# PHYSICAL AND NUTRITIONAL REQUIREMENTS OF HOUSE-DUST MITE DERMATOPHAGOIDES PTERONYSSINUS AND ITS FUNGAL ASSOCIATION

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BEHAVIORAL FACTORS HOUSE-DUST MITE ABSTRACT: The physical structure and nutritional component of the substrate were found to be decisive factors for the growth and survival of the house-dust mite, Dermatophagoides pteronyssinus. To elucidate the physical limits of the food environment, population growth of D. pteronyssinus was investigated on rearing media as well as on natural substrate. A more rapid reproduction of D. pteronyssinus on fibrous house-dust, as compared to rearing media consisting of non-fibrous fine particles, indicated that the mite prefers an aerated over a dense substrate. This was shown to be related to the antagonistic effects of the endemic mold, Aspergillus penicilloides, with which the mite establishes a symbiotic relationship. The fibrous component of the mattress microhabitat is a natural biotope for D. pteronyssinus. Deeper mattress fibres may provide a protective niche for mites during winter. They may also provide protection from other adverse environmental conditions.

CONDITIONS
DU MILIEU
ACARIEN
DES POUSSIÈRES

RÉSUMÉ: La structure physique du milieu nutritif comme la nature chimique des constituants sont des facteurs décisifs dans la survie et le développement de l'Acarien des poussières de maison. Afin de déterminer les limites physiques propres de l'environement nutritif, la croissance des populations de *D. pteronyssinus* fut testée à la fois sur un substrat de culture et sur le milieu naturel. Une colonisation plus rapide des substrats fibreux indique que l'Acarien s'accomode plutôt d'un milieu aéré et peu dense. Ceci est démontré en relation avec des effets antagonistes de la moisissure endémique *Aspergillus penicilloides* avec laquelle l'Acarien établit une relation de symbiose. Le composant fibreux du microhabitat matelas se précise être un biotope naturel pour *D. pteronyssinus*. Les fibres profondes du matelas fourniraient un refuge pour les acariens durant l'hiver, ainsi qu'une protection contre d'autres conditions nuisibles.

# Introduction

As suggested by RODRIGUEZ and BLAKE (1979), discordant results from nutritional studies of *D. pteronyssinus* may be caused by variable effects of

fungi, since specific fungi were shown to be an important factor in the house-dust ecosystem. The most xerophilic members of the Aspergillus glaucus (Eurotium) and A. restrictus groups, such as A. amstelodami and especially A. penicilloides, enhance growth of D. pteronyssinus (BRONSWIJK and SINHA,

 Laboratorium of Ecology and Biogeography, University of Louvain-la-Neuve, Belgium. Acarologia, t. XXVIII, fasc. 4, 1987. 1973; LUSTGRAAF, 1977). These fungi represent the commonest species found in house dust with relative humidities of 50-80 % (Bronswijk, 1981). However, these fungi also appear to exert antagonistic effects on house-dust mite growth. Such adverse effects by endemic fungi on stored-product mites have already been demonstrated (SOLOMON et al., 1964). Xerophilic fungi play an important role by predesting the substrate (BRONSWIJK and SINHA, 1973; LUSTGRAAF, 1978), while it has been found that lipids have both beneficial and harmful influences on the growth of D. pteronyssinus (SAINT GEORGES-GRIDELET, 1984). Inhibitory effects of Aspergillus penicilloides may be caused mainly by high relative humidities (≥ 80 %) and components of the substrate (LUSTGRAAF, 1978). In uncrowed cultures of D. pteronyssinus reared on high metabolic nutrients, the physical structure of the substrate appears to have some influence on mite growth (SAINT GEORGES-GRIDELET, 1980).

Further work may explain the relation between laboratory data and field observations. According to Wharton (1976), nutritional studies on housedust mites have been limited to the search for diets suitable for their culture. Although further research was done concerning diet improvement (RODRI-GUEZ and BLAKE, 1979), very few experiments involving media requirements of D. pteronyssinus were performed on the natural dust substrate. Experiments were conducted mainly under conditions that are optimal for this species, e.g 75 % relative humidity and 25° C temperature. Contrary to general opinion, good growth and survival of D. pteronyssinus populations may occur on some suitable substrates at as low a humidity (such as 64 % RH) as noted for the species D. farinae, and this should be related to fungal metabolism (SAINT GEORGES-GRIDELET, 1984). Since the natural housedust substrate consists of highly complex material, some of its components probably also explain the survival and growth of the mite in the the natural environment where living conditions often lie below optimal or even critical values.

