probably through fungal mycelia ingestion. Smrž et al. (2016) stated that chitinolytic enzymes seemed to be unnecessary in the digestion of spore contents, and spore walls were mechanically broken by *T. putrescentiae* chelicerae so that their contents could be digested. The buccal apparatus of mites can break fungal cell walls (spores and/or hyphae) allowing them to feed on different fungal diets (Smrž et al. 2016; Silva et al. 2018).

Fungal pigmentation could interfere on mite palatability, as mites were observed to be grazing more on white hyphae than pigmented ones. For instance, the consumption

of white mycelium of *F. guttiforme*, *T. mentagrophytes*, *M. canis*, and *M. gypseum* was observed (Fig. 2). Fungal pigments are known as environmental stress protectors, and the ingestion of fungal conidia by amoeba was higher when conidia were devoid of the fungal spore pigment (Hillmann et al. 2015). In fact, the melanized surface of some fungal conidia has been found to protect against the model amoeba *Dictyostelium discoideum* (Novohradská et al. 2017).

Although mites increased their populations at the expense of most fungi tested, dispersal was selective and might vary according to mite species (Hubert et al. 2003). For example, mites had high population growth on *Alternaria* sp., *M. canis*, *M. gypseum*, and *A. chevalieri* in the first phase, but were not able to disperse these fungal species.

We conclude that *T. putrescentiae* dispersed and sustained its population growth on human pathogenic fungi as well as on fungi isolated from stored food, and could act as a dissemination vector for medically important fungi. According to our results, we can partly consider that *T. putrescentiae* preferentially disperses the fungal species that provide it with best fitness. Cell morphology (filamentous × yeast-like fungi) did not influence *T. putrescentiae* fitness, but there was a differential effect associated to fungal species. Although we were not able to explain this differential effect, we suggest it might be related to fungal cell composition, to the potential resistance to cell wall hydrolysis by chitinases, and to the presence of pigments.

Acknowledgements The authors thank Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES-Brazil) and Universidade Federal do Rio Grande do Sul (UFRGS) for the doctoral scholarship granted to the first author. We are grateful to UNIVATES—Universidade do Vale do Taquari for the opportunity to carry out the practical part of this study. Noeli Juarez Ferla, Patricia Valente and Maria Lúcia Scrofernecker are supported by a CNPq productivity research scholarship.

Author contributions GLS, IZE, FD, DH, DMP performed the research, GLS, PV, MLS and LJ prepared the manuscript, GLS, MJM, NJF and OSS conceived and designed the study. GLS, LJ, PV and NJF analyzed the data.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Research involving human participants and/or animals For this type of study formal consent is not required.

References

- Anagnostou C, Dorsch M, Rohlf M (2010) Influence of dietary yeasts on *Drosophila melanogaster* life-history traits. *Entomol Exp Appl* 136:1–11
- Arlian L, Geis D, Vyszenski-Moher D, Bernstein I, Gallagher J (1984) Antigenic and allergenic properties of the storage mite *Tyrophagus putrescentiae*. *J Allergy Clin Immunol* 74:166–171
- Armitage DM, George CL (1986) The effect of three species of mites upon fungal growth on wheat. *Exp Appl Acarol* 2:111–124
- Ayres M, Ayres Júnior M, Ayres DL, Santos AS (2007) BioEstat 5.0: aplicações estatísticas nas áreas das ciências biológicas e médicas. Belém: MCT; IDSM; CNPq
- Botha CJ, Truter M, Sulyok M (2018) Multimycotoxin analysis of South African *Aspergillus clavatus* isolates. *Mycotoxin Res* 34:91–97
- Bottalico A, Perrone G (2002) Toxigenic *Fusarium* species and mycotoxins associated with head blight in small-grain cereals in Europe. *Eur J Plant Pathol* 108:611–624
- Colloff MJ (2009) Dust mites. CSIRO Publishing, Springer, Collingwood, Dordrecht

