

Tyrophagus putrescentiae mites grown in dog food cultures and the effect mould growth has on mite survival and reproduction

Michael S. Canfield* and William J. Wrenn†

*Animal Dermatology Clinic, 2965 Edinger Avenue, Tustin, CA 92780, USA

†Orange County Vector Control District, 13001 Garden Grove Boulevard, Garden Grove, CA 92813, USA

Correspondence: Michael S. Canfield, Animal Dermatology Clinic, 2965 Edinger Avenue, Tustin, CA 92780, USA. E-mail: drmcandfield@tampabay.rr.com

Present address: Michael S. Canfield, Florida Veterinary Specialists, 3000 Busch Lake Boulevard, Tampa, FL, 33614, USA.

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None.

Abstract

The purposes of this study were to determine whether the storage mite, *Tyrophagus putrescentiae*, could survive and thrive on dog food and if mould growth was important to their survival. All of the chambers ($n = 42$) were started with 10 female mites and evaluated every other day for mite survival and for the spontaneous development of mould. Ten chambers tested the effect of low moisture on mite survival. Eight chambers were used as positive and negative controls ($n = 4$ each); positive control mites were fed Fleischmann's® yeast and negative controls had no food source. Three dog foods were evaluated in the same manner. Four chambers had food but mould development was limited by replacing the food kernel every 48 h and four chambers were allowed to grow mould. Mites grown in chambers without moisture died from desiccation within 5 days. The termination point was day 34 when all mites in the negative control group (moisture but no food) died. Although *T. putrescentiae* survived and grew on all three commercial dog foods, there was no statistically significant difference in mites counts among the dog foods ($P < 0.10$). Mite counts in the 'no' mould and mould groups ranged from 8 to 11 and 144 to 245, respectively, and differences were significant ($P < 0.0001$). This study found that *T. putrescentiae* is a fungivorous storage mite that can grow and flourish on dog food. The study demonstrated that the presence of mould positively influences mite viability, while low relative humidity can result in detrimental consequences for *T. putrescentiae*.

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Introduction

There has been increasing interest in storage mites in recent years concerning their allergenicity in both humans and animals.¹ Anaphylaxis has been reported in sensitized humans consuming storage mite and house dust mite-contaminated goods.^{2–4} Reports in the human literature have also indicated that allergen sensitization to storage mites is no longer limited to those individuals with occupational exposure as previously reported.^{5,6} In addition, it has been purported that storage mite sensitivity in dogs may be as important, if not more important than house dust mite sensitivity.⁷ Antigenic and allergenic properties of *T. putrescentiae* have been researched, as well as its cross antigenic and allergenic properties with *Dermaphagoides farinae*.^{8,9}

Tyrophagus putrescentiae (Schrank, 1781) is a small species (0.28–0.41 mm) with a translucent body and virtually colourless chelicerae and limbs (Figure 1).¹⁰ The mite's life cycle requires 2–3 weeks under ideal environmental conditions (23 °C and a relative humidity of 87%).¹⁰ *Tyrophagus putrescentiae* is frequently found in stored food with relatively high protein, fat and moisture content.^{10,11} Foodstuffs such as dried eggs, dried bananas, cheeses, ham, fishmeal, wheat spillage, oats, flour and copra have been documented with *T. putrescentiae* contamination.² This mite is also a frequent contaminant of mycology laboratory cultures.^{10,12} *Tyrophagus putrescentiae* is a fungivorous mite and is referred to as the mould mite, grocery mite and cheese mite. The purposes of this study were to determine whether: (i) *T. putrescentiae* mites survive and reproduce on commercial dry dog foods; (ii) differences were noted among the foods utilized; and (iii) spontaneous mould development influenced mite viability.