The present study attemps to extend the latter works on nutritional requirements of the house-dust mite, *D. pteronyssinus*, taking into account the physical structure of nutritional media as well as

that of its natural substrate, and considering the actual influence of the endemic fungi.

The results of four experiments were analyzed, each experiment arising from the results of the previous experiment.

#### MATERIALS AND METHODS

A series of two tests were conducted both on high nutritional media and on pooled house-dust to define the general effect of the physical structure.

These were followed by another series of two tests using the mattress-dust microcosm, the most common microhabitat of D. pteronyssinus (BRONSWIJK, 1981) (although long pile carpetings also appear to be a favoured microhabitat for the Dermatophagoides spp., mainly observed in North American countries according to Arlian et al., 1979-1980 and in New Zealand according to ABBOT et al., 1981). Different types of mattress-dust were used first to verify the specific role of fibrous components in any mattress microhabitat. In order to specify some influences of the endemic fungi, a second mattress-dust test was performed at a higher relative humidity than the optimal one, to induce antagonistic effects of endemic fungi on the natural substrate and to compare with similar conditions occurring on a loose rearing medium.

In all experiments, the fungal growth was estimated by observing the extent of mycelial development and the presence of conidiophores on the substraste according to the method of LUSTGRAAF (1978).

## 1. Media rearing.

Three nutrient substrates were selected for mite rearing: a mixture of human skin scales and dried yeast (3:1 w/w), a mixture of wheat germs/casein/dried yeast (2:1:1 w/w) and dried *Daphnia* (SPIEKSMA, 1969; BRONSWIJK, 1972). Wheat germ and *Daphnia* substrates were tested in a coarse and fine form. Coarse media were prepared by grinding with mortar as pestle, resulting in particles 10 to  $450 \, \mu \text{m}$  (with  $20 \, \% < 100 \, \mu \text{m}$  and  $7 \, \% < 50 \, \mu \text{m}$  — mean range  $100\text{-}150 \, \mu \text{m}$ ). Powdery media were

obtained by using an electric Vibromill® ultragrinder (particles of 5 to 150  $\mu$ m with 65-95 % < 50 µm). According to the usual mode of preparation (Spieksma, 1967), skin scales constitute a fine material (particles of 5 to 115 µm with 10 % < 50 μm). It represents a semi-natural medium and, in a way, a reference nutrient. The proportions of different particles were recorded by using calibrated sieves and counting them under a stereomicroscope. For each substrate and for each type of medium, four replicates of 300 mg each were conducted. Five males and five females (young adults taken from nymphal molts) were transferred to each culture cell (diameter 12 mm, height 44 mm). To prevent mite escape, vials were stoppered using 50 µm mesh nylon bolting silk (40 mm in diam.) and placed at optimal thermohygrometric conditions (25° C; 75 % RH). According to the extraction method using ethanol (Gridelet and Lebrun, 1973; Bronswijk et al., 1978), mite countings were made after eight weeks of incubation or about three life cycles of D. pteronyssinus. At the conclusion of incubation, all media rearing can immediately be fixed at the same time by immersing in alcohol. The use of noncorrosive liquids such as alcohol permits a distinction to be made between dead mites and mites which were alive before extraction from house-dust (damaged and dessicated specimens as opposed to undamaged and fully distented specimens).

