

Ecological Relationships between Xerophilic Fungi and House-Dust Mites (Acarida: Pyroglyphidae)

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Summary. At 75 and 80% relative humidity (RH), on a wheat germ flake medium, *Aspergillus penicilloides* grew abundantly and suppressed the population growth of *Dermatophagoides pteronyssinus*. At 71% RH, *A. penicilloides* grew moderately and was only antagonistic to *D. pteronyssinus* when the fungus was previously incubated on the medium.

On a human dander medium and on mattress dust, *A. penicilloides* grew moderately at 71% and 75% RH and stimulated the development of *D. pteronyssinus* populations. Also a moderate growth of *Eurotium repens* on human dander positively influenced *D. pteronyssinus*. *Wallemia sebi* and *Penicillium brevicompactum* grew slightly or did not grow at all at 75% RH. No effect was observed on *D. pteronyssinus*.

It appears that xerophilic fungi may stimulate, and occasionally may reduce, the growth of house-dust mite populations in the natural environment.

Introduction

House dust is a dry habitat in which only a few xerophilic organisms can live. Among these are the house-dust mites (Acarida: Pyroglyphidae) (Bronswijk, 1973) and xerophilic fungi (Lustgraaf, 1977; Lustgraaf and Bronswijk, 1977; Lustgraaf and Jorde, 1977). In Western Europe, the most abundant house-dust inhabitants are the mite *Dermatophagoides pteronyssinus* and fungi belonging to the *Aspergillus glaucus* and *A. restrictus* groups. House-dust mites are associated with house-dust allergen(s) (Voorhorst et al., 1969). In the house-dust environment, human skin scales provide food for both mites and fungi (Bronswijk and Sinha, 1973).

Little is known about the ecological relationships between house-dust mites and xerophilic fungi. Bronswijk and Sinha (1973) found that *D. pteronyssinus*

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grew better on human skin scales which had been predigested by a member of the *A. glaucus* group, *Eurotium (Aspergillus) amstelodami* Mangin.

The aim of this study is to define more precisely the relationships between xerophilic fungi and house-dust mites. For this purpose the fungal flora was studied in laboratory rearings of house-dust mites. Also experiments are described in which the effect of some xerophilic fungi on the growth of *D. pteronyssinus* populations is determined at varying substrate and air humidity conditions.

Materials and Methods

Two *Dermatophagoides pteronyssinus* (Trouessart 1897) rearings of 7 and 14 months respectively, and a 27 month house-dust rearing of *D. pteronyssinus* and *Euroglyphus maynei* (Cooreman 1950) were examined. The rearings were kept at 25° C in closed vessels containing a saturated NaCl solution (75% relative humidity (RH), Winston and Bates, 1960). *D. pteronyssinus* was fed with wheat germ flakes, yeast powder, casein, dried fish meal, and dried *Daphnia*, in varying proportions. The house-dust rearing originated from the ocean steamer "Balong" (Nedlloyd lines, Rotterdam, Captain J.W. Koning).

Mites and rearing medium particles (equal in size to the mites) were inoculated on to malt extract agar of Oxoid (MEA) containing 0 or 40% (w/w) sucrose. Part of the mite inoculum was surface-disinfected in 0.2% HgCl₂ for 2 min and was washed subsequently in sterile distilled water for 30 min. The fungal outgrowth was analyzed after 7 to 9 days of incubation at 25° C. The significance of the difference with respect to the contamination with fungi is tested with the χ^2 test at $\alpha=0.05$ (Siegel, 1956). Tests for sterility were performed.

Six replicate samples of 0.02 g dust (divested of sand and fibers) were examined for mites using a flotation technique (Bronswijk, 1973) and a stereomicroscope (25 $\times$). Mites were embedded in Hoyers' medium (Baker and Wharton, 1952) and studied under a phase-contrast microscope.

