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The Influence of Environmental Temperature and Humidity on Temporal Decomposition of Cockroach Allergens Bla g 1 and Bla g 2 in Feces

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ABSTRACT The aim of the study was to establish a model of the environmental fate of German cockroach (*Blattella germanica* L.) allergens Bla g 1 and Bla g 2 in feces under controlled and field conditions. Temporal decline (3, 6, and 9 mo) of allergens Bla g 1 and Bla g 2 in the feces protected from cleaning was measured under laboratory and experimental household conditions. The influence of environmental temperature (15, 20, 25, 30, and 35°C) and moisture (53, 75, 85, and 100% RH) on allergen degradation was estimated for 3, 6, and 9 mo. Bla g 1 was more stable than Bla g 2 and the proteins. The proteins and Bla g 2 contents were correlated negatively with the decomposition time; Bla g 1 was not. However, when the content of Bla g 1 in control and exposed tubes was compared, the decrease after exposure was significant at exposure in 35°C, 53 and 100% RH. In laboratory, the shortest half-life (16–38 d) of Bla g 2 was at high temperature and humidity (100% RH at 35°C), whereas the longest half-life (340 d) was at 25°C and 85% RH. In the apartment, the half-life was 406 d. The results indicate that Bla g 1 and Bla g 2 allergens can persist in feces for several months under usual household humidity and temperature.

KEY WORDS cockroach, allergens, decomposition, Bla g 1, Bla g 2

To date, eight different human IgE-binding compounds have been characterized in the German cockroach (Gore and Schal 2007, Gustchina et al. 2005, Wunschmann et al. 2005). Cockroach allergens are recognized as being important in the pathogenesis of asthma in the human population not only in the United States, but also in temperate, tropical, and subtropical climates (Gore and Schal 2007). Sarpong et al. (1996) discovered that African-American race and low socioeconomic status were factors significantly increasing the risk for sensitization to cockroach allergens in asthmatic children. Cockroaches pose a serious medical hazard in food-processing factories (Stejskal and Verner 1996), farms (Zurek et al. 2003, Stejskal et al. 2006), and urban buildings (Schal and Hamilton 1990, Wang and Bennet 2009).

Most cockroach allergens are associated primarily with the cockroach alimentary tract (Gore and Schal 2004a), and a large amount is excreted in their feces. After defecation, feces accumulate in huge quantities in cockroach refuges and aggregations (Stejskal 1997), thereby creating hidden dangerous allergen reservoirs. From these foci, and even after removing the

feces, allergens are gradually released via air movement into the home environment, where they cause chronic allergenic problems long after the extermination of the originating cockroach population.

Because cockroach allergens in households are one of the current strongest risk factors predictive of allergic sensitization (Cohn et al. 2006), a significant reduction of exposure to cockroach allergens is required to improve the health of inner city populations (Sever et al. 2007). Cockroaches have been traditionally controlled by insecticide baits (Appel 1990, Gore and Schal 2004b) and residual sprays (Stejskal et al. 2009) with varying degrees of success. Arbes et al. (2004) were the first to show that cockroach control alone can significantly reduce cockroach allergens in infested homes. Although there is evidence that professional control of cockroaches has the potential to reduce their allergens (Arbes et al. 2004, Wang and Bennet 2009), the previous studies showed that after successful control of cockroach population, the allergens persisted in the environment (Gergen et al. 1999, Eggleston et al. 1999, Williams et al. 1999). The reasoning for this was that successful removal of cockroach allergens from the infested environment is not easy to accomplish with remedial sanitation (Gore and Schal 2007). Making the issue even more fuzzy, Zhang et al. (2005) discovered that a pest control based on

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boric acid insecticides may not only fail to suppress allergens, but may increase their production. From the above, it is clear that there is boiling debate and controversy over the efficacy of various methods of cockroach allergen mitigation. Gore and Schal (2007) reviewed multiple methodological problems that are encountered in field studies trying to separate the role of cleaning and pest control on allergen removal. In addition, many factors potentially responsible for allergen decay or stability have not been included in the studies at all. For example, no published study has included the effect of environmental physical factors on allergen mitigation after cockroach population extermination. Therefore, it has been felt by the authors that there is a need to establish a comparative baseline model of the temporal decay of allergens under various environmental temperature and humidity regimes in the absence of cleaning.

The objective of this study was to determine the stability of Bla g 1 and Bla g 2 allergens and proteins in cockroach feces under controlled moisture and temperature regimes in laboratory and household conditions.