# 2. House-dust substrates.

Tests were carried out on pooled house-dust collected from different types of houses at different places so as to obtain an average culture medium considered representative of house-dust. Dust sampled represented various biotopes of the house: the floor, the carpet, and the mattress. Dust samples were gathered, then passed through three sieves (mesh sizes 1.6, 0.5 and 0.05 mm respectively) so as to separate the dust into three classes, fibres (synthetic and natural plant fibres-animal hairs), coarse particles (food particles, insect fragments, pieces of wood, sand) and fine particles (ashes, dander, powdered foodstuffs). Each type of dust was well mixed with fine needles to obtain homogeneous material as much as possible without loosing

the structure. From each type of dust, after homogenization, 21 mg of 500 mg were set up in the vials. Each aliquot was heated for 48 h at 45° C prior to inoculation of D. pteronyssinus to prevent possible pre-existent mite-infestation (KINNAIRD, 1974). Dust units were then placed for 24 h at optimal relative humidity to rehydrate. Ten males and ten females (young adults) were transferred on each culture cell. Seven series of three replicates each were placed at optimal relative humidity during the 14-week incubation period, which was longer than in previous tests since the mite life cycle should be longer on house-dust than on more nutritional rearing media. For the different types of dust, mites were counted every two weeks, using the alcohol suspension-method, to determine the gradual growth of population of each stase.

#### 3. Mattress microhabitat.

In the first trials, dusts from six mattresses in different houses were taken using a conventional vacuum cleaner (500 watts) with separate paper bags. The six houses investigated had been selected in order to obtain three mattress dusts essentially composed of fine synthetic and animal fibres (woolen mattresses) and three mattress dusts mostly containing plant fibres (vegetable horsehair mattresses). From each dust, after homogenizing the sample, three units of 25 mg were spread out, then exposed for 72 h to a 15 w UV light (Germicidal) placed 10 cm away. Agitation of dust particles (3 times a day) was done to ensure complete UV exposure. This procedure establishes a standard microcosm and reduces possible pre existent infestation (mite, fungi) to a low level without changing the chemical nature of the substrate which may be possible using a heat treatment (LEACH, 1971). Ten males and ten females (young adults) were then transferred to each dust unit. Microcosm were incubated at 25° C and 75 % relative humidity for 14 weeks to compare degrees of colonization. At the end of this period, fibrous and fine parts of each sample were separated (fibres removed with fine forceps) for mite extraction according to the methods of BRONSWIJK et al. (1978).

The second trials were performed on pooled

mattress-dust from different houses with various beddings so that dust samples would be as representative as possible of the mattress environment. After being homogenized with fine needles, pooled dust was divided among units of 25 mg each, spread out, then exposed for 72 h to a 15 w UV light placed 10 cm away with the same precautions as discussed above. Aliquots of 25 mg each would be sufficient to support growth of the house-dust mite during 8 weeks; lower quantities of dust might ensure rapide colonization. Ten males and ten

females (young adults) were transferred to each dust microcosm; they were placed at optimal temperature (25° C) but at a higher relative humidity (85  $\pm$  2%) to induce high antagonistic effects of the endemic mold, *Aspergillus penicilloides*, and probably those of other dust-inhabiting mesohygrophilic fungi. The same procedure was followed using dried *Daphnia* (coarse form) to make some comparison between the natural substrate and a nutritional medium.

#### RESULTS AND DISCUSSION

# 1. Mite growth on culture media.

D. pteronyssinus did not thrive on media containing a high proportion of fine particles (65 to 95 % < 50  $\mu$ m) except for skin scales, probably because of a peculiar nutritive value and aerated loose substrate (Table 1). On micronized substrates, the decrease of mite populations and the high mortalities of adults and immatures coincided with excessive fungal growth (dense mycelium with many conidiophores) of endemic Aspergillus (A. penicilloides<sup>1</sup>). This phenomenon, well marked on glucidic culture media such as wheat germs (Lust-

TABLE 1. Number of *D. pteronyssinus* reared on different food media for eight weeks at 25° C and 75 % RH <sup>1</sup>.

| Media         | Mean nu            |                           |                |  |
|---------------|--------------------|---------------------------|----------------|--|
| Media         | Live specimens     | Dead specimens            | Total          |  |
| Wheat germs   |                    |                           |                |  |
| coarse        | $2247 \pm 483^{1}$ | $20 \pm 5  (9 \pm 2)^2$   | $2267 \pm 486$ |  |
| fine          | $53 \pm 46$        | $65 \pm 10 \ (61 \pm 24)$ | $118 \pm 39$   |  |
| Dried Daphnia |                    |                           |                |  |
| coarse        | $1731 \pm 117$     | $196 \pm 52 \ (10 \pm 2)$ | $1927 \pm 161$ |  |
| fine          | $86 \pm 8$         | $13 \pm 5 \ (13 \pm 5)$   | $99 \pm 10$    |  |
| Skin scales   |                    |                           |                |  |
| (fine)        | $2541 \pm 690$     | $22 \pm 9  (9 \pm 4)$     | $2563 \pm 689$ |  |

<sup>1.</sup> Mean and SE for 4 replicates under each set of conditions using 5 pairs of young adults.