Three replicate samples of 0.02 or 0.005 g medium (dust samples divested of sand and fibers) were examined for fungi by a dilution plate count technique (Bronswijk et al., in press) using MEA with 0% (to isolate hydrophilic and mesophilic fungi), 40% (to isolate mesophilic, xerophilic and hydrophilic fungi), or 64% (to isolate xerophilic and mesophilic fungi) sucrose. Two duplicate experiments were performed. The null hypothesis of no difference between the means of each 6 replicates is tested with the randomization test at $\alpha=0.05$ (Siegel, 1956).

Twenty mites from a rearing of *D. pteronyssinus* which had been contaminated with *Aspergillus penicilloides* Speg. (*A. restrictus* group) were inoculated into flasks, each containing 0.02 g wheat germ flakes/casein/yeast powder (1:1:1 w/w) medium. Prior to the inoculation of the mites, 9 of a total of 18 flasks were incubated for one week with *A. penicilloides* at 25° C, 3 of them in a closed vessel containing saturated SrCl₂·6H₂O (71% RH, Winston and Bates, 1960), 3 of them in a vessel with saturated NaCl (75% RH), and 3 of them in a vessel with saturated (NH₄)₂SO₄ (80% RH). After inoculation of the mites, the flasks were (re)placed at the above-mentioned humidities. The mites were counted under a stereomicroscope (10–25 $\times$) after 8 weeks of incubation at 25° C. Two duplicate experiments were performed, and details were as described above.

Twenty mites (*D. pteronyssinus*) were inoculated into flasks each containing 0.005 to 0.01 g mattress dust. The dust had been sterilized before in saturated propylene oxide for 40 h (Solomon et al., 1964). The mites were incubated at 71% RH. Prior to the inoculation of mites, 24 flasks were incubated at 75% RH for 0, 8, 16, or 24 weeks, 12 of them with *A. penicilloides*. Three replicate flasks were taken at each incubation time. Further details were as described above.

Twenty-five mites were inoculated into flasks, each containing 0.01 g human skin scales/yeast powder (3:1 w/w) medium. The mites were incubated at 71% RH. Prior to the inoculation of mites, 21 of a total of 36 flasks were incubated for 6 weeks at 25° C and 75% RH with *A. penicilloides*, *Eurotium (Aspergillus) repens* Corda (*A. glaucus* group), *Wallemia sebi* (Fr.)v. Arx, *Penicillium brevicompactum* Dierckx, and without fungi. In the remaining flasks these same fungi were inoculated more heavily, but no previous incubation had been conducted. Three replicate flasks were taken for each type of fungus. Skin scales were collected from the heads of students and from healthy skin parts of leg-ulcer patients. Further details were as described above.

Results

Fungi in Rearings of House-Dust Mites

Seven months prior to this investigation, numerous mites were observed in the 14-month rearing of *D. pteronyssinus*. At that time, a sample of mites was taken from it and transferred to fresh medium in order to initiate the 7-month rearing. At the beginning of this investigation nearly all the mites in the 14-month rearing were found dead, only some larvae and nymphs survived. The 7-month rearing was flourishing and contained a dense population at all stages. Freshly collected, the house-dust sample of *D. pteronyssinus* and *E. maynei* contained 3 mites per 0.5 g dust. After 19 months of incubation the culture was flourishing, although no precise counts were made; 8 months later only dead mites were found (25 ± 7 specimens per 0.02 g dust).

Three or less diaspores of mesophilic and hydrophilic fungi were isolated from 600 μ g of medium of both the 7- and 14-month *D. pteronyssinus* rearings, and the house-dust rearing. *P. chrysogenum* Thom and yeasts were mainly determined among the fungi. Table 1 shows that xerophilic fungi were isolated from these rearings 3×10^2 to 3×10^4 times more frequently than mesophilic and hydrophilic ones. *A. penicilloides* was the most abundant species. The house-dust rearing also contained considerable numbers of *E. halophilicum* Christensen et al. cells. Table 1 shows also that the 14-month rearing contained significantly more *A. penicilloides* diaspores than the 7-month rearing. Observations made at a dilution of 10^{-1} showed an absence of xerophilic fungi in 200 μ g of the original food ingredients.