Materials and methods

Contaminant *T. putrescentiae* mites were collected from locally maintained Trombiculid mite cultures to establish seed cultures of *T. putrescentiae* mites. The mites were contaminants in an acarologist's trombiculid cultures which were harvested and cultivated for the purposes of this study. Four clear 946 mL plastic chambers with press-on lids were utilized as culture chambers. Six millimetre holes in the lids were covered with 8 mm pieces

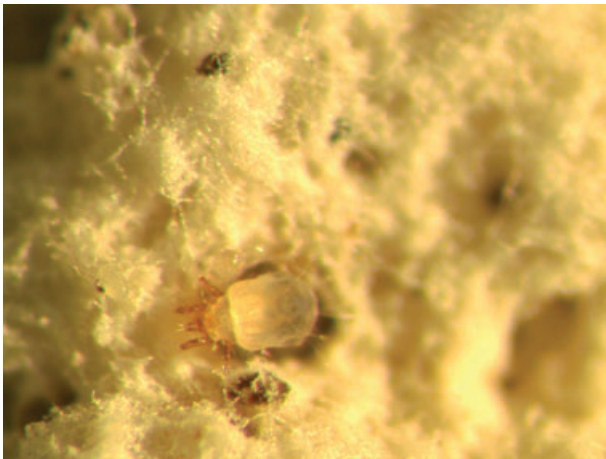


Figure 1. *Tyrophagus putrescentiae* mite, translucent body, with virtually colourless chelicerae and limbs.

of 0.1 μm filter paper held in place by rubber cement at the periphery of the filter paper. Four-hundred millilitres of substrate was added to the chambers to maintain the microenvironments' humidity. The substrate contained an inorganic mixture of plaster of Paris (DAP Products Inc., Baltimore, MD, USA) and activated charcoal which served to maintain the chamber humidity. The seed cultures were maintained within a dark laboratory cabinet maintained at 13–21 °C minimum and 22–32 °C maximum temperature with an average relative humidity of 75% (range 66–84%) and were provided Fleishmann's® yeast as food.¹³ The mean relative humidity of the culture was 83.5% (range 80–87%) throughout the initial rearing period and during the study itself. Weekly, the cultures were evaluated for viable mites under a stereomicroscope, distilled water was added to maintain the humidity and yeast was replenished if needed. The seed cultures were maintained until adequate numbers of mites were reared to perform the remainder of the study. After

3 weeks of seed culture incubation, 42 separate mite cultures were established as shown in Figure 2.

During the preliminary work for this study, the realization that the mould growth would envelope the kernel of food and that the mites would traverse above and below the mould growth prompting the development of a chamber which could be rotated under the stereomicroscope in nearly a 360° fashion without disturbing the mites or displacing the kernels of food. Therefore, the authors designed a culture system for this project that allowed for mite containment, maintenance of the microenvironment, anchoring of the food kernel, replacement of the food kernel and microscopic examination of the culture chambers. Clear 250 mL polystyrene containers with press-on lids (Dynalab Corp., Rochester, NY, USA) were utilized as culture chambers. Six millimetre holes in the lids were covered with 8 mm pieces of 0.1 μm filter paper held in place by rubber cement at the periphery of the filter paper.

Sixty millilitres of substrate was added to 32 of the 42 chambers to maintain the microenvironment's humidity. The substrate was prepared by combining plaster of Paris and activated charcoal in a 9 : 1 ratio followed by the addition of a sufficient quantity of distilled water to form a pourable suspension. A 22-gauge needle-hub was embedded into the substrate before it set. In the 10 cultures without substrate, the 22-gauge needle hubs were anchored to the chambers with a small amount of epoxy. To avoid potential toxicity from epoxy vapours, the chambers were assembled 4 weeks prior to the study allowing fume dissipation.

