

Risk Evaluation of Spatial Distribution of Faecal Mice Contaminants in Simulated Agricultural and Food Store

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Abstract.- We studied spatial distribution of faeces of a wild strain of house mice after invasion into a new environment. In a simulated small store room, we evaluated the density of faeces and their relative allocation in the following three sectors: shelter, food + water and remaining area. Individual positions of a total of 9, 809 deposited faeces were recorded. The spatial distribution of faeces was not random but aggregated. The average relative proportion of faeces in the three sectors was: 7.0% at food+water; 15.7% at shelter; and 76.7% in remaining area. Although the relative proportion of the total deposited faeces was lowest around food, the risk of food spoilage remains high due to high faeces numbers produced per mouse and day. Even a single mouse invasion into a simulated store caused serious floor contamination (97.3 faeces/m²) as the average daily defecation rate was 102.2 ± 5.7 faeces/individual (range: 48-156).

Keywords: Agricultural stores, food safety, risk evaluation, faecal contamination, *Mus musculus*.

INTRODUCTION

Rodents are abundant in grain stores, food stores and industry premises, warehouses and retail stores (Knote, 1988; Rustamani *et al.*, 2005; Stejskal *et al.*, 2015). For agricultural production stored in Asia, it was documented that rodents and arthropods are more damaging (cca 5 % annual losses) than birds (cca 0.8% losses) (Hafiz, 1983). A specific pattern of seed injury is produced by rodent gnawing (Stejskal *et al.*, 2014). In addition to economic losses on stored agricultural products, rodents cause injury to packages, construction materials and electric circuits, due to the enormous hardness of their teeth (Buckle and Smith, 1994; Frydova *et al.*, 2013). Rodents have a capacity to seriously contaminate the environment by pathogens and parasites (*e.g.* Reeves and Cobb, 2005) and stored commodities and processed food by urine, hairs and faeces (LaVoie *et al.*, 1991; Hussain, 2002; Stejskal and Aulicky, 2014). Hussain (2002) was one of few researchers who precisely described the risk and extent of rodent faecal filth found in cereal commodities sampled from real-world warehouses. Rodent faeces are not only appalling

physical contaminants but they contain pathogens, toxinogenic fungi and allergens (Hollander *et al.*, 1997; Meerburg and Kijlstra, 2007; Mushtaq-ul-Hassan *et al.*, 2008; Stejskal *et al.*, 2005). When agricultural and food sanitation EU officers trace faeces or rodent activity inside warehouses and grain stores, they impose a high penalty and close the facility for unsanitary conditions if there are serious violations of Regulation EC 852/2004.

This work was initiated due to recent emerging mice risks as pests and contaminators in agricultural stores and warehouses. The main criterion for defining risk is the probability of a contamination of stored commodity and food. To calculate risk, estimates are needed for at least three basic risk parameters: i) contamination rate, ii) distribution of contaminants, and iii) the efficacy of cleaning and disinfestation treatments. Although mice are well studied pests, there is surprisingly little experimentally substantiated information that can help to establish risk of contamination of food according to the above mentioned three parameters. There are published reports on parameters i) and iii); that include rodent control and mouse and defecation dynamics (LaVoie *et al.*, 1991; Frantz and Davis, 1991; Aulicky *et al.*, 2010; Frynta *et al.*, 2012). The published info on faecal contamination rate of the main three rodent species (*Mus musculus* Linnaeus, 1758, *Rattus norvegicus* (Berkenhout,

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1769), *R. Rattus* (Linnaeus, 1758) is summarized in the Table I. But there is no data on distribution of mouse faeces (*i.e.*, risk parameter ii) in the habitat of mice. In our opinion, the lack of experimental knowledge on spatial distribution of mouse faeces within a facility prevents construction of a more or less objective method to evaluate overall contamination risk of the facility. Hence, any judgments on contamination risk and extent, even if made by trained agriculture and public health inspectors, can be considered as subjective and based only on personal experience and perception with the legal consequences for potential law suits.

Table I.- Contamination potential of stores and warehouses by faeces of various rodent species.