All 71 living mites and medium particles of both the 7- and the 14-month rearing were contaminated with *A. penicilloides*, but only 4 of 88 mites and

Table 1. Xerophilic fungi in rearings of house-dust mites (Acarida: Pyroglyphidae)

Type of rearing	Quantity investigated (in μ g)	Number of diaspores ^a	
		<i>Aspergillus penicilloides</i>	<i>Eurotium halophilicum</i>
7-month rearing of <i>D. pteronyssinus</i> on a wheat germ flake medium	6	102 (73–171) ^c	0
14-month rearing of <i>D. pteronyssinus</i> on a wheat germ flake medium ^b	6	1016 (655–1348) ^c	0
27-month rearing of <i>D. pteronyssinus</i> and <i>E. maynei</i> on house dust	60	78 (25–132)	7 (1–14)

^a Mean and range from 2 duplicate experiments of 3 replicates each

^b One colony of *A. gracilis* Bain. and one colony of an undetermined member of the *A. glaucus* group were also observed

^c Probability of the difference $P=0.002$

Table 2. Contamination with fungi of *D. pteronyssinus* and medium particles from mite rearings. Living mites and particles were inoculated on MEA to isolate hydrophilic and mesophilic fungi, and on MEA with 40% sucrose to isolate mesophilic and xerophilic fungi

Inoculum Type	Age of rearings in months	No. of inocula with hydrophilic and mesophilic fungi		No. of inocula with mesophilic and xerophilic fungi	
		No. tested	No. positive	No. tested	No. positive ^e
Mites	14	16	0	12	12
Mites	7	24	2 ^a	18	18
Medium particles	14	24	1 ^b	21	21
Medium particles	7	24	1 ^c	20	20
Mites	14	19	0	19	15
surface-disinfected Mites	7	24	1 ^d	24	9
surface-disinfected					

^a *Penicillium* sp.

^b *P. chrysogenum*

^c *A. yeast*

^d *P. brevicompactum*

^e *A. penicilloides*

medium particles were positive for mesophilic and hydrophilic fungi (Table 2). Surface-disinfected mites from the 14-month rearing were associated more frequently with *A. penicilloides* than those from the 7-month rearing ($\chi^2=5.8$, $P<0.01$, Table 2).

The medium particles of the 14-month rearing showed a brown discoloration. Fungi could not be traced in the rearings with the unaided eye, however, microscopical observations revealed masses of *A. penicilloides* conidia on all medium particles. Numerous conidia were also present on the outer surface of the mites and in the digestive system and fecal pellets (Fig. 1). No mycelium was found to be associated with the mites.

The Growth of D. pteronyssinus at Different Level of Relative Humidity in the Presence of A. penicilloides

D. pteronyssinus mites, which were contaminated with *A. penicilloides*, built up larger populations at 71% RH than at 75 or 80% RH (Fig. 2). This figure also shows that at 71% RH, the mite growth was negatively affected by the preincubation with *A. penicilloides*. No such significant difference existed at 75 or 80% RH. On the wheat germ flakes/casein/yeast powder medium, *A. penicilloides* developed a dense mycelium with conidiophores at 75 and 80% RH. At 71% RH, the medium remained granular, although on the glass surface of the flasks some hyphae and incomplete conidiophores were seen.