All culture chambers were housed within the same dark laboratory cabinet. Environmental temperature was controlled by the heating and cooling system for the laboratory to mimic changes in temperature as expected to occur in a southern California home or factory environment. A thermometer housed with the cultures recorded temperatures ranging from 13–21 °C (50–70 °F) to

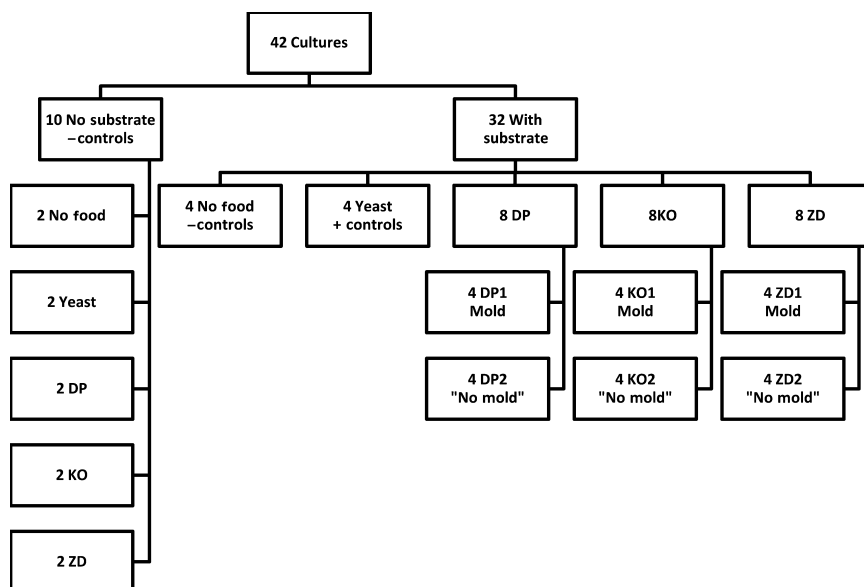


Figure 2. Forty-two chambers divided into respective groups. DP1, KO1 and ZD1 represent the mould group while DP2, KO2 and ZD2 represent the 'no-mould' group where DP is the Royal Canin Veterinary Diet® canine potato and duck formula, KO is the Eukanuba Response® KO and ZD is the Hill's Prescription Diet z/d® ultra.

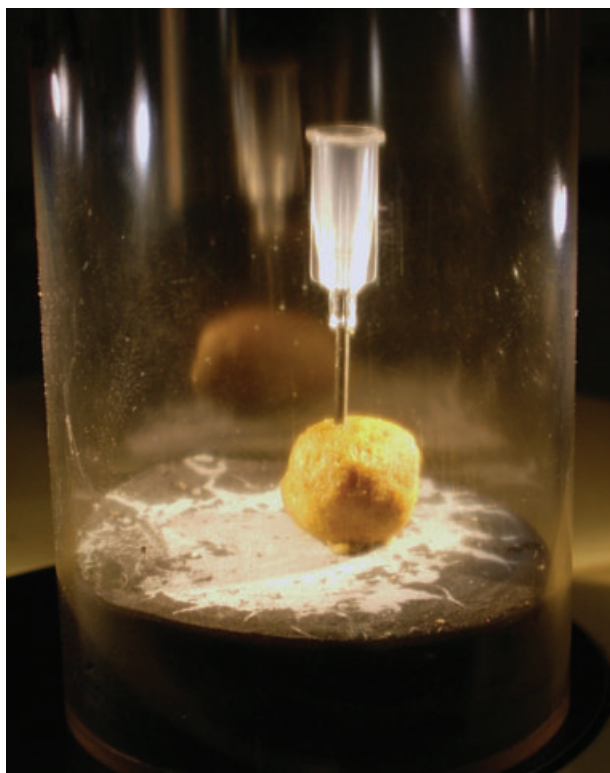


Figure 3. Chamber with substrate, food kernel and needle anchor.

22–32 °C (60–80 °F) throughout the study. Relative humidity in the chambers was not measured throughout the study but was assumed to be similar to the mean relative humidity of 83.5% measured in the seed cultures housed in the same laboratory.