- Deschuyffeleer N, Audenaert K, Samapundo S, Ameye S, Eeckhout M, Devlieghere F (2011) Identification and characterization of yeasts causing chalk mould defects on par-baked bread. *Food Microbiol* 28:1019–1027
- Dobrosavljevic DD, Popovic ND, Radovanovic SS (2007) Systemic manifestations of *Cheyletiella* infestation in man. *Int J Dermatol* 46:397–399
- Druzhinina IS, Komoni-Zelazowska M, Kredics L, Hatvani L, Antal Z, Belayneh T, Kubicek CP (2008) Alternative reproductive strategies of *Hypocrea orientalis* and genetically close but clonal *Trichoderma longibrachiatum*, both capable of causing invasive mycoses of humans. *Microbiology* 154:3447–3459
- Duek L, Kaufman G, Palevsky E, Berdicevsky I (2001) Mites in fungal cultures. *Mycoses* 44:390–394
- Erbán T, Hubert J (2008) Digestive function of lysozyme in synanthropic acarid mites enables utilization of bacteria as a food source. *Exp Appl Acarol* 44:199–212
- Erbán T, Klimov PB, Smrž J, Phillips TW, Nesvorná M, Kopecký J, Hubert J (2016) Populations of stored product mite *Tyrophagus putrescentiae* differ in their bacterial communities. *Front Microbiol* 7:1046
- Franz J-T, Masuch G, Musken H, Bergmann K-C (1997) Mite fauna of German farms. *Allergy* 52:1233–1237
- Franzolin MR, Gambale W, Cuero RG, Correa B (1999) Interaction between toxigenic *Aspergillus flavus* Link and mites (*Tyrophagus putrescentiae* Schrank) on maize grains: effect on fungal growth and aflatoxin production. *J Stored Prod Res* 35:215–224
- Frisvad JC, Smedsgaard J, Larsen TO, Samson RA (2004) Mycotoxins, drugs and other extrolites produced by species in *Penicillium* subgenus *Penicillium*. *Stud Mycol* 49:201–241
- Glass NL, Donaldson GC (1995) Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. *Appl Environ Microbiol* 61:1323–1330
- Gow NAR, Latge JP, Munro CA (2017) The fungal cell wall: structure, biosynthesis, and function. *Microbiol Spectr*. <https://doi.org/10.1128/microbiolspec>
- Griffiths DA, Hodson AC, Christensen CM (1959) Grain storage fungi associated with mites. *J Econ Entomol* 52:514–518
- Hillmann F, Novohradská S, Forberger T, Heinekamp T, Westermann M, Winckler T, Brakhage AA (2015) Virulence determinants of the human pathogenic fungus *Aspergillus fumigatus* protect against soil amoeba predation. *Environ Microbiol* 17:2858–2869
- Hubert J, Stejskal V, Kubátová A, Munzbergová Z, Vánová M, Zd'árková E (2003) Mites as selective fungal carriers in stored grain habitats. *Exp Appl Acarol* 29:69–87
- Hubert J, Jarosik V, Mourek J, Kubatova A, Zdarkova E (2004) Astigmatid mite growth and fungi preference (Acar: Acarida): comparisons in laboratory experiments. *Pedobiologia* 48:205–214
- Hubert J, Nesvorná M, Ságová-Marečková M, Kopecký J (2012) Shift of bacterial community in synanthropic mite *Tyrophagus putrescentiae* induced by *Fusarium* fungal diet. *PLoS ONE* 7:e48429
- Hubert J, Nesvorná M, Kopecký J (2014) The effect of *Tyrophagus putrescentiae* on *Fusarium poae* transmission and fungal community in stored barley in a laboratory experiment. *Insect Sci* 21:65–73
- Katoh Y, Kuninaka A, Yoshino H, Takatsuki A, Yamasaki M, Tamura G (1978) Scanning electron microscopy of giant cells of *Penicillium citrinum*. *J Gen Appl Microbiol* 24:233–240
- Kheradmand K, Kamali K, Fathipour Y, Goltapeh EM (2007) Development, life table and thermal requirement of *Tyrophagus putrescentiae* (Astigmata: Acaridae) on mushrooms. *J Stored Prod Res* 43:276–281
- Kopecký J, Nesvorná M, Mareckova-Sagova M, Hubert J (2014) The effect of antibiotics on associated bacterial community of stored product mites. *PLoS ONE* 9:e112919
- Kucerova Z, Horak P (2004) Arthropod infestation in samples of stored seeds in the Czech Republic. *Czech J Genet Plant Breed* 40:11–16
- Maraun M, Martens H, Migge S, Theenhaus S, Scheu S (2003) Adding to “the enigma of soil animal diversity”: fungal feeders and saprophagous soil invertebrates prefer similar food substrates. *Eur J Soil Biol* 39:85–95
- Matsumoto T, Hisano T, Hamaguchi M, Miike T (1996) Systemic anaphylaxis after eating storage-mite-contaminated food. *Int Arch Allergy Immunol* 109:197–200
- Mediavilla A, Angulo J, Rodero JM, Dominguez E, Galán C, Infante F (1996) Fungal contamination of potential medical interest in Spanish grain stores. *J Invest Allergol Clin Immunol* 6:196–201
- Mohapatra D, Kumar S, Kotwaliwale N, Singh KK (2017) Critical factors responsible for fungi growth in stored food grains and non-chemical approaches for their control. *Ind Crops Prod* 108:162–182
- Mullen GR, O'Connor BM (2009) Mites. In: Mullen GR, Durden L (eds) *Medical and veterinary entomology*, 2nd edn. Academic Press, London, pp 423–482
- Naidu J, Singh SM (1994) *Aspergillus chevalieri* (Mangin) Thom and Church: a new opportunistic pathogen of human cutaneous aspergillosis. *Mycoses* 37:271–274