Rodent pest species	Faecal contaminants	Reference
House mouse (<i>Mus musculus</i>)	72 (24-116) per 24 h	Frynta <i>et al.</i> (2012)
Norwegian rat (<i>Rattus norvegicus</i>)	37 (16-55) per 24 h	Frantz and Davis (1991)
Roof rat (<i>Rattus rattus</i>)	59 (31-126) per 24 h	Frantz and Davis (1991)
	108 (76-15) per 36 h	Aulicky <i>et al.</i> (2010)

This work is the first attempt to experimentally estimate an amount and proportion of deposited faeces by a wild strain of house mouse (*M. musculus*) in enclosed habitat conditions. The study was performed in a simulated store/warehouse room that included shelter, food and a water resource, and the remaining unobstructed free space was surrounded by wall intersections.

MATERIALS AND METHODS

Experimental animals and ethical note

Laboratory born descendants of wild house mice (*i.e.*, F2 generation bred in laboratory) belonging to the western subspecies *Mus musculus domesticus* Schwarz et Schwarz (1943) were used as experimental subjects. Mice were kept and bred in pairs in plastic cages (300 x 180 x 150 mm). Water and cereal based food (ST1 mice and rat breeder pelleted diet; VELAZ, Czech Republic) were provided *ad libitum*. The animals were kept in a breeding room under an artificial light:dark 12:12 h regime. The experiments were performed

exclusively during the subjective dark phase of the day in a specially designed experimental enclosure (floor proportions: 2.5 x 1.75 m) illuminated with a 40 W red light bulb. Any harm to experimental animals was avoided, and only non-invasive methods for sample collection were used. The experiments were performed in accordance with the Czech law implementing all corresponding European Union regulations and were approved by the Institutional Animal Care and Use Committee (Meerburg *et al.*, 2008).

Experimental design

Since it is difficult to perform experiments with stored product in the field it is common to use a simulated store or simulated warehouse as described in Hawkin *et al.* (2011). The experiments were performed in the experimental room (enclosure) at the accredited facility of Crop Research Institute, Prague, Czech Republic. The enclosure size (4.2 m² floor area) simulated the common small territory (Pocock *et al.*, 2004) in stores and warehouses. The enclosure contained shelter (wooden box with plastic roof; black colour; 18 x 18 cm and height 6.5 cm; circular entry of approximately 3 cm in diameter), food (served in small pots with a 1.5 cm diameter and 1-3 cm length) and a water resource (250 ml plastic cylinder with metallic feeding pump). The enclosure was equipped by semitransparent glass and red light illumination for direct observation without disturbing animals as well as camera (Fig. 1) and recording system (Panasonic super Dynamics II WV GP460). We used two designs that differed in proximity of shelter to the water and food resources in the enclosure as follows: Design A, “water + food” sector was next to the “shelter” sector; and Design B, “water + food” sector was on the opposite side of the room from the “shelter” sector. After each experiment, the arena was mechanically cleaned and washed with water containing detergent and hot steam. A new shelter in addition to fresh food and water was provided for each design and tested mouse. To quantitatively evaluate the spatial allocation of faeces, the floor of the enclosure was graphically divided into 15 equal rectangles (Fig. 2). Faeces load (= faecal pellets/droppings) on each rectangle was counted inside the shelter and on the



Fig. 1. The enclosure was equipped by semitransparent glass and red light illumination (for direct observation without disturbing animals) as well as camera and recording system.

food. Separately were evaluated 3 sectors of special interest as follows: 1) “shelter” sector (= 1 hatched rectangle); 2) “food + water” sector (= 1 dotted rectangle); and 3) “remaining area” sector (= 13 white rectangles along walls and in the middle of the room). Explored was faecal distribution of newly immigrated (invaded) individuals rather than established mice populations because the latter is more common in warehouses (Kent, 1959; Knot, 1988). We have been observed faecal distribution of an individual mouse for 96 hours after its introduction into the enclosure. We tested for each design in 12 replications (each design with 6 females and 6 males). In our experiment, we simulated distribution of a newly introduced single individual (not an established population), which is the common pattern of infestation in warehouses.