Three dry commercial dog foods commonly used for ‘elimination trial’ were used in this study: Royal Canin Veterinary Diet® Canine potato and duck formula (DP) (Royal Canin, St. Charles, MO, USA), Eukanuba Response® KO (KO) (Iams; The Procter & Gamble Company, Cincinnati, OH, USA), and Hill’s Prescription Diet z/d® ultra (ZD) (Hill’s Pet Nutrition, Topeka, KS, USA). Food kernels were drilled with an 18-gauge needle. The 18-gauge needle passing through the centre of a kernel of food was aligned with the 22-gauge needles in the chambers. The needles served as anchoring devices to prevent kernel movement thus minimizing damage to the mites during handling, microscopic examination, and replacement of the food kernels (Figure 3). The 42 chambers were divided into groups as shown in Figure 2 and each chamber was inoculated with 10 large female mites, assuming that the largest mites were females and would likely be carrying fertilized eggs (Figure 4). The 10 chambers with no substrate were subdivided into five groups of two and served as negative controls. Of the remaining 32 chambers with substrate, four contained only substrate and served as negative controls, and four were supplied 10 grains Fleishmann’s® yeast as a mite food supply and served as positive controls. The chambers without substrate were incorporated to validate the importance of the substrate in maintaining a suitable micro environment, most notably high relative humidity.



Figure 4. A mite mounted at the time the study cultures were established demonstrating multiple eggs.

The 24 remaining chambers were divided into three groups of eight. The three groups of eight were then subdivided into six groups of four. These subgroups were designated as mould groups (DP1, KO1 and ZD1) and ‘no mould groups’ (DP2, KO2 and ZD2). The mould developed spontaneously on the food kernels in the high relative humidity of the microenvironment. The chambers were examined microscopically every other day for mite survival. On evaluation days, the food in the ‘no-mould’ groups was exchanged with a fresh kernel of food to decrease the amount of mould growing within the chamber. If mites were present on the kernel upon removal, the mites were transferred with a pencil brush back to the chamber. The chambers were incubated together until the termination point of the study, day 34, when all of the mites in the chambers with substrate and no food supply had expired naturally.

Once the termination point was reached, the 28 chambers (four control and 24 dog food chambers)



Figure 5. New methylene blue stain uptake by the filter paper with the non-staining mite in the foreground.

with live mites were filled with approximately 100 mL of 90% ethanol and 5 mL of new methylene blue stain and allowed to soak for 10–15 min. The contents were then filtered through 0.1 µm filter paper in a Büchner funnel and flask under negative pressure. The stain imparted colour to the filter paper increasing the contrast between the mites and filter paper (Figure 5). The filter paper was examined under a stereomicroscope and mite numbers were tallied. In addition, kernels of food were evaluated for mites by gently tearing the kernels open with metallic probes followed by exploration of the interior structure of the kernels for mites. The empty chambers were then evaluated for mites following filtration of the contents. All developmental stages of mites other than eggs were counted. The resultant 28 separate mite totals were utilized for statistical analysis. The remaining 14 chambers (10 non-substrate controls and four substrate but no food controls) were evaluated for live mites and none were detected.

A two-way analysis of variance was performed utilizing the 28 data points from the three dog food types under two conditions (mould versus 'no-mould'). The statistical analysis was implemented with SAS (Statistical Analysis System) software.

Results

All of the mites in the 10 chambers with no substrate expired by day 5, and the mites appeared turgid and dehydrated (Figure 2). Moulds with similar gross colony morphology developed spontaneously on all of the food kernels in the mould group. The kernels in the mould group developed an almost sponge-like character. Mould development was minimal in the 'no-mould' groups. Figure 6 and Table 1 show the mean number of mites counted from the experimental and positive

Table 1. Mean number of mites produced in the positive controls with yeast and substrate, mould groups and 'no mould' groups with standard deviations in parenthesis