- Nesvorná M, Gabrielova L, Hubert J (2012) Suitability of a range of *Fusarium* species to sustain populations of three stored product mite species (Acari: Astigmata). *J Stored Prod Res* 48:37–45
- Novohradská S, Ferling I, Hillmann F (2017) Exploring virulence determinants of filamentous fungal pathogens through interactions with soil amoebae. *Front Cell Infect Microbiol* 7:497
- Núñez NK, da Cunha AA, dos Santos Dutra M, Barbosa GL, Morassutti AL, de Souza RG, Vargas MHM, Antunes GL, Silveira JS, Silva GL, Pitrez PM (2016) Acute and chronic exposure to *Tyrophagus putrescentiae* induces allergic pulmonary response in a murine model. *Asia Pac Allergy* 6:48–55
- O'Connor BM (1982) Evolutionary ecology of astigmatid mites. *Annu Rev Entomol* 27:385–409
- Osborne LE, Stein JM (2007) Epidemiology of *Fusarium* head blight on small-grain cereals. *Int J Food Microbiol* 119:103–108
- Otonelli CDS, Magagnin CM, Castrillón MR, Mendes SD, Heidrich D, Valente P, Scroferneker ML (2014) Antifungal susceptibilities and identification of species of the *Sporothrix schenckii* complex isolated in Brazil. *Med Mycol* 52:56–64
- Pankiewicz-Nowicka D, Boczek J, Davis R (1984) Food selection in *Tyrophagus putrescentiae* (Schränk) (Acari: Acarididae). *J Georgia Entomol Soc* 19:317–321
- Ploetz RC (2006) *Fusarium*-induced diseases of tropical, perennial crops. *Phytopathology* 96:648–652
- Racovitza A (1969) The influence of various moulds on the multiplication of some mycophagous mites. *J Gen Microbiol* 57:379–381
- Rajkowska K, Kunicka-Styczyńska A (2018) Typing and virulence factors of food-borne *Candida* spp. isolates. *Int J Food Microbiol* 279:57–63
- Reuter M, Bell G, Greig D (2007) Increased outbreeding in yeast in response to dispersal by an insect vector. *Curr Biol* 17:81–83
- Ribes JA, Vonover-Sams CL, Baker DJ (2000) Zygomycetes in human disease. *Clin Microbiol Rev* 13:236–301
- Rodrigues JG, Potts MF, Patterson CG (1984) Mycotoxin-producing fungi: effects on stored product mites. In: Griffiths DA, Bowman CE (eds) *Proceedings of 6th international congress of acarology*, Chichester, vol. 1. pp 343–350
- Rosa CAR (1986) Perfil bioquímico e sérico de suínos intoxicados experimentalmente com citrinina. Rio de Janeiro, Dissertação de Mestrado. Instituto de Veterinária da Universidade Federal Rural do Rio de Janeiro
- Rybanska D, Hubert J, Markovic M, Erban T (2016) Dry dog food integrity and mite strain influence the density-dependent growth of the stored-product mite *Tyrophagus putrescentiae* (Acari: Acaridida). *J Econ Entomol* 109:454–460
- Samson RA, Seifert KA, Kuijpers AFA, Houbraken JAMP, Frisvad JC (2004) Phylogenetic analysis of *Penicillium* subgenus *Penicillium* using partial β -tubulin sequences. *Stud Mycol* 49:175–200
- Samson RA, Visagie CM, Houbraken J, Hong SB, Hubka V, Klaassen CH, Perrone G, Seifert KA, Susca A, Tanney JB, Varga J, Kocsubé S, Szigeti G, Yaguchi T, Frisvad JC (2014) Phylogeny, identification and nomenclature of the genus *Aspergillus*. *Stud Mycol* 78:141–173
- Silva GL, Esswein IZ, Radaelli TFS, Rocha MS, Ferla NJ, Silva OS (2018) Influence of various diets on development, life table parameters and choice oviposition test of *Tyrophagus putrescentiae* (Acari: Acaridae): an illustration using scanning electron microscopy (SEM). *J Stored Prod Res* 76:77–84
- Sinha RN (1966) Feeding and reproduction of some stored-product mites on seed-borne fungi. *J Econom Entomol* 59:1227–1232
- Smrž J (2003) Microanatomical and biological aspects of bacterial associations in *Tyrophagus putrescentiae* (Acari: Acaridida). *Exp Appl Acarol* 31:105–113
- Smrž J, Čatská V (2010) Mycophagous mites and their internal associated bacteria cooperate to digest chitin in soil. *Symbiosis* 52:33–40
- Smrž J, Svobodová J, Čatská V (1991) Synergetic participation *Tyrophagus putrescentiae* (Schränk) (Acari, Acaridida) and its associated bacteria on the destruction of some soil micromycetes. *J Appl Entomol* 111:206–210
- Smrž J, Soukalová H, Čatská V, Hubert J (2016) Feeding patterns of *Tyrophagus putrescentiae* (Sarcoptiformes: Acaridae) indicate that mycophagy is not a single and homogeneous category of nutritional biology. *J Insect Sci* 16:94
- Solarz K, Senczuk L, Maniurka H, Cichecka E, Peszke M (2007) Comparisons of the allergenic mite prevalence in dwelling sand certain outdoor environments of the Upper Silesia (southwest Poland). *Int J Hyg Environ Health* 210:715–724
- Stejskal V, Hubert J (2008) Risk of occupational allergy to stored grain arthropods and false pest-risk perception in Czech grain stores. *Ann Agric Environ Med* 15:29–35