GRAAF, 1978), was here more pronouced, relative to the fine structure of the substrate. It would seem that the change in physical structure of the substrate explains the adverse fungal effects upon D. pteronyssinus growth. Since the pulverization process increases the hygrometric capacity of a material as well as the availability of its nutrients (SNOW et al., 1944), it was found to promote a rapid development and therefore an inhibitory fungal proliferation on the more nutrient media. This resulted in rapid extinction of the mite population on such a high nutritional and pulverulent substrate.

### 2. Differential colonization on house-dust substrate.

The physical structure of the natural house-dust substrate appeared to have similar effects on colonization of mites (Fig. 1). A low development of *D. pteronyssinus* with low egg production and low immature populations was observed in fine dust, with mite populations decreasing and dying after 4-6 weeks (more than 10 weeks according to SPIEKSMA, 1967). Mites were found to reproduce successfully on coarse and fibrous dusts. A faster growth was noted on the latter media, which promoted high and rapid densities of larval and nymphal stases. The marked growth and reproduction of *D. pteronyssinus* occurring on fibrous dust demonstrated

<sup>2.</sup> Mean percentage of mortality.

<sup>1.</sup> Identification of Aspergillus penicilloides on malt extract agar with 64 % sucrose; agar media of choice for isolation of xerophilic fungi (Lustgraaf, 1977).

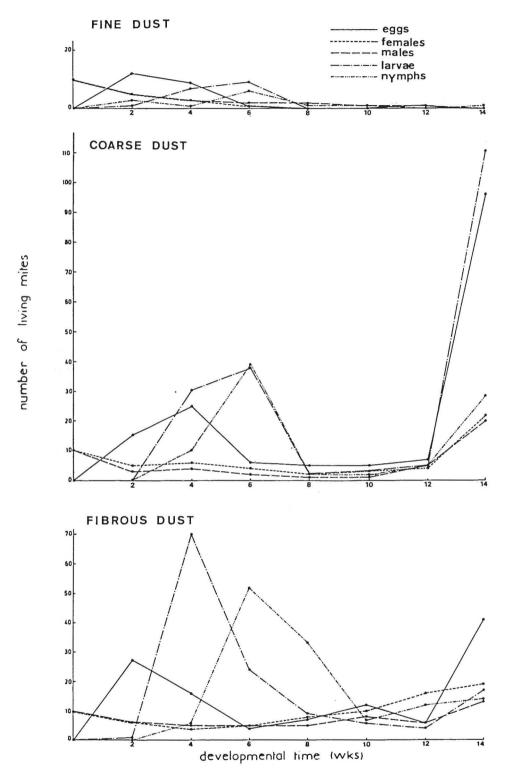


Fig. 1: Development of *D. pteronyssinus* in fine, coarse and fibrous house-dust over a 14 week period at 25° C and 75 % RH (10 pairs of adults in each of 3 replicates).

the suitability of this substrate for the mite. A peculiar heterogeneity of results for coarse dust trials was noted in spite of having homogenized the substrate prior to the setting of mite cultures. Therefore, the last high value of density for eggs and larvae (mainly for one replicate) should not be representative. Any favourable effect would probably be due to an occasional occurence of one of the nutrient component in that substrate, since the substrate appeared not to be homogenous. The more rapid reproduction recorded on fibrous dust as compared to fine dust proved that the mite prefers an aerated substrate. This is in accordance with field observations of high mite densities on loose pellets of fibrous mattress dust (FAIN, 1966; GRIDELET-DE SAINT GEORGES, 1976). Under natural conditions, mattress dust should represent a more adequate substrate for mite growth due to its loose physical structure as well as its proteinous value because of the presence of human dander.