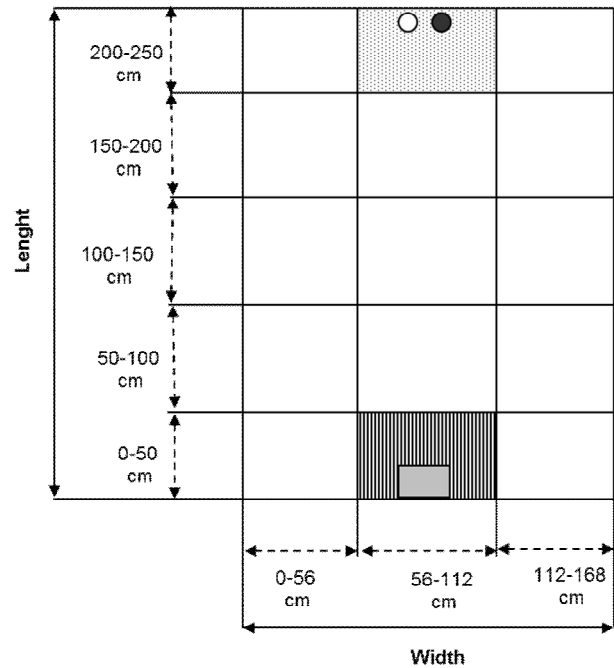


Fig. 2. The floor of the experimental room (enclosure) was graphically divided into 15 regular rectangles and three sectors of interest where faeces were counted: 1) “shelter” sector (=1 hatched rectangle); 2) “food + water ” sector (=1 dotted rectangle); and 3) “remaining area” sector (=13 white rectangles along walls and in the middle of the room). Legend: Black and white points represent position food (white point) and water (black point).

The four year study on warehouse (supermarket) mouse infestations by Knot (1988) showed that most of the mouse interceptions in warehouses have been recognised as individual migrants from colonies established outside warehouses.

Statistical analysis

The effect of explanatory variables on the distribution of faeces using Generalised Linear Models with the negative binomial error structure (GLM-nb) due to high overdispersion was tested. Comparison of dropping density among places was done using the Generalised Estimating Equations with Poisson error (GEE-p) structure due to the repeated measurements on the same individual. GEE is an extension of GLM used for inferences on non-independent data by specifying correlation structure in residuals (Pekár and Brabec, 2012). The

analyses were performed within R (R Development Core Team 2011) using MASS (Venables and Ripley, 2002) and geepack (Yan, 2002) packages.

RESULTS

In the entire experiment, the positions of 9 809 deposited faeces were recorded and evaluated. The number of faeces produced by experimental mouse ranged from 48 to 156.5 pellets per day (males: 100.4 ± 9.5 (average \pm SE); females: 104.0 ± 6.8 pellets per day). During 4 days of exposure, the average contamination rate was 102.2 ± 5.7 faeces/day/mouse resulting in the average contamination of enclosure floor by 97.3 faeces/m²/mouse. Figure 3 shows comparison of the mean number of faeces (per rectangle) at different positions of food+water in the room. The faeces were not randomly distributed in the enclosure because their distribution was highly aggregated (coefficient of aggregation, $\theta = 0.46$; Fig. 3). The distribution of faeces was not significantly affected by the sex (GLM-nb, $X^2_1 = 0.1$, $P = 0.79$) or by the position of the water+food (GLM-nb, $X^2_1 = 0.01$, $P = 0.96$). The faeces were mainly distributed along the walls (Fig. 3), and their density differed significantly among sectors (GEE-p, $X^2_2 = 184.1$, $P < 0.0001$; Fig. 4). The density of faeces in the “remaining area” was significantly lower than in the “shelter” area (contrasts, $P < 0.0001$). The density of faeces in the water+food sector was significantly lower than that in the shelter area (contrasts, $P < 0.0001$).