	Mean mite numbers at study conclusion		
	Positive controls	Mould group	'No mould' group
	305 (47)		
DP		245 (103)	11 (5)
KO		289 (121)	5 (7)
ZD		144 (53)	8 (8)

DP, Royal Canin Veterinary Diet® canine potato and duck formula; KO, Eukanuba Response® KO; ZD, Hill's Prescription Diet z/d® ultra. Note the negative controls failed to sustain mites and were not tallied.

control groups at the termination of the experiment with standard deviations in parenthesis. The mean mite number differences between mould conditions were statistically significant ($P < 0.0001$) while the differences between the types of foods were not statistically significant ($P > 0.10$). Mites were observed to have material presumed to be ingesta passing through their digestive tracts which matched the colour of the mould within their chambers.

Discussion

Tyrophagus putrescentiae mites were unable to survive in the chambers without substrate, likely due to hydric stress and lack of shelter. Relative humidity and temperature are pivotal environmental factors affecting mite survivability, fecundity and development.^{14–16} Mites in the chambers without substrate crowded together as described by Cutcher in mites exposed to a desiccating atmosphere.¹⁷ The chambers with substrate were designed to mimic home or factory environmental conditions without specialized equipment,

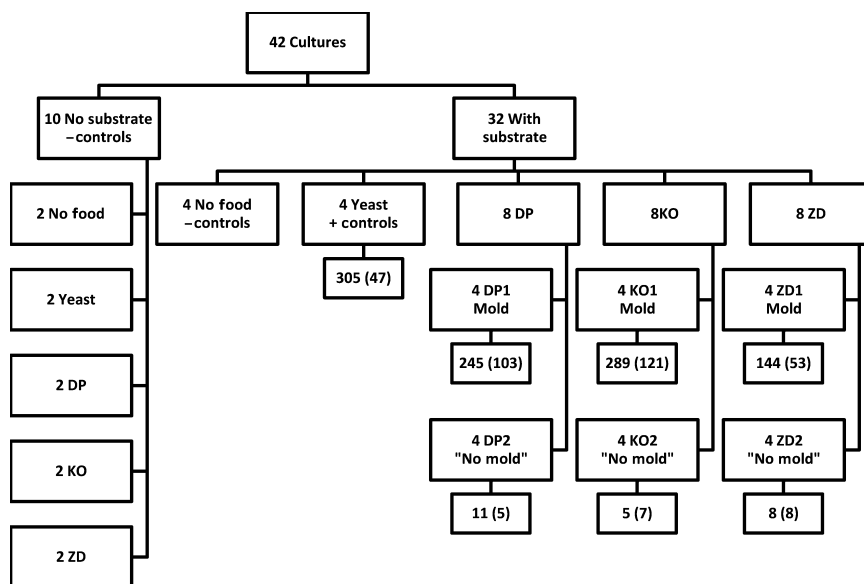


Figure 6. Mean number of mites counted at the termination of the study within their respective groups with standard deviations in parenthesis. The numbers were rounded to the nearest integer. DP1, KO1 and ZD1 represent the mould group while DP2, KO2 and ZD2 represent the 'no-mould' group where DP is the Royal Canin Veterinary Diet® canine potato and duck formula, KO is the Eukanuba Response® KO and ZD is the Hill's Prescription Diet z/d® ultra. Note the negative controls failed to sustain mites and were not tallied.

and the substrate served as a water reservoir to maintain the relative humidity. Other investigators have evaluated *T. putrescentiae* to determine optimal rearing conditions.¹⁸ The mites housed on substrate and provided yeast were utilized as positive controls as the seed cultures were acclimated to yeast and yeast is known as an acceptable food source for rearing *T. putrescentiae* mites.¹³

