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Monitoring of *Rattus norvegicus* based on non-toxic bait containing encapsulated fluorescent dye: Laboratory and semi-field validation study

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ABSTRACT

We tested the applicability of fluorescent non-toxic bait to the wild Norway rat (*Rattus norvegicus*). We described the temporal dynamics of the production of fluorescent faeces after fluorescent bait consumption in the laboratory and, subsequently, tested the applicability of this monitoring method in a wild rat colony in an outdoor enclosure. In the laboratory experiment, no effect of animal sex on total faeces production was found ($P > 0.05$). The first fluorescent faeces were detected after 6–7 h; production peaked from 9 to 15 h; and the last detectable faeces were observed 26–27 h after bait administration. The proportion of highly fluorescent faeces to total produced faeces increased to over 80% during the peak period. In the semi-field experiment, 40% of collected faeces in the enclosure were fluorescent and 78.6% of the fluorescent faeces were deposited on the surface on the floor, i.e., only small proportion of fluorescent faeces were found hidden in the shelters, thereby no for monitoring purposes. Both laboratory and semi-field studies indicated a good potential (e.g., palatability and high production of fluorescent faeces per 24 h and their dispersion out of the hidden places) of the use of fluorescent bait for monitoring *R. norvegicus* in rodent control practices.

1. Introduction

Rodents have enormous potential to cause multiple types of damage by feeding on crops and commodities and through transmission of pathogens. Environmental contamination by rodent faeces (Stejskal and Aulicky, 2014; Aulicky et al., 2015) constitutes a risk because droppings may contain medically and veterinarily important bacteria, parasites, toxigenic fungi and allergens (Hollander et al., 1997; Wildey, 2002; Stejskal et al., 2005; Meerburg and Kijlstra, 2007). Wildey (2002) documented that rodents infested about 70% of the grain stores in the UK irrespective of type showing that this group of pests belongs among the most persistent stored product pests. In Europe there are three main rodents species occurring stores and food premises (Klosterman and Mager, 2014; Stejskal et al., 2015). The ranking of pest rodents according to their world-wide importance proposed by Capizzi et al. (2014) placed the Norway rat (*Rattus norvegicus* Berk.) species together with the house mouse (*Mus musculus* L.) second after the black rat (*Rattus rattus* L.). Because of the significance of *R. norvegicus*, this species must be systematically controlled using an integrated pest control (IPM) approach. However, efficient control of *R. norvegicus* is becoming increasingly difficult because of the physiological (e.g., Endepols et al., 2011; Buckle et al., 2013; Esther et al., 2014) and behavioural resistance (Brunton et al., 1993; Buckle and Prescott, 2011) of this species to toxic anticoagulant baits and because of the continuing trend of limiting various active ingredients and formulations of toxic baits in the EU.

Traditionally, in the grain stores, food industry premises and urban sewers, toxic baits have been used for both rodent control and monitoring (e.g., see Patergnani et al., 2010; Mughini et al., 2012). The rate of bait consumption indicates population trends or the efficacy of the treatment. However, the indoor use of toxic baits has now been completely prohibited in the food industry or restricted to occasional and temporally/spatially limited use

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(Klosterman and Mager, 2014). In these restricted situations the only remaining available chemical control is a permanent outdoor barrier-toxic-baiting. However, even the strategy of outdoor barrier toxic baiting is now also under legislation pressure due to the risk of secondary intoxication of wildlife animals in the surrounding environment (e.g., Newton et al., 1990). These new demands inevitably have triggered wider usage of either mechanical traps, tracking dusts or non-toxic baits for permanent monitoring.

Fluorescent capsules consist of a layer of a polymer shell enclosing one or more fluorescent materials such as fluorescent microspheres, while the technology of encapsulation of fluorescent particles was patented in 1999. This new technology has been quickly adopted by medicine and pharmacy. Connected with this trend, a relatively new sub-group of non-toxic dusts and baits, based on fluorescent UV (black light)-visible dye has emerged on the market recently in USA and Europe. After the ingestion of these baits, encapsulation protects dye during its passage through the digestive tract and rodents produce coloured and UV-luminescent faeces that are easily detectable from a distance of 5 m under dark conditions (Frynta et al., 2012). Their other advantage is that there is no false positivity concerning the presence of faeces in dirty conditions where small particles may falsely mimic rodent or cockroach faeces (Stejskal, 1997; Frynta et al., 2012; Varadinova et al., 2015). Because there is no requirement regarding the registration of these products in most EU countries (biocide – Regulation (EU) No 528/2012, or plant protection products – Regulation (EC) No 1107/2009), the information on their use labels is generally very essential. Furthermore, as these formulations are relatively new, there has been very little material to support their use published by scientists or technical experts. In fact, there are few published reports regarding either experience in pest control practice (e.g., Corrigan, 2010) or scientific information on physiological (e.g., defecation rates; Frynta et al., 2012) or behavioural characteristics of rodents, or descriptions of the distribution of rodent faeces after bait ingestion in their home range (e.g., Stejskal and Aulicky, 2014).