- Takeuchi A, Shimizu-Ibuka A, Nishiyama Y, Mura K, Okada S, Tokue C, Arai S (2006) Purification and characterization of an α -amylase of *Pichia burtonii* Isolated from the traditional starter “Murcha” in Nepal. *Biosci Biotechnol Biochem* 70:3019–3024
- Theron-De Bruin N, Dreyer LL, Ueckermann EA, Wingfield MJ, Roets F (2018) Birds mediate a fungus-mite mutualism. *Microb Ecol* 75(4):863–874
- Vanhaelen M, Vanhaelen-Fastré R, Geeraerts J (1980) Occurrence in mushrooms (Homobasidiomycetes) of cis and trans-octa-1,5-dien-3-ol, attractants to the cheese mite *Tyrophagus putrescentiae* (Schrank) (Acarina, Acaridae). *Experientia* 36:406
- Varga J, Rigó K, Molnár J, Tóth B, Szencz S, Téren J, Kozakiewicz Z (2003) Mycotoxin production and evolutionary relationships among species of *Aspergillus* section *Clavati*. *Antonie Van Leeuwenhoek* 83:191–200
- Walsh TJ, Dixon DM (1996) Chapter 75: spectrum of mycoses. In: Baron S (ed) *Medical microbiology*, 4th edn. University of Texas Medical Branch at Galveston, Galveston, TX
- Watanabe M, Yonezawa T, Lee K, Kumagai S, Sugita-Konishi Y, Goto K, Hara-Kudo Y (2011) Molecular phylogeny of the higher and lower taxonomy of the *Fusarium* genus and differences in the evolutionary histories of multiple genes. *BMC Evol Biol* 11:322
- White TJ, Bruns T, Lee S, Taylor JW (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) *PCR protocols: a guide to methods and applications*. Academic Press Inc., New York, pp 315–322

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Affiliations

Guilherme Liberato da Silva^{1,2}  · **Isadora Zanatta Esswein**¹ · **Daiane Heidrich**³ · **Fabíola Dresch**¹ · **Mônica Jachetti Maciel**¹ · **Danielle Machado Pagani**² · **Patrícia Valente**² · **Maria Lúcia Scroferneker**² · **Liana Johann**¹ · **Noeli Juarez Ferla**¹ · **Onilda Santos da Silva**²

¹ Laboratório de Acarologia Tecnovates, Univates – Universidade do Vale do Taquari, Lajeado, RS 95914-014, Brazil

² Departamento de Microbiologia, Imunologia e Parasitologia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS 90050-170, Brazil

³ Centro de Ciências Médicas, Univates – Universidade do Vale do Taquari, Lajeado, RS 95914-014, Brazil