As there was a different number of rectangles in each sector, the percentage of faeces distribution was as follows: $76.73 \pm 2.87\%$ (average \pm SE) of faeces (range = 32.46–96.88%) in the “remaining area”; $15.65 \pm 2.62\%$ of faeces (range = 2.84–63.61%) in the “shelter” sector; $7.00 \pm 1.25\%$ of faeces (range = 0–21.30%) in the “food+water” sector; $11.16 \pm 2.75\%$ of faeces (range = 0–62.30%) directly inside the shelter; and $0.74 \pm 0.31\%$ of faeces (range = 0–4.56%) in the pot with food pellets.

DISCUSSION

After invasion of a new place (*e.g.*, store in a warehouse or a supermarket), mice inevitably start

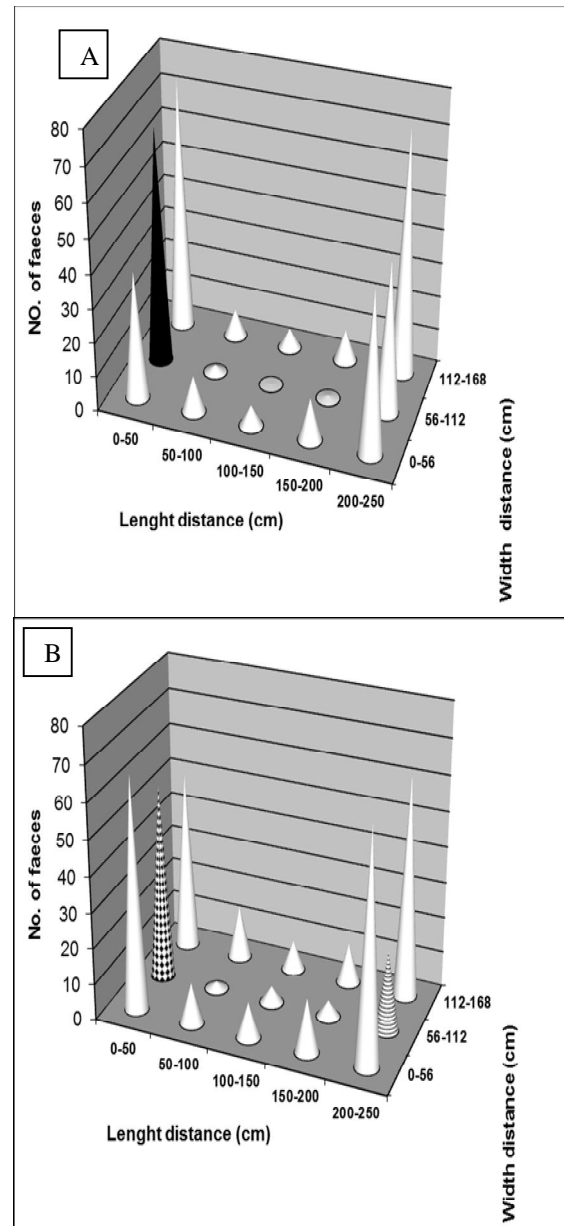


Fig. 3. Comparison of the mean number of faeces (per rectangle) at different positions of food + water in the room. A, Experiments in which food and water were placed next to the shelter. B, Experiments in which food and water were placed in the opposite side from the shelter. Legend: Black bars - No. of faeces at the sector containing shelter + food + water. Dotted bars - No. of faeces at the sector containing shelter. Dashed bars - No. of faeces at the sector containing food + water. White bars - No. of faeces on each of the remaining (empty) sectors in the experimental room.

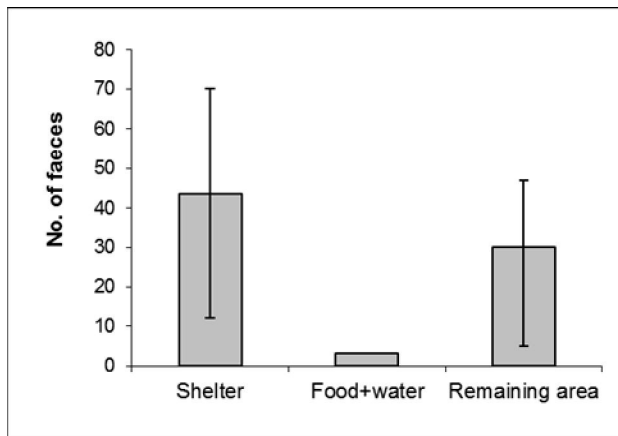


Fig. 4. Comparison of the mean number (i.e., density per rectangle) of faeces among three sectors. Whiskers are quartiles.