Therefore, in this paper, we explored the applicability of fluorescent non-toxic bait to the Norway rat, *R. norvegicus*. Our aims were to (1) describe the temporal dynamics of the production of fluorescent faeces after fluorescent bait consumption in the laboratory; and (2) examine using of the fluorescent bait in a wild rat population under semi-field conditions, simulated using a large outdoor arena with shelters.

2. Material and methods

2.1. Experimental animals

The wild founders of the experimental colony were six Norway rats (*Rattus norvegicus*) trapped in agricultural facilities in the Central Bohemian region (Czech Republic). The animals were kept in quarantine for at least three months before being transferred to an outdoor enclosure that simulated the natural conditions of wild rat populations. The experimental rats belonged to the second or third generation born in the enclosure.

The enclosure consisted of an oval arena (ellipse 4.5 m × 4 m), provided with a concrete and stony floor, concrete and sheet metal wall (2.5 m high), water container and food pot (Fig. 1a, b). Four horizontal tunnels (20 cm in diameter, 30 cm long) from the arena into the concrete wall were provided with nest sites (17 × 17 cm) that allowed the experimenter to inspect the nest (Fig. 1b). Food (Ssniff, Germany) and water were provided ad libitum. The arena allowed free movement, reproduction and social interaction among the rats. With the exception of the experimental protocol and the necessary reduction of population numbers to fulfil welfare requirements, the catching and marking of animals born in the arena was avoided to prevent disturbance and/or possible habituation. This rule was strictly applied for four months prior to semi-field experiment.

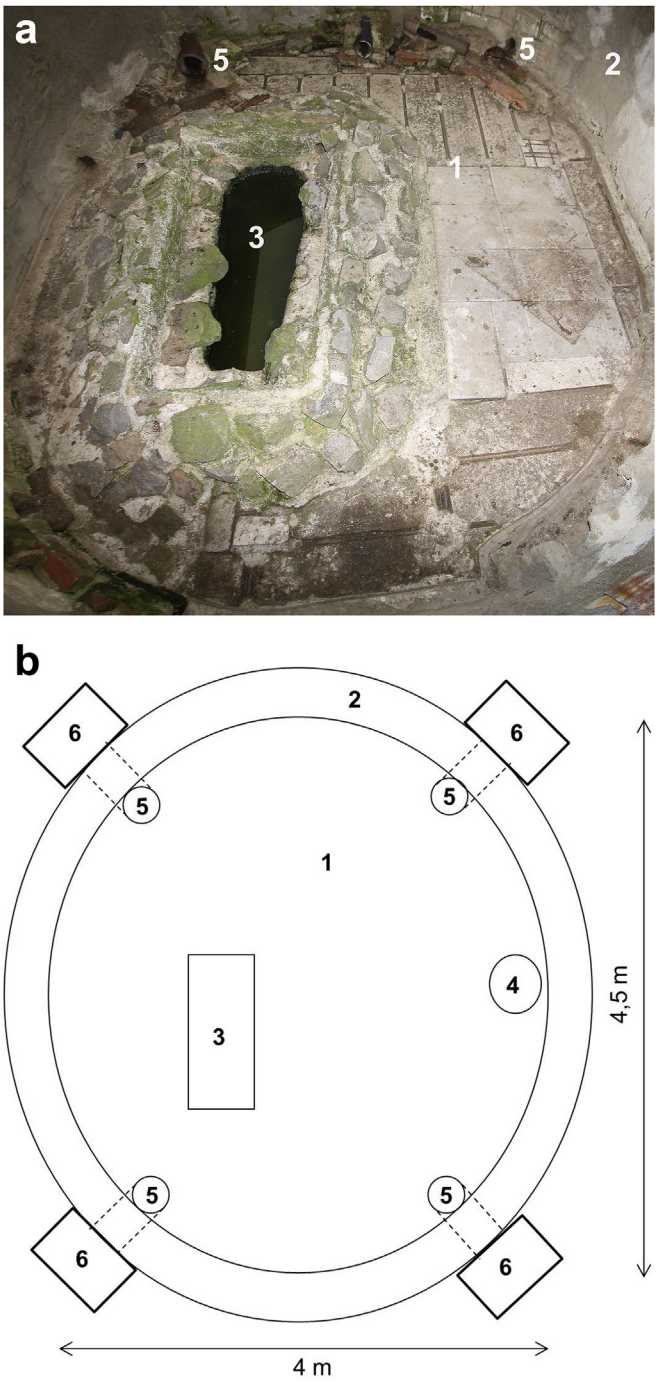

Fig. 1. The detailed view of the arena (a) bird's eye view; (b) schematic outline of the arena. 1 – concrete and stony floor (ellipse 4.5 m × 4 m), 2 – concrete and sheet metal wall (2.5 m high), 3 – water container, 4 – foot pot, 5 – four horizontal tunnels to nests, 6 – hidden nest sites at the end of horizontal tunnels (accessible for control from the outside of the enclosure).

2.2. Laboratory experiment

The adult rats were transported and housed solitarily (22 males and 11 females) in the wire mesh cages (20 × 30 × 24 cm) with free