Materials and Methods

Preparation of Samples. Feces were produced by a laboratory strain of *Blattella germanica* L., the strain originated from the Crop Research Institute (Prague, Czech Republic), in an environmental chamber maintained at light 12:12 D:L photoperiod, $27 \pm 1^\circ\text{C}$, and 65% RH (Stejskal 1997). The diet consisted of dehydrated food for rabbits (name: KK1/RA, ZZN Rakovník a.s., Rakovník, Czech Republic) and dogs (name: Baron, Provini Pet Food, s.r.o., Praha, Czech Republic) in the proportion 1:4 (dry weight). The age of the collected cockroach males ranged from 7 to 18 d. Approximately 50 males were transferred to empty 1-L plastic chambers (Alliachem, s.r.o. Chropyně, Czech Republic). The feces were collected in 24-h intervals using a hairbrush. The procedure was repeated five times after a 5-d period. The collected feces were stored in 50-ml Greiner centrifuge tubes (catalogue T1818, Sigma-Aldrich, St. Louis, MO) in a deep freezer (-40°C) until used. Just before the experiment, the feces were properly mixed altogether per all collected samples to obtain a large homogenous sample that was then lyophilized in a PowerDry LL3000 (Thermo, Shanghai, China). After the feces were lyophilized, the material was separated and weighted into samples of 50 ± 0.5 mg weight in 600- μL Eppendorf tubes using a Mettler AE 240 microbalance (Mettler-Toledo, Columbus, OH). In five samples, the number of feces was counted. The unlocked tubes were covered by muslin fixed with an elastic band. The samples were kept in various conditions according to the experimental design. For every condition and decomposition period, 10 replicates were prepared.

Decomposition of the Feces. As an undecomposed control, 16 tubes were stored in the freezer during the whole experiment. In every experimental condition, the tubes were exposed for 3, 6, and 9 mo with 10

replicates per treatment and decomposition period, except for the baseline characteristic, where there were 16 altogether. After finishing the decomposition period in each condition, the tubes were locked and stored in the freezer until they were analyzed.

The effect of temperature on allergen degradation was evaluated in five temperature-controlled regimes ($15, 20, 25, 30$, and $35 \pm 0.5^\circ\text{C}$) in thermostats. The tubes were placed into bags in desiccators. A saturated solution of KCl provided $\approx 85\%$ RH in all temperature regimes in the desiccators. The effect of humidity on allergen degradation was evaluated in four moisture regimes. Four humidity regimes were evaluated as follows: 53, 75, 85, and 100%. They were achieved by maintaining desiccators containing saturated solutions of $\text{Mg}(\text{NO}_3)_2$, NaCl, KCl, and distilled water, respectively. The desiccators were placed in controlled temperature at $25 \pm 0.5^\circ\text{C}$. The thermostats had 12:12 DL photoperiod.

Decomposition under natural, household conditions was observed in an occupied volunteer city house in Tuchomerice, near Prague. The start of the experiment was in July 2006, and experiment was terminated on April 2007. The temperature and moisture conditions were monitored by Tinytalk temperature and humidity sensors (Gemini Data Loggers, Chichester, United Kingdom). The temperature was $20 \pm 1.6^\circ\text{C}$ (mean $\pm$ SD; minimum 16°C ; median 20°C ; maximum 28°C), and the humidity was $48 \pm 9\%$ (mean $\pm$ SD; minimum 26%; median 47%; maximum 73%).

Proteins and Allergen Contents After Decomposition. Five hundred microliters of cold physiological solution (0.9% wt:vol of NaCl) containing 0.1% Triton X-100 was added to each Eppendorf tube containing cockroach feces. Each sample was mixed for 1 min using a MS1 Minishaker (IKA, Staufen, Germany), and the mixtures were kept in a rack on ice. The Eppendorf tubes in a rack were shaken on a PST-60 HL temperature-controlled shaker (Biosan, Riga, Latvia) at 4°C using a ES 500 thermostat (Nuve, Ankara, Turkey) for 12 h. The extracts were centrifuged ($14,000 \times g$, 4°C , Jouan MR23i), and 200 μL of the homogenate was transferred into Eppendorf tubes. The homogenates were diluted one-fifth by the addition of 800 μL of cold physiological solution. The total protein content of each sample was determined using Bradford reagent (catalogue B6916, Sigma-Aldrich, St. Louis, MO) at 595 nm (Multiskan Ascent, Thermo, Shanghai, China). The protein standard (catalogue P0834, Sigma-Aldrich) was used for calibration.

The content of allergens Bla g 1 and Bla g 2 was determined using Indoor Biotechnologies enzyme-linked immunosorbent assay kits (Indoor Biotechnologies, Inc., Charlottesville, VA), according to the manufacturer's instructions. Bound immunocomplex was detected by peroxidase-conjugated goat anti-rabbit IgG (A6670, Sigma-Aldrich, St. Louis, MO) and followed by the tetramethylbenzidine Microwell peroxidase substrate system (catalogue 50-76-00, Kirkegaard & Perry Laboratories, Gaithersburg, MD). The reaction was terminated by tetramethylbenzidine Stop solution (catalogue 50-85-04, Kirkegaard & Perry