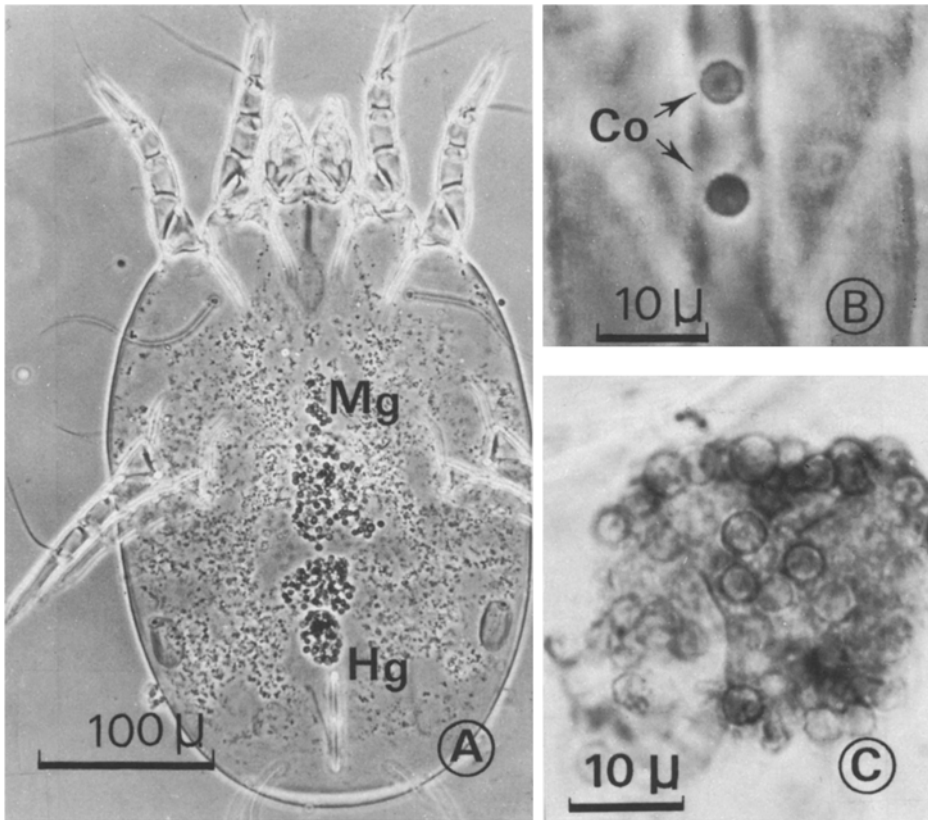


Fig. 1A-C. *A. penicilloides* conidia in the digestive system and fecal pellets of juveniles of *D. pteronyssinus*. **A** Total view on a nymph. **B** Dorsal view on esophagus. **C** Fecal pellet. *Co* conidia, *Mg* midgut, *Hg* hindgut

The Growth of D. pteronyssinus in Mattress Dust with A. penicilloides Added

Less diaspores of *A. penicilloides* were counted in mattress dust in equilibrium with 75% RH at longer incubation periods (Fig. 3). No viable cells were recovered from the mattress dust incubated without *A. penicilloides*. In none of the samples with *A. penicilloides* added were any other fungal species found. A thin mycelium was observed on the dust particles and on the glass walls of the flasks with *A. penicilloides*. *D. pteronyssinus*, when transferred to these substrates, developed significantly better on the mattress dust preincubated with *A. penicilloides* (Fig. 4).

The Growth of D. pteronyssinus on Human Skin Scales with Xerophilic Fungi Added

The population growth of *D. pteronyssinus* was enhanced significantly on human dander/yeast powder which had been preincubated with *A. penicilloides* or *E. re-*

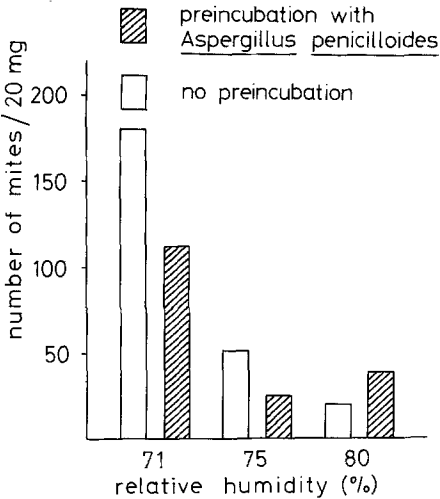


Fig. 2. Number of *D. pteronyssinus* on wheat germ flakes/casein/yeast powder after 8 weeks of incubation at 25° C and 71, 75 or 80% relative humidity, together with *A. penicilloides*. Cultures started with 20 mites. Two duplicate experiments of 3 replicates each were performed with and without preincubation of the culture medium with the fungus, at 25° C and different air humidities. Probabilities of the difference: 71–75% and 71–80% without preincubation $P=0.002$, 71–75% with preincubation $P=0.004$, 71–80% with preincubation $P=0.02$, 71% without preincubation – 71% with preincubation $P=0.009$