to gnaw on food and food packages, and they contaminate the environment by hairs, urine and faeces. According to Frynta *et al.* (2012), a single mouse kept in small cage produces cca 70 faeces per day. In the present study the mice free movement (in the experimental room - not cage) resulted in the production of even more faeces (cca 100 faeces per day and mouse). We also found that even four days of mouse activity may result in heavy contamination of the experimental room (97.3 faeces/m²/individual), which is quite alarming for the food industry, supermarkets and retail food stores because even occasional invasion of a single mouse may result in dangerous faecal contamination within a short time period. Our results demonstrating high mouse contamination potential were in concordance with the results reported by LaVoie *et al.* (1991) for farm storage. After 150 days of simulated grain storage, these authors recovered 220 faeces and 611 hairs per m² of grain surface, and they found 65% of the grain surface contaminated by urine. In a previous study (Stejskal and Aulicky, 2014), it was also described high faecal contamination potential (6.90 faeces/m² of grain surfaces and 34.80 faeces/m² of grain conveyor belts) of a related commensal rodent species (roof rat) within only a month exposure period under real world farm conditions.

Several authors (Hurst, 1987; Kitaoka, 1995; Gray *et al.*, 2002; Frynta *et al.*, 2010; Volfova *et al.*, 2011) have studied spatial activity and behaviour of

various commensal rodents in an open field and arena with factors that may change the behaviour (social composition, shelter and food). Gray *et al.* (2002) found that animals in laboratory enclosures spend more time active in the areas containing food resources patrolling for intruders and depositing fresh urine as territorial scent marks, but these researchers did not focus on faecal distribution. Although limited in extent, our experiment therefore provides the first insight into spatial distribution of house mouse faeces in a simulated store room. Not surprisingly, the spatial dispersion of faeces was not regular or random, but it was aggregated. We did not expect the relative low (cca 7%) proportion of faeces allocated around the “food+water” sector in comparison with the remaining areas of the experimental room. The highest proportion of faeces was found along the wall intersection followed by the areas in and around the shelter and around the food and water. As mentioned above, we did not find any published comparative data on mice or other commensal rodents. Nevertheless, from the hygienic and sanitation point of view, it is interesting to notice that the mouse faeces distribution pattern differs from the pattern of another serious urban pest, namely cockroach (*Blattella germanica* L.). Cockroach faeces usually accumulate directly inside the shelter area because cockroaches generally tend to aggregate and defecate when they are at rest (Stejskal, 1997; Varadinova *et al.*, 2015). In contrast, our results indicated that the newly invading mouse mostly defecates outside the shelter when moving, which was in agreement with the behavioural observation of Frynta *et al.* (2012), who reported the wild strain of *M. musculus* defecates mainly during the “active” night period.

Practical conclusions and implications

Invasion of a single mouse into a warehouse may cause serious contamination and hygienic violation because the average daily defecation rate was approximately 100 faeces per pest individual. Although the relative proportion of total deposited faeces was low around food, the risk of food spoilage still remains high due to high faeces number (approximately 70-100) produced per mouse and day. This result implies the

implementation of strict prophylactic rodent control systems in food production, storage and distribution facilities based on mouse-proof construction, sensitive rodent monitoring and early warning system. However, there is another aspect coming from our work that may not be as obvious. Agricultural, food and hygienic inspectors are not the only ones sensitive to faecal contamination around food as the public is also sensitive. It therefore attracts much sanitation and control attention of food facility managers once the contamination is discovered. However, our work implies that it should be taken into account that contamination around food may represent only 7% of total faecal deposits concurrently present in the facility. Therefore, in such cases, effort should also be made to discover and remove the hidden faecal deposits along the wall intersections (usually hidden bellow shelves and pallets) and around mouse shelters. Without removing the hidden faecal deposits, there is a risk that the hidden and uncleaned accumulations of faeces will gradually deplete and become part of the airborne dust containing extremely stable allergens as described by Hollander *et al.* (1997).