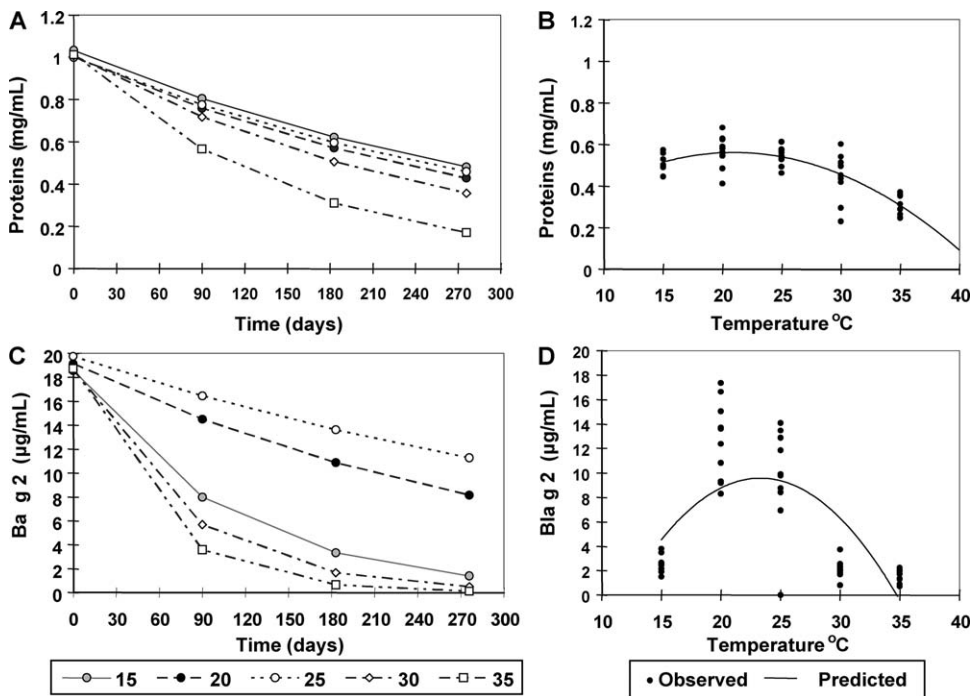


Fig. 1. The effect of decomposition time on proteins and the Bla g 2 allergen in the feces during 9 mo in controlled temperature regimes. (A) The decomposition of proteins according to concentration (A); $A = A_0 \times \exp^{(\lambda \times t)}$ model; only fits are shown. The parameters of the model are given in Table 2. (B) The relationships between temperature and protein concentration after 9 mo of decomposition of feces at different temperatures. The fit is a second order polynomial model: concentration = $at^2 + bt + c$, where t is temperature and the parameters are as follows: $a = -0.001 \pm 0.0002$; $b = 0.055 \pm 0.012$; and $c = -0.010 \pm 0.136$. (C) The decomposition of Bla g 2 according to the concentration (A); $A = A_0 \times \exp^{(\lambda \times t)}$ model; only fits are shown. The parameters of the model are given in Table 2. (D) The relationships between temperature and Bla g 2 concentration after 9 mo of decomposition of feces at different temperatures. The fit is a second order polynomial model: concentration = $at^2 + bt + c$, where t is temperature and the parameters are the following: $a = 0.073 \pm 0.013$; $b = 3.41 \pm 0.64$; and $c = -30.2 \pm 7.5$.

Laboratories), and the absorbance was measured on an enzyme-linked immunosorbent assay reader (Multiskan Ascent, Thermo, Shanghai, China) at 405 nm. The readings were corrected using blanks and quantified according to the calibration curve. The following parameters represented in the feces extract were used in the analyses: 1) mg of proteins per ml; 2) U of Bla g 1 per ml; and 3) μg of Bla g 2 per ml.

Data Analyses. At the start of the analysis, the correlation among total proteins, Bla g 1, Bla g 2, decomposition time, and environmental factors (e.g., temperature and relative humidity) was estimated using Pearson correlation coefficients (P_c). If the correlation between allergen or protein content and decomposition time was significant, the regression was performed. If not, the differences between control and exposed feces in the contents of proteins or allergens were compared by t test. For decomposition of proteins and allergens, the exponential decay function was expected to be $Y = A_0 \times \exp^{(\lambda \times t)}$, where t is time (0, 3, 6, 9 mo). To compare the various regimes, the amounts of proteins and allergens were compared at 9 mo of degradation by polynomial regression or by analysis of variance (ANOVA) test with Tukey's post

hoc comparison. The analyses were done in XL Stat 2007 (Addinsoft, New York, NY).

Results

Baseline Characteristics. One tube of 50 mg mass of feces contains 744 ± 16 fecal pellets. The concentration of the proteins and allergen content in the control tubes was 1.05 ± 0.06 mg/ml total proteins, 321 ± 60 U/ml Bla g 1, and 19 ± 2 μg /ml Bla g 2.

The Effect of Temperature on Feces Decomposition. The contents of proteins, Bla g 1, and Bla g 2 were negatively correlated with temperature ($P_c = -0.65$, $P < 0.001$; $P_c = -0.24$, $P = 0.004$; and $P_c = -0.28$, $P < 0.001$, respectively). Proteins and Bla g 2 were correlated with decomposition time ($P_c = -0.34$, $P < 0.001$; $P_c = -0.29$, $P < 0.001$), whereas Bla g 1 was not ($P_c = -0.03$, $P = 0.72$). There were significant correlations between total proteins and Bla g 1 ($P_c = 0.53$, $P < 0.001$), proteins and Bla g 2 ($P_c = 0.54$, $P < 0.001$), and Bla g 1 and Bla g 2 ($P_c = 0.50$, $P < 0.001$).