The study accomplished its goals by demonstrating that *T. putrescentiae* mites survive and reproduce on various dry dog foods. It also demonstrated that the presence of mould positively influences mite viability, reinforcing that *T. putrescentiae* is a fungivorous mite.¹³ It is possible that the mould served as a source of shelter and free water for the mites, rather than a food supply. However, material visible within many of the mites was the colour of the mould in their respective chambers, suggesting ingestion. The study demonstrated no statistical differences in mite counts between the three foods (Table 1). This study may provide insight as to why some investigators have been able to identify storage mites while others have been less successful.^{18–20} Our study suggests that dry dog food stored in a sealed container without the benefit of added moisture may not support mite development readily. However, observations during this study show that dry food will act to absorb moisture from the environment (substrate) and spontaneously develop mould. We hypothesize that this series of events benefits the mite in that the food absorbs the environmental moisture which helps prevent hydric stress to the mites, provides free water, serves as shelter and provides a food supply. The mould is a likely requirement for survival in addition to the environmental requirements. *Tyrophagus putrescentiae* mites have been grown on moist dog food previously, but dry dog food has not been evaluated in a similar fashion to the authors' knowledge.²¹

Ideally, relative humidity, fecundity and longevity would have been recorded separately, as valuable information could have been attained. Running totals of mite numbers throughout the study might have been useful but with the mites habitually moving under the fungal growth on the food this became a daunting task and created concerns for damaging the mites in the process.¹³ Future studies utilizing this novel culture system may yield important insights into mite reproduction and survival under varying conditions and may provide insight regarding mite control.

The conclusions that can be drawn from this study are limited and certainly the number of replicates was low due in part to the complexity of the study design. This study does not address the question of whether exposure to this mite's allergens occurs from the raw ingredients entering the food or from environmental contamination with mites, not unlike the house dust mites. Likewise, it is unknown whether processing of food ingredients containing mites might affect their antigenicity. The increasing prevalence of storage mite allergy is being recognized as a domestic and occupational problem after having been previously thought to be primarily an occupational problem.⁹ The storage mites certainly warrant additional studies.

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Resumen El propósito de este estudio fue determinar si el ácaro de almacén, *Tyrophagus putrescentiae* puede sobrevivir y proliferar en comida para perro y si el crecimiento de moho era importante en su supervivencia. Todas las cámaras ($n = 42$) se iniciaron con diez hembras y se evaluaron en días alternos para la supervivencia de ácaros y para el desarrollo espontáneo de moho. En diez cámaras se probó el efecto de baja humedad en la supervivencia de los ácaros. Ocho cámaras se utilizaron como controles positivos y negativos ($n = 4$ cada uno); los ácaros control positivo fueron alimentados levadura de Fleischmann® y los controles negativos no tuvieron fuente de alimentación. Se evaluaron tres comidas de perro de la misma forma. Cuatro cámaras tenían comida pero el crecimiento de moho fue limitado al reemplazar los gránulos de comida cada 48 horas y en cuatro cámaras se permitió el crecimiento de moho. Los ácaros metidos en cámaras sin humedad murieron por desecación en cinco días. El punto de terminación fue el día 34 cuando todos los ácaros en el grupo control negativo (humedad sin comida) murieron. Aunque *T. putrescentiae* sobrevivió y creció en las tres comidas comerciales, no hubo diferencia estadística significativa en el recuento de ácaros entre las comidas ($P < 0.10$). El recuento de ácaros en los grupos sin moho y con moho variaron de 8–11 y de 144–245 respectivamente y las diferencias fueron significativas ($P < 0.0001$). Este estudio demuestra que *T. putrescentiae* es un ácaro fungívoro que puede crecer y proliferar en comida para perro. El estudio también demuestra que la presencia de moho influye positivamente en la viabilidad de los ácaros, mientras que una baja humedad relativa puede causar un detrimento en la viabilidad de *T. putrescentiae*.