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REFERENCES

- AULICKY, R., FRANKOVA, M., RODL, P., ELIASOVA, B., FRYNTA, D. AND STEJSKAL, V., 2010. Fluorescent non-toxic bait as a new method for black rat (*Rattus rattus*) monitoring. In: *10th International Working Conference on Stored Product Protection, Julius-Kühn-Archiv, No. 425*:950-952.
- BUCKLE, A.P. AND SMITH, R.H., 1994. *Rodent pests and their control*. CAB International, Wallingford and United Kingdom.
- FRANTZ, S.C. AND DAVIS, D.E., 1991. Bionomics and integrated pest management of commensal rodents. p. 243-313. In: *Ecology and management of food industry pests* (ed. J.R. Gorham), FDA Tech. Bull. 4. Association of Official Analytical Chemists, Arlington.
- FRYDOVA, B., SEPITKA, J., STEJSKAL, V., FRYDA, J. AND LUKES, J., 2013. Nanoindentation mapping reveals a gradient in mechanical properties of dental enamel in rat incisors. *Comput. Meth. Biomech. Biomed. Eng.*, **16**:290-291.
- FRYNTA, D., VOLFOVA, R., FRANKOVA-NOVAKOVA, M. AND STEJSKAL, V., 2010. Oestrous females investigate the unfamiliar male more than the familiar male in both commensal and non-commensal populations of house mice. *Behav. Process.*, **83**:54-60.
- FRYNTA, D., ELIASOVA, B., FRANKOVA, M., AULICKY, R., RODL, P. AND STEJSKAL, V., 2012. Production of UV-light-detectable faeces from house mice (*Mus musculus*) after consumption of encapsulated fluorescent pigment in monitoring bait. *Pest Managem. Sci.*, **68**:355-361.
- GRAY, S.J., JENSEN, S.P. AND HURST, J.L., 2002. Effects of resource distribution on activity and territory defence in house mice, *Mus domesticus*. *anim. Behav.*, **63**:531-539.
- HAFIZ, A., 1983. Losses incurred in stored food grains by insect pests - a review. *Pakistan J. agric. Res.*, **4**:198-207.
- HAWKIN, K.J., STANBRIDGE, D.M. AND FIELDS, P.G., 2011. Sampling *Tribolium confusum* and *T. castaneum* in mill and laboratory settings: differences between strains and species. *Can. Entomol.*, **5**:5040-517.
- HOLLANDER, A., VAN RUN, P., SPITHOVEN, J., HEEDERIK, D. AND DOEKES, G., 1997. Exposure of laboratory animal workers to airborne rat and mouse urinary allergens. *Clin. Exp. Allergy*. **27**:617-626.
- HUSSAIN, I., 2002. Occurrence of rodent filth in grain commodities sampled from ration shops, Rawalpindi (Pakistan). *Pakistan J. Zool.*, **34**:239-242.
- HURST, J.L., 1987. The functions of urine marking in a free-living population of house mice, *Mus domesticus* Ruddy. *Anim. Behav.*, **35**:1433-1442.
- KENT, N.L., 1959. Contamination of stacked grain by rats and mice. *Scott. Agric.*, **39**:98-101.
- KITAOKA, A., 1995. Effect of a shelter on open field activity of rats (*Rattus norvegicus*) and mice (*Mus musculus*). *Jap. Psychol. Res.*, **37**:201-209.
- KNOTE, C.E., 1988. Stopping house mice building infestations through exterior control. In: *Proc. Vertebr. Pest Conf.* (eds. A. Crabb and R. Marsh), Vol. 13, University of California and Davis, pp. 107-111.
- LAVOIE, G.K., MITCHELL, G.C. AND BORNSTEIN, M.S., 1991. Contamination and consumption of stored wheat by a closed population of *Mus musculus*. In: *Proc. 11th Int. Cong. Plant Protection* (ed. E.D. Magalona), vol I, October, 1987 Manila, Philippines, pp. 45-51.
- MEERBURG, B.G. AND KIJLSTRA, A., 2007. Role of rodents in transmission of *Salmonella* and *Campylobacter*. *J. Sci. Fd. Agric.*, **87**:2774-2781.
- MEERBURG, B.G., BROM, F. AND KIJLSTRA, A., 2008. Perspective: the ethics of rodent control. *Pest Managem. Sci.*, **64**:1205-1211.