The amount of proteins decreased with increasing decomposition time according to the exponential decay function in all temperature regimes (Fig. 1A). The

λ parameters of the decomposition model showed differences between decomposition at 35°C and other temperatures. The half-life ($T_{1/2}$) of proteins was between 109 and 253 d (Table 1). After 276 d of decomposition, the protein concentration decreased to 17–56%. The relationship between protein content and temperature was fitted by polynomial regression model ($R^2 = 0.66$, Fig. 1B).

The amount of Bla g 1 was unchanged after exposition at temperatures from 15 to 30°C; there were no significant differences in Bla g 1 between control and exposed tubes at the compared temperatures (Table 2). In contrast, the concentration of Bla g 1 was significantly lower after exposure to 35°C than in control; it was 70% of baseline Bla g 1 content (Table 1).

The concentration of Bla g 2 decreased with increasing time in all temperature regimes (Fig. 1D). After 276 d of decomposition, the Bla g 2 concentration decreased to 1–57% of baseline (Table 1). Both parameters λ and $T_{1/2}$ of Bla g 2 were influenced by temperature (Table 2). The $T_{1/2}$ of Bla g 2 was highest at 20 and 25°C, in which the $T_{1/2}$ were 513 and 407 d, respectively. At 15, 30, and 35°C, the $T_{1/2}$ ranged from 39 to 76 d. It is also clearly illustrated by the differences in concentration of Bla g 2 among temperature regimes after 9 mo of decomposition. The relationship between Bla g 2 content and temperature was fitted by polynomial regression model ($R^2 = 0.49$, Fig. 1D).

The Effect of Moisture on Allergen Stability. There was significant negative correlation among moisture and proteins, Bla g 1, and Bla g 2 ($P_c = -0.74$, $P < 0.001$; $P_c = -0.27$, $P = 0.003$; and $P_c = -0.58$, $P < 0.001$, respectively). Total proteins and Bla g 2 were correlated with decomposition time ($P_c = -0.27$, $P = 0.003$; $P_c = -0.23$, $P = 0.012$, respectively), whereas Bla g 1 was not ($P_c = -0.15$, $P = 0.11$). There were significant correlations between total proteins and Bla g 1 ($P_c = 0.55$, $P < 0.001$), total proteins and Bla g 2 ($P_c = 0.80$, $P < 0.001$), and Bla g 1 and Bla g 2 ($P_c = 0.66$, $P < 0.001$).

The amount of proteins decreased with increasing decomposition in all moisture regimes compared (Fig. 2A). The λ parameters of the decomposition model showed differences between decomposition at 53 and 75% from 85 and 100% moisture regimes, but there was a shorter $T_{1/2}$ of proteins in the 100% moisture regime (Table 3). The amount of proteins decreased to 46–84% of baseline content after 276 d of decomposition (Table 4). The protein concentration was significantly different among moisture regimes (ANOVA, $F_{(3, 36)} = 98.6$; $R^2 = 0.89$, $P < 0.0001$). Tukey's comparison showed two separated groups, i.e., the protein contents at 53 and 75% moisture levels were higher than contents at 85 and 100% moisture levels (Fig. 2B).

The concentration of Bla g 1 was not influenced by exposure of the feces to moisture regimes at 75 and 85% RH, but there were differences between control and exposed feces at 53 and 100% RH (Table 3). At 53% RH, the Bla g 1 content decreased to 86% of baseline, and at 100% RH, the increase was to 10% of baseline (Table 4).

Table 1. The effect of temperature and decomposition time on total proteins, Bla g 1, and Bla g 2 in the feces under controlled temperatures

Factor	Time (days)	Temperature (°C)					
		15		20		25	
		Mean $\pm$ SD ^a	%	Mean $\pm$ SD ^a	%	Mean $\pm$ SD ^a	%
Proteins ($\mu\text{g/ml}$)	0	1.05 $\pm$ 0.06	100	1.05 $\pm$ 0.06	100	1.05 $\pm$ 0.06	100
	90	0.75 $\pm$ 0.07	78	0.63 $\pm$ 0.12	76	0.61 $\pm$ 0.04	78
	183	0.62 $\pm$ 0.09	60	0.50 $\pm$ 0.13	57	0.60 $\pm$ 0.04	60
	276	0.51 $\pm$ 0.04	47	0.57 $\pm$ 0.08	43	0.54 $\pm$ 0.04	46
Bla g 1 (U/ml)	0	321 $\pm$ 84	100	321 $\pm$ 84	100	321 $\pm$ 84	100
	90	270 $\pm$ 42	84	481 $\pm$ 148	148	259 $\pm$ 58	81
	183	300 $\pm$ 68	93	202 $\pm$ 63	63	335 $\pm$ 84	104
	276	270 $\pm$ 86	86	419 $\pm$ 104	131	353 $\pm$ 97	110
Bla g 2 ($\mu\text{g/ml}$)	0	18.79 $\pm$ 2.63	100	18.79 $\pm$ 2.63	100	18.79 $\pm$ 2.63	100
	90	6.55 $\pm$ 1.42	35	18.5 $\pm$ 7.03	98	19.02 $\pm$ 1.89	102
	183	4.28 $\pm$ 0.91	23	3.2 $\pm$ 1.8	17	14.17 $\pm$ 1.85	75
	276	2.57 $\pm$ 0.68	14	12.6 $\pm$ 3.2	67	9.60 $\pm$ 4.11	51

^a The data are expressed as the means and standard deviations from 10 replicates for every treatment.