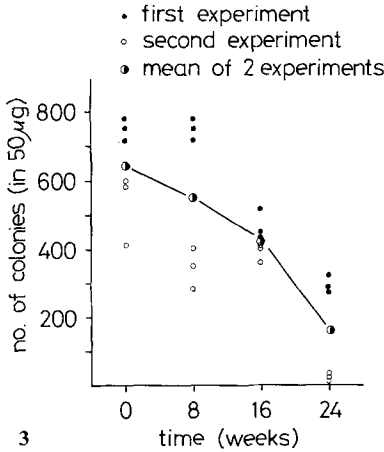


Fig. 3. Number of diaspores of *A. penicilloides* in mattress dust after incubation at 25° C and 75% relative humidity. Two duplicate experiments of 3 replicates each were performed

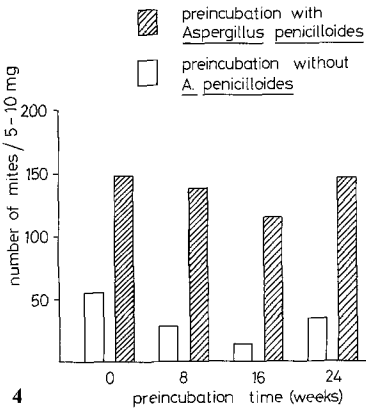


Fig. 4. Number of *D. pteronyssinus* in mattress dust after 8 weeks of incubation at 25° C and 71% relative humidity. Two experiments of 3 replicates each were performed, after preincubation for 0 to 24 weeks at 25° C and 75% relative humidity with and without *A. penicilloides*. Cultures started with 20 mites. Probabilities of the difference between preincubation with and without *A. penicilloides*: 0 weeks $P>0.05$, 8 weeks $P=0.002$, 16 weeks $P=0.002$, 24 weeks $P=0.004$

Table 3. Number of *D. pteronyssinus* on human skin scales/yeast powder after 8 weeks of incubation at 25° C and 71% relative humidity. Cultures started with 25 mites after preincubation of the culture medium for 6 weeks at 25° C and 75% relative humidity with and without fungi

Origin of the skin scales	Species of fungi added	Number of mites ^a	
		Mean ^b	Range
Head	<i>Aspergillus penicilloides</i>	148	(112–210)
Head	<i>Eurotium repens</i>	205	(158–270)
Head	<i>Wallemia sebi</i>	74	(48–171)
Head	<i>Penicillium brevicompactum</i>	103	(36–158)
Head	No fungi	80	(2–133)
Leg	<i>Aspergillus penicilloides</i>	102	(91–134)
Leg	No fungi	115	(7–195)

^a Two duplicate experiments of 3 replicates each^b Probabilities of the difference: *A. penicilloides* – no fungi $P=0.01$, *E. repens* – no fungi $P=0.004$, *W. sebi* – no fungi $P>0.05$, *P. brevicompactum* – no fungi $P>0.05$, *A. penicilloides*/leg skin scales – no fungi/leg skin scales $P>0.05$ **Table 4.** Number of *D. pteronyssinus* on human dander/yeast powder after 8 weeks of incubation at 25° C and 71% relative humidity. Cultures started with 25 mites after addition of fungi to the culture medium

Species of fungi added	Number of mites ^a	
	Mean ^b	Range
<i>Aspergillus penicilloides</i>	85	(33–150)
<i>Eurotium repens</i>	5	(0–16)
<i>Wallemia sebi</i>	18	(0–29)
<i>Penicillium brevicompactum</i>	19	(0–53)
No fungi	17	(3–58)