Contrary to the rearing media, however, in none of the pooled dust units was any hyphal growth noticed after a 14-week period of incubation at the mite optimal 75 % RH.

# 3. Specific role of fibrous component in mattress microhabitat.

After colonization of D. pteronyssinus on six different mattress dusts during 14 weeks, higher proportions of each stase, mostly larvae and eggs, were actually recorded in fibrous parts, with more than 60 % of the total number in each of the whole mattress-dust units (Table 2). Much higher mite densities were recorded in these fibrous mattressdusts as compared to those recorded in fibrous parts of pooled dust from the previous test. The fibrous component of mattress-dusts which yielded high numbers of eggs and immatures in this study gave evidence of an ideal biotope for growth and reproduction of D. pteronyssinus. A more pronounced occurence of mite refuge in mattress-dust composed of fine synthetic and proteinaceous fibres than on vegetable horsehair material could be partly explained by the loose and more aerated substrate. Similar results were found by SAINT GEORGES-GRIDELET et al. (1987). In the latter study,

a piece of ticking (partly synthetic) was added in order to approach the real conditions of mattress microhabitat. The high concentrations of immature populations and eggs as well as accumulation of fecal pellets in fibrous mattress-dust and ticking also show that the fibrous component of the mattress constitutes the more specific habitat for D. pteronyssinus. As noted for the pooled-dust trials, no visible fungal was found in any of the mattressdusts at 75 % RH. LUSTGRAAF (1978) reported that only a few diaspores of A. penicilloides are counted in mattress-dust at such low relative humidity, although mattress-dust improves significantly as a substrate for D. pteronyssinus after preincubation with A. penicilloides. In other respects, A. penicilloides appears to promote the appearance of antagonistic effects as shown by a sharp decline in the growth of D. pteronyssinus after too many spores were added to the natural substrate (SAINT GEORGES-GRIDELET, 1981).

Table 2. Mean percentage of *D. pteronyssinus* stages in fibrous part of six different mattress dusts after fourteen weeks at 25° C and 75 % RH using ten pairs of young adults on each replicate of 25 mg.

| Mite           | Woollen mattresses 1 |      | Vegetable horsehair <sup>2</sup><br>mattresses |      |      |      |
|----------------|----------------------|------|--|------|------|------|
| stage          | 1                    | 2    | 3  | 4    | 5    | 6    |
| Adults         | 52.3 <sup>3</sup>    | 49.8 | 58.1   | 59.3 | 42.1 | 40.6 |
| Nymphs         | 67.9                 | 60.4 | 58.9   | 57.2 | 52.8 | 48.1 |
| Larvae 5       | 71.7                 | 63.2 | 75.4   | 66.0 | 45.5 | 55.2 |
| Eggs 5         | 90.8                 | 90.5 | 88.0   | 61.4 | 62.6 | 87.7 |
| Total number 4 | 1694                 | 1258 | 1417   | 1082 | 1389 | 1711 |

- 1. composed of fine synthetic and animal proteine fibre.
- 2. composed of plant fibres.
- 3. mean of 3 replicates.
- 4. mite number in the whole substrate (fibrous + fine parts)/replicate.
- 5. stases more frequent on woollen-mattresses fibres than on vegetable horsehair-mattresses fibres (P < 0.05).

# 4. Antagonistic effects of endemic fungus on the rearing medium and in the mattress microhabitat.

Comparing the natural substrate and *Daphnia* rearing medium at high air humidities provided additional support for the unpredictable role of endemic fungi influencing the growth and survival of *D. pteronyssinus*. At 75 % RH, xerophilic fungi

were not found to be abundant or even visible in culture media nor in dust samples, although general occurence of the mite in any substrate has been observed (Bronswijk and Sinha, 1973; Lust-GRAAF, 1978) — a reason why conflicting views have been expressed as to the role of xerophilic fungi in the ecology of house-dust mites. At higher humidities a more intense fungal activity would increase appearance of antagonistic effects such as those frequently observed on glucidic media (Lust-GRAAF, 1978). A relative humidity of 85 % or higher can be temporarily encountered in natural conditions in temperate and tropical climates (RIJC-KAERT et al., 1981). The latter results of mite culture trials at such high 85 % RH (Table 3) would show the different development of D. pteronyssinus growth on the natural substrate as compared to that on a nutritional medium. A low but distinct postembryonic development occurred after eight weeks of incubation on mattress-dust (only a few adult mites of second generation). On dried Daphnia medium, the mite growth was inhibited and seemed