^b The fits are fits of a nonlinear regression model for total proteins and Bla g 2. The parameters of the model are given in Table 2. For Bla g 1, the means $\pm$ standard deviations are presented, and the means were calculated per exposure times, which are not significantly different.

Table 2. The overview of the fit of decomposition models of the amount of proteins, Bla g 1, and Bla g 2 in various temperature regimes

Parameters		Temperature (°C)				
		15	20	25	30	35
Protein concn (mg/ml) ^a	R ²	0.904	0.724	0.805	0.831	0.882
	A ₀	1.034 ± 0.017	1.003 ± 0.032	0.99 ± 0.024	1.007 ± 0.027	1.012 ± 0.03
	λ	-0.003 ± 0.0001	-0.003 ± 0.0003	-0.003 ± 0.0002	-0.004 ± 0.0003	-0.006 ± 0.0004
	T _{1/2}	250 ± 14	225 ± 25	245 ± 21	184 ± 15	107 ± 9
Bla g 1 concn (U/ml) ^b	T	1.74	-1.04	0.18	1.04	4.20
	df	43	43	43	43	43
	P	0.088	0.305	0.860	0.303	<0.001
	R ²	0.927	0.332	0.559	0.933	0.952
Bla g 2 concn (μg/ml) ^a	A ₀	19 ± 0.50	19 ± 1	20 ± 1	19 ± 0.5	19 ± 0.5
	λ	-0.009 ± 0.001	-0.003 ± 0.001	-0.002 ± 0.0003	-0.013 ± 0.001	-0.018 ± 0.002
	T _{1/2}	74 ± 6	224 ± 68	340 ± 59	53 ± 5	38 ± 4

^a The proteins and Bla g 2 amounts decreased according to the nonlinear regression model: concentration = A₀ × Exp^(λ × t); the R² and parameters A₀, λ, and T_{1/2} of proteins (days) (mean ± standard deviation) are estimated.

^b The Bla g 1 concentration was not influenced by decomposition time, and, therefore, the control and exposed concentration was compared by *t* test.

The concentration of Bla g 2 decreased after exposure to various moisture regimes (Fig. 2C); after 276 d, the decrease was to 45–61% of baseline concentrations at 53, 75, and 85% RH regimes. At 100% RH, the decrease was to 2% of baseline after 90 d of decomposition (Table 4). It is clearly illustrated by the T_{1/2}, when the lowest T_{1/2} value is 16 d for Bla g 2 at 100% RH. When the concentration of Bla g 2 at the end of decomposition was compared (ANOVA *F*_(3, 35) = 88.7;

R² = 0.88, *P* < 0.0001), there were significant differences among moisture regimes. Tukey's comparison showed significant differences of concentration at 100% RH from the other humidity regimes and another difference between the 75 and 53% RH regimes (Fig. 2D).

Stability of Allergen in the Household. In the feces decomposed in the experimental house, total proteins and Bla g 2 were negatively correlated with decom-