^a Two duplicate experiments of 3 replicates each^b Probabilities of the difference: *A. penicilloides* – no fungi $P=0.009$, *E. repens* – no fungi $P>0.05$, *W. sebi* – no fungi $P>0.05$, *P. brevicompactum* – no fungi $P>0.05$

pens (Table 3). Preincubation with *W. sebi* or *P. brevicompactum* had no significant effect. At 75% RH, these fungi either did not grow or grew very poorly, while *A. penicilloides* and *E. repens* formed a thin mycelium on the medium particles. *A. penicilloides* did not positively affect the mite growth on skin scales from the leg (Table 3). Without prior incubation of the dander with the fungi, only *A. penicilloides* supported the mite growth (Table 4). In the flasks with *A. penicilloides*, most of the mites were concentrated at sites with an excess of fungus inoculum. Hyphal growth was observed on the human dander particles at these sites. The human dander in the flasks with *E. repens* was joined together to form a solid mass, a result of abundant hyphal growth. No hyphae were observed in the flasks with *W. sebi* and *P. brevicompactum*.

Discussion

Compared to the numbers of mesophilic and hydrophilic fungi, numerous diaspores of *A. penicilloides* were found in the house-dust mite rearings. This observation suggests that the xerophilic fungus is able to colonize the rearing media at optimal conditions for mite growth. *A. penicilloides* might be responsible for the observed decline of an older *D. pteronyssinus* rearing by its poisoning of the medium with its toxic metabolites. This finding can be compared with the report of Solomon et al. (1964) on antagonism between the fungal species *W. sebi* and *A. restrictus*, and flour mites (*Acarus siro* L.).

On wheat germ flakes/casein/yeast powder, *D. pteronyssinus* developed dense populations at 71% RH, but not at 75 and 80% RH. Obviously the observed abundant growth of *A. penicilloides* at 75 and 80% RH hindered the population development of the mites. Usually a RH of 75 to 80% is maintained to rear house-dust mites (Voorhorst et al., 1969; Bronswijk and Sinha, 1973). I think it is better to maintain 71 to 73%, especially when media such as wheat germ flakes are used.

Members of the *A. glaucus* and *A. restrictus* groups are a common cause of spoilage of stored grain where they break down fatty substances of the kernels (Sauer and Christensen, 1969). Human skin scales must contain at least 10% fat (Carruthers, 1962). This percentage is toxic to *D. farinae* Hughes 1961 (Matsumoto, 1975). Bronswijk and Sinha (1973) found that only partly defatted human dander is suitable for the nutrition of *D. pteronyssinus*. The observed beneficial effect of *E. repens* and *A. penicilloides* on the mite growth may be due to fat digestion of the human dander by the fungi. Possibly because the leg skin scales had a lower fat content than the dander (Marples, 1965), the growth of *A. penicilloides* on this substrate did not positively affect the mites.

Conidia of *A. penicilloides* were observed to pass through the digestive tract of the mites without incurring morphological damage. Griffith et al. (1959) reported that 78% of the conidia of the related species *A. restrictus* from fecal pellets germinated. Apparently the conidia were only digested to a minor degree by the mites. The growth of *D. pteronyssinus* which we observed on human dander at the inoculum masses of *A. penicilloides* probably did not result from the use of conidia as a food source but from the digestion of the human dander fats by the fungal growth. However, heavy inoculation followed by abundant hyphal growth can inhibit mite development, as was seen with *E. repens*.

Since the dilution plate count technique is not effective for the measurement of spore germination and hyphal growth (Parkinson et al., 1971), the counted number of diaspores of *A. penicilloides* in mattress dust decreased at longer incubation times at 75% RH. Mattress dust improved significantly as a substrate for *D. pteronyssinus* after preincubation with *A. penicilloides*. Since the ratio of pyroglyphid mites to xerophilic fungi in this experiment is about as high as that found in the dust of occupied mattresses in July in the Netherlands (2×10^{-3} ; Lustgraaf, unpublished), it is concluded that xerophilic fungi can also aid the growth of house-dust mites in the natural environment. In this way the fungi indirectly stimulate the formation of house-dust allergens.

The antagonistic effects of xerophilic fungi on house-dust mites were observed after abundant fungal growth. Occasionally when high air humidities (80% or higher) occur in house dust for months (i.e. in cellars or rooms with moist walls), antagonistic effects of fungi to house-dust mites are probable.

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