Table 3. Number of *D. pteronyssinus* reared on dried *Daphnia* and on mattress dust after eigth weeks at 25° C and 85 % RH using ten pairs of young adults on each replicate of 25 mg.

|                    | Dried Daphnia | Mattress dust |  |  |  |
|--------------------|---------------|---------------|--|--|--|
| Living             |               |               |  |  |  |
| ♂                  | 3             | 8             |  |  |  |
| Q-+ E              | 0             | 3             |  |  |  |
| <sup>∓</sup> ~no E | 3             | 6             |  |  |  |
| Tn                 | 0             | 22            |  |  |  |
| PN                 | 3             | 110           |  |  |  |
| L                  | 4             | 62            |  |  |  |
| Е                  | 2             | 96            |  |  |  |
| Dead               |               |               |  |  |  |
| ð                  | 8             | 3             |  |  |  |
| <b>б</b><br>Р<br>И | 7             | 2             |  |  |  |
| N                  | 21            | 23            |  |  |  |
| L                  | 76            | 72            |  |  |  |
| Total              | 127           | 407           |  |  |  |
| S                  | 22.42         | 51.28         |  |  |  |
| M                  | 88            | 25            |  |  |  |
| E_OV               | I             | 37            |  |  |  |
| nE/Q               | 11            | 39            |  |  |  |

S : Standard deviation.

M : Mean percentage of mortality

F: Fecundity OV: mean percentage of ovigerous females Fecundity nE/OV: mean number of eggs produced/female

TN: Tritonymp, PN: Protonymph, L: Larva, E: Egg.

to be stopped at the larval or protonymphal stase. On the natural substrate, fecundity was still appreciable and mortality not too high. On both substrates A. penicilloides mycelia were largely present but were less dense, with few conidiophores on mattress-dust substrate. From previous studies (SAINT GEORGES-GRIDELET, unpublished), conidiophores of Aspergillus growing from the substrate seemed more numerous in dust units without mite inoculation than when mites were growing on it, probably because mites feed upon many of these conidiophores, since passage of viable spores through the digestive tract of D. pteronyssinus has been shown (LUSTGRAAF, 1978). So even at higher relative humidities, fungal metabolism would be less marked on the natural substrate, which is physically and chemically more heterogeneous than a culture medium since Aspergillus sp. grew moderately on it. Therefore, in the house-dust environment, D. pteronyssinus benefited from the effects of A. penicilloides. Few fungal colonies are needed for optimal growth of D. pteronyssinus, as shown by effectively controlling D. pteronyssinus populations using a fungicide on mattress-dust (SAINT GEORGES-GRIDELET, 1981; LEBRUN and SAINT GEORGES, 1984). A persistent control seems essential for optimizing acaricide efficiency by applying sufficient concentration. Nevertheless, the acaricide control could be proved despite the protective role of mattress ticking (SAINT GEORGES-GRIDELET, 1987).

In conclusion, this study has shown importance that different substrates have in the growth and survival of D. pteronyssinus which seemed mainly related to the importance of the endemic fungus. Mattress-dust fibres would provide an aerated substrate essential for optimal D. pteronyssinus development. These fibres would contribute to maintaining the equilibrium in the mattress ecosystem by commensal hosts since better control of the symbiotic endemic mold could occur. The more rigid fibres of ticking as well as the deeper fibres of mattress where dander accumulates would also provide a shelter against chemical and physical control and would lessen seasonal fluctuations of outdoor temperature and humidity. Field observations show migration of D. pteronyssinus into bedding and mattresses in the presence of various

stresses (Dusbabek, 1979; Carswell *et al.*, 1982). These characteristics of the microhabitat should be taken into account when controlling house-dust mite populations.

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