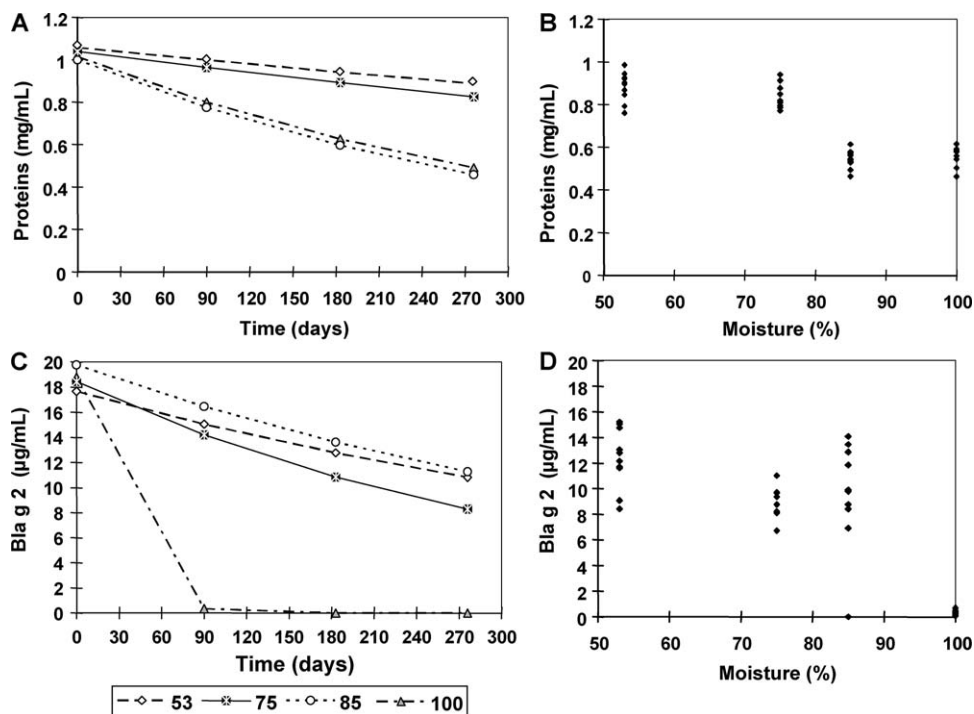


Fig. 2. The effect of moisture on total proteins and Bla g 2 decomposition. (A) The decomposition of proteins according to the model concentration = A₀ × Exp^(λ × t) model; only fits are shown. The parameters of the model are given in Table 3. (B) The relationships between temperature and protein concentration after 9 mo of decomposition of feces at different moisture regimes. (C) The decomposition of Bla g 2 according to the concentration (A); A = A₀ × Exp^(λ × t) model = A₀ × Exp^(λ × t) model; only fits are shown. The parameters of the model are given in Table 3. (D) The relationship between moisture and Bla g 2 concentration after 9 mo of decomposition of feces at different moisture regimes.

Table 3. The overview of the decomposition models of the amount of proteins, Bla g 1, and Bla g 2 in various moisture regimes and in the experimental house

Parameters		Relative humidity %				Experimental house
		53	75	85	100	
Proteins concn (mg/ml) ^a	R^2	0.577	0.677	0.805	0.829	0.633
	A_0	1.06 ± 0.013	1.04 ± 0.013	0.99 ± 0.024	1.014 ± 0.022	1.072 ± 0.014
	λ	-0.0006 ± 0.0001	-0.0008 ± 0.0001	-0.003 ± 0.0002	-0.0026 ± 0.0002	-0.0008 ± 0.0001
	$T_{1/2}$	$1,094 \pm 165$	830 ± 98	245 ± 21	263 ± 23	882 ± 118
	T	2.095	-1.250	0.18	18.527	-1.735
Bla g 1 concn (U/ml) ^b	df	44	42	43	43	44
	P	0.042	0.218	0.860	<0.0001	0.090
	R^2	0.425	0.809	0.559	0.971	0.454
Bla g 2 concn (μ g/ml) ^a	A_0	17.66 ± 0.704	18.43 ± 0.472	20 ± 1	18.79 ± 0.389	17.84 ± 0.65
	λ	-0.0018 ± 0.0003	-0.0029 ± 0.0002	-0.002 ± 0.0003	-0.045 ± 0.016	-0.0017 ± 0.0003
	$T_{1/2}$	390 ± 84	239 ± 21	340 ± 59	16 ± 9	406 ± 81

^a The proteins and Bla g 2 amounts decreased according to the nonlinear regression model $\text{concentration} = A_0 \times \text{Exp}^{(\lambda \times t)}$; the R^2 and parameters A_0 , λ , and $T_{1/2}$ of proteins (days) (mean $\pm$ standard deviations) are estimated.

^b The Bla g 1 concentration was not influenced by decomposition time, and, therefore, the control and exposed concentration was compared by t test.

position time ($P_c = -0.81$, $P < 0.001$; $P_c = -0.65$, $P < 0.001$). The proteins were correlated with Bla g 2 ($P_c = 0.48$, $P = 0.001$). The correlations between the decomposition time and Bla g 1 were not significant ($P_c = 0.16$, $P = 0.29$). There were no significant correlations between total proteins and Bla g 1 ($P_c = 0.11$, $P = 0.47$), Bla g 1, and Bla g 2 ($P_c = -0.005$, $P = 0.98$).

The amount of proteins decreased with increasing decomposition time (Fig. 3A). The $T_{1/2}$ of proteins was fitted as 882 d (Table 3). After 276 d, the total proteins decreased to 80% of baseline (Table 4).

The Bla g 1 concentration remained constant under field conditions (Table 4). There was no significant difference in content of Bla g 1 between exposed and control tubes $T_{(1, 44)} = -1.173$; $P = 0.09$ (Table 3).

There was a decrease of Bla g 2 after the exposure (Fig. 3B). The $T_{1/2}$ of Bla g 2 was fitted at 406 d (Table 3). After 276 d, content of Bla g 2 decreased to 64% of baseline (Table 4).

Discussion

Knowledge of the effect of various physical conditions or allergen management strategies (Eggleston and Arruda 2001, Gore and Schal 2007, Nalyanya et al. 2009) on cockroach allergens from indoor environment is important in designing more effective allergen mitigation programs. In this study, we observed the effect of temperature and moisture on the environmental fate of proteins and allergens in cockroach feces and, consequently, validated the laboratory model under field conditions.