Zusammenfassung Die Ziele dieser Studie waren es zu untersuchen, ob die Futtermittelmilbe *Tyrophagus putrescentiae* in Hundefutter überleben und sich vermehren kann und ob Schimmelwachstum wichtig sind für ihr Überleben. Alle Kammern ($n = 42$) wurden mit zehn weiblichen Milben bestückt und jeden zweiten Tag auf lebende Milben und auf die spontane Entwicklung von Schimmel untersucht. In zehn Kammern wurde der Einfluss von wenig Feuchtigkeit auf das Überleben der Milben getestet. Acht Kammern wurden als Positiv- und Negativkontrollen (jeweils $n = 4$) verwendet; Den Milben der Positivkontrolle wurden Fleischmann's® Hefe gefüttert und die Negativkontrollen hatten keine Futterquelle. Es wurden drei Hundefutter auf dieselbe Art und Weise untersucht. Vier Kammern hatten Futter, aber das Schimmelwachstum wurde limitiert indem die Futterkörner alle 48 Stunden ausgetauscht wurden und in vier Kammern wurde das Schimmelwachstum erlaubt. Milben, die in den Kammern ohne Feuchtigkeit wuchsen, starben innerhalb von fünf Tagen an Austrocknung. Den Schlusspunkt bildete der Tag 34 an dem alle Milben in der Negativkontrollgruppe (Feuchtigkeit aber kein Futter) tot waren. Obwohl *T. putrescentiae* überlebte und in allen drei kommerziellen Hundefuttern wuchs, bestand kein statistisch signifikanter Unterschied bezüglich der Anzahl der Milben zwischen den Hundefuttern ($P < 0.10$). Die Milbenzahlen in der „kein“ Schimmel und in den Schimmelgruppen rangierten zwischen 8 und 11 beziehungsweise zwischen 144 und 245, wobei diese Unterschiede signifikant waren ($P < 0.0001$). In dieser Studie wurde herausgefunden, dass *T. putrescentiae* eine Schimmel-liebende Futtermittelmilbe ist, die in Hundefutter wachsen und gedeihen kann. Die Studie zeigte, dass die Präsenz von Schimmelpilzen das Überleben der Milben positiv beeinflusst, während niedrige Luftfeuchtigkeit nachteilige Konsequenzen für *T. putrescentiae* hat.

Les buts de cette étude étaient de déterminer si l'acarien de stockage, *Tyrophagus putrescentiae* peut survivre et se reproduire dans les aliments pour chien et si le développement de moisissures est important pour leur survie. Toutes les chambres ($n = 42$) ont été pourvues de 10 acariens femelles. Le nombre d'acariens et le développement de moisissures ont été évalués tous les deux jours. Dix chambres ont permis de tester l'effet de la faible humidité sur la survie des acariens. Huit chambres ont servi de témoins positifs et négatifs (4 de chaque); les acariens contrôles positifs étaient nourris avec des levures Fleischmann's® et les contrôles négatifs n'avaient pas de source de nourriture. Trois aliments pour chiens ont été évalués de la même façon. Quatre chambres avaient de la nourriture mais le développement de moisissures était limité par le remplacement de l'aliment tous les 2 jours. Quatre chambres permettaient la croissance des moisissures. Les acariens élevés dans les chambres sans moisissures sont morts par dessiccation au bout de cinq jours. Le moment déterminant était le jour 34 au cours duquel tous les acariens du contrôle négatif (moisissure mais pas nourriture) sont morts. Bien que *T. putrescentiae* survive et se développe avec les trois aliments industriels, il n'y avait pas de différence statistiquement significative entre les comptages d'acariens des trois groupes ($P < 0.10$). Les comptages d'acariens dans le groupe « pas de moisissure » et « présence de moisissures » ont varié de 8 à 11 et 144 à 245 respectivement, avec différence significative ($P < 0.0001$). Cette étude a montré que *T. putrescentiae* est un acarien de stockage fungivore capable de croître et de prospérer sur les aliments pour chien. L'étude a montré que la présence de moisissures influençait positivement la viabilité des acariens alors qu'une humidité relative faible peut entraîner des conséquences néfastes pour *T. putrescentiae*.