- MUSHTAQ-UL-HASSAN, M., HUSSAIN, I., SHEHZADI, B., SHAHEEN, M., MAHMOOD, M.S., RAFIQUE, A. AND MAHMOOD-UL-HASSAN, M., 2008. Occurrence of some zoonotic microorganisms in faecal matter of house rat (*Rattus rattus*) and house mouse (*Mus musculus*) trapped from various structures. *Pakistan Vet. J.* **28**:171-174.
- PEKAR, S. AND BRABEC, M., 2012. *Modern analysis of biological data. 2. Linear models with correlation in R*. Masaryk University Press, Brno [in Czech].
- POCOCK, M., SEARLE, J. AND WHITE, P., 2004. Adaptations of animals to commensal habitats: population dynamics of house mice *Mus musculus domesticus* on farms. *J. anim. Ecol.*, **73**:878–888.
- R DEVELOPMENT CORE TEAM, 2011. *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org/>
- REEVES, W. AND COBB, K.D., 2005. Ectoparasites of house mice (*Mus musculus*) from pet stores in South Carolina, U.S.A. *Comp. Parasitol.*, **72**:193-195.
- RUSTAMANI, M.A., KAKAR, A.W., KHOOHARO, A.A., DARS, F., ANSARI, S.A. AND BALOCH, H.B., 2005. Grain losses caused by house rat *Rattus rattus* L. and its control. *Pakistan J. Zool.*, **37**:33-38.
- STEJSKAL, V., 1997. Distribution of faeces of the German cockroach, *Blattella germanica*, in a new refuge. *Ent. Exp. Appl.*, **84**:201–205.
- STEJSKAL, V. AND AULICKY, R., 2014. Field evidence of roof rat (*Rattus rattus*) faecal contamination of barley grain stored in silos in the Czech Republic. *J. Pestic. Sci.*, **87**:117–124.
- STEJSKAL, V., AULICKY, R. AND KUCEROVA, Z., 2014. Pest control strategies and damage potential of seed-infesting pests in the Czech stores – a review. *Pl. Protect. Sci.*, **50**: 165–173.
- STEJSKAL, V., HUBERT, J., AULICKY, R. AND KUCEROVA, Z., 2015. Overview of present and past and pest-associated risks in stored food and feed products: European perspective. *J. Stored Prod. Res.* <http://dx.doi.org/10.1016/j.jspr.2014.12.006>.
- STEJSKAL, V., HUBERT, J., KUBATOVA, A. AND VANOVA, M., 2005. Fungi associated with rodent feces in stored grain environment in the Czech Republic. *J. Pl. Dis. Prot.*, **112**:98-102.
- VARADINOVA, Z., FRYNTA, D., AULICKY, R. AND STEJSKAL, V., 2015. Detection of cockroach faeces: consumption of fluorescent bait and production of UV-light-detectable faeces from German cockroach, *Blattella germanica*. *Ent. Exp. Appl.*, **155**: 167–175.
- VENABLES, W.N. AND RIPLEY, B.D., 2002. *Modern applied statistics with S*. Fourth Edition. Springer, New York.
- VOLFOVA, R., STEJSKAL, V., AULICKY, R. AND FRYNTA, D., 2011. Presence of conspecific odours enhances responses of commensal house mice (*Mus musculus*) to bait stations. *Int. J. Pest Manage.*, **57**:35-40.
- YAN, J., 2002. Geepack: Yet another package for generalized estimating equations. *r-News*, **2**:3:12-14.

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