Our results showed that the contents of proteins and Bla g 2 were correlated negatively to the decomposition time, whereas Bla g 1 was not. However, when the content of Bla g 1 in control and exposure tubes was compared, the decrease after exposure was significant at exposure in 35°C and 100% RH. The decrease was observed also at 53% RH, but it was on the border of significance (Table 4). In all other conditions, we did not find any significant decrease of Bla g 1 during our experiments.

The comparison of allergens and proteins in temperature regimes 15, 30, and 35°C, and constant moisture regimes 53, 75, and 100% RH, and experimental apartment showed that the $T_{1/2}$ of Bla g 2 is much lower than that of total proteins (Table 1–4). This indicates that Bla g 2 is decomposed more rapidly than total proteins. Our only hypothetical explanation was that decomposition was associated with the activity of microbial flora and/or proteolytic activity in feces. It is known that there are high levels of active proteases in the gut of cockroaches, which could be still active in the feces (Vinokurov et al. 2007, Sudha et al. 2008), and after defecation the feces are colonized by coprophagous microflora. It is supported by scanning electron microscopy study showing increasing fungal colonization and partial rupture of fecal membrane by the penetration of microflora mycelium (Krizkova-Kudlikova et al. 2006).

We intended our experiment to be a simulation modeling the environmental fate of cockroach allergens after successful extermination of the cockroach population under different environmental conditions in the absence of cleaning and with reduced air movement. We completely excluded cleaning and limited air movement by enclosing the feces in open Eppendorf tubes. We found that temperature and humidity influenced the contents of Bla g 1 and Bla g 2 in different ways, showing that Bla g 1 (Gore and Schal 2005) is more stable and likely more risky than Bla g 2. This is in concordance with the findings from the household that most cockroach-allergic individuals (33–77%) have detectable IgE antibodies to Bla g 1 (Eggleston et al. 1998, Gore and Schal 2007).

Our data demonstrated that within the range of usual household physical temperature and humidity, the correlation between decomposition time and Bla g 1 content in the feces was missing, whereas with Bla g 2 there were significant correlations. Occupants consider normal and comfortable conditions when the temperature and relative humidity are maintained within the ranges of 15–22°C and 25–50% RH (<http://www.fcs.uga.edu/pubs/current/B924.html>). How-

Table 4. The effect of moisture and decomposition time on total proteins, Bla g 1, and Bla g 2 in the feces decomposed under controlled temperature and in the experimental house

Factor	Time (days)	Relative humidity %												Experimental house	
		53				75				85				100	
		Mean $\pm$ SD ^a	Fit ^b	%		Mean $\pm$ SD ^a	Fit ^b	%		Mean $\pm$ SD ^a	Fit ^b	%		Mean $\pm$ SD ^a	Fit ^b
Proteins (mg/ml)	0	1.05 $\pm$ 0.06	1.06	100		1.05 $\pm$ 0.06	1.04	100		1.05 $\pm$ 0.06	1.00	100		1.05 $\pm$ 0.06	1.07
	90	1.01 $\pm$ 0.06	1.00	94		0.94 $\pm$ 0.05	0.96	93		0.63 $\pm$ 0.05	0.80	80		1.03 $\pm$ 0.05	1.00
	183	0.94 $\pm$ 0.05	0.94	89		0.87 $\pm$ 0.06	0.89	86		0.66 $\pm$ 0.08	0.64	64		0.98 $\pm$ 0.05	0.93
	276	0.88 $\pm$ 0.07	0.89	84		0.85 $\pm$ 0.06	0.82	79		0.57 $\pm$ 0.05	0.51	51		0.81 $\pm$ 0.04	0.86
Bla g 1 (U/ml)	0	321 $\pm$ 84	321 $\pm$ 84	100		321 $\pm$ 84	339 $\pm$ 55	100		321 $\pm$ 84	321 $\pm$ 84	100		321 $\pm$ 84	321 $\pm$ 84
	90	253 $\pm$ 53				308 $\pm$ 34				259 $\pm$ 58				364 $\pm$ 123	355 $\pm$ 82
	183	278 $\pm$ 34	277 $\pm$ 59	86		389 $\pm$ 79				335 $\pm$ 84				417 $\pm$ 90	
	276	299 $\pm$ 77				351 $\pm$ 56				353 $\pm$ 97				339 $\pm$ 92	
Bla g 2 (μ g/ml)	0	18.79 $\pm$ 2.63	17.66	100		18.79 $\pm$ 2.63	18.43	100		18.79 $\pm$ 2.63	18.79	100		18.79 $\pm$ 2.63	17.4
	90	12.02 $\pm$ 2.81	15.05	85		13.62 $\pm$ 1.59	14.20	77		19.02 $\pm$ 1.89	16.45	83		13.22 $\pm$ 3.34	15.3
	183	12.53 $\pm$ 2.32	12.76	72		10.46 $\pm$ 1.36	10.85	59		14.17 $\pm$ 1.85	13.61	69		11.74 $\pm$ 1.52	13.1
	276	12.37 $\pm$ 2.33	10.82	61		8.94 $\pm$ 1.20	8.28	45		9.60 $\pm$ 4.11	11.26	57		13.08 $\pm$ 1.19	11.1

^a The data are expressed as the means and standard deviations from 10 replicates for every treatment.

^b The fits are fits of a nonlinear regression model for total proteins and Bla g 2. The parameters of the model are given in Table 3. For Bla g 1 the means $\pm$ standard deviations are presented; the means were calculated per exposure times, which did not significantly differ.

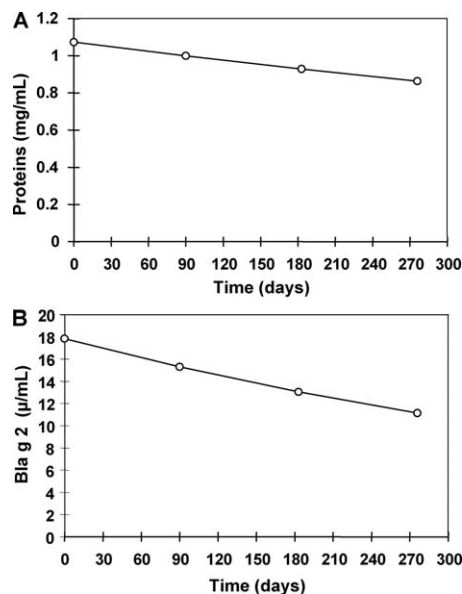


Fig. 3. The effect of exposure in experimental house on total proteins and Bla g 2 levels. (A) The decomposition of proteins according to the concentration (A); $A = A_0 \times \text{Exp}^{-(\lambda \times t)}$ model; only fits are shown. (B) The decomposition of Bla g 2 according to the model concentration = $A_0 \times \text{Exp}^{-(\lambda \times t)}$ model; only fits are shown. The parameters of both models are given in Table 3.

ever, it should be stressed that although Bla g 1 is stable under such normal conditions, it degrades substantially under extreme conditions (i.e., 35°C and 100% RH) (Tables 2 and 3). The highest decrease was at 100% RH, when the concentration decreased to 10% of baseline. In households, there are many microhabitats that differ dramatically from the average household temperature and humidity (e.g., kitchen, boiler rooms, sinks, and refrigerators). Our model highlights that the existence of microhabitats may cause spatiotemporal variations in allergen decay, even within an apartment. The methodical implication is that the selection of different sampling sites for allergens in the household makes a comparison of the results of various allergen mitigation studies difficult, if not impossible (Gore and Schal 2007).

The laboratory model showing the long-term stability of Bla g 1 in feces was confirmed in the validation household study, in which the Bla g 1 persisted for 9 mo in our experiments. Although the decrease of Bla g 2 was apparent both in the laboratory and in the household (Table 4), the correlation in the household was not significant. For controlled humidity at 75 and 85% RH and temperatures of 20 and 25°C, the $T_{1/2}$ of Bla g 2 ranged from 224 to 340 d.

In low-income housing, where the risk of allergen contamination is high, kitchen dust samples from 33% of the sampled apartments had Bla g 1 at level ≥ 8 U/g (Wang et al. 2008). In our experiments, we used 50 mg of feces formed from 744 fecal pellets and 321 U/ml Bla g 1. It means that such amount of feces is presented in 40 g of dust in such highly contaminated kitchen.

What is the practical message of our findings for allergen risk assessment and mitigation? Our data clearly show a significant potential for the long-term persistence of cockroach allergens Bla g 1 and Bla g 2 under normal household temperature and humidity. This is, however, in strong contrast to the findings of many recent field studies (Arbes et al. 2004, Gore and Schal 2007, Wang and Bennet 2009, Nalyanya et al. 2009) that documented a significant allergen decline after cockroach extermination. We have to point out that in our experiments (i.e., both laboratory and household validation) the allergens were protected in feces inside opened plastic vials covered by the mesh. So the discrepancy may be explained in that in the real world cockroach feces stay unprotected in open space exposed to other environmental factors. For example, in the case of poor sanitation, the bioavailability of allergens may be decreased by environmental dust or grease. Conversely, air movement, air conditioning, ventilation, and daily cleaning and sanitation may remove allergens. It is documented that proper sanitation not only removes allergens (Eggelston et al. 1999, McConnell et al. 2003, Gore and Schal 2007), but also prevents the resurgence of cockroach populations by limiting food resources and enhancing the efficacy of blaticide sprays and baits (Schal 1988; Rivault and Cloarec 1995, 1997; Eggelston and Arruda 2001). Moreover, the findings of Gore and Schal (2007) that Bla g 1 production in *B. germanica* is modulated in relation to food intake may imply that the cleaning and removal of food debris from the cockroach-infested environment may have an impact on the production and composition of cockroach allergens. The long life of Bla g 1 and Bla g 2 under undisturbed environments implies that house cleaning or cockroach allergen sampling must target to places where cockroach feces are mostly found – cockroach harborages. Without removing the feces, they will continue to be the source of allergens in settled and air dust.

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