

Evaluation of indoxacarb gel bait performance after long-term aging on *Ctenolepisma longicaudatum* (Escherich, 1905) (Zygentoma, Lepismatidae)

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Ctenolepisma longicaudatum (Escherich, 1905) (Zygentoma, Lepismatidae) is an indoor nuisance and a potential threat to cultural heritage objects. The sustained effect of insecticidal bait application was investigated by collecting old and potentially degraded bait from previously conducted pest control experiments. The collected bait was compared with fresh bait in no-choice laboratory tests. The different insecticidal bait treatments decimated *C. longicaudatum* populations significantly with no difference between 43-month-old and fresh bait. Across all different levels of degradation 79–97% of the individuals died within 18 days. The study documents a sustained effect of bait and should direct pest control technicians towards a single bait application when managing *C. longicaudatum* populations. This will reduce the amount of bait used and the number of man-hours needed to decimate or eradicate a local population. The knowledge generated in this study may therefore directly contribute to a reduction of human exposure to harmful pesticidal substances in the indoor environment.

Key words: Zygentoma, Lepismatidae, *Ctenolepisma longicaudatum*, Long-tailed silverfish, insecticidal bait, pest control, IPM (Integrated Pest Management).

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Introduction

Ctenolepisma longicaudatum (Escherich, 1905), (Zygentoma, Lepismatidae), also known as the giant-, gray- or long-tailed silverfish, has recently invaded Europe and become a common and unwanted pest in European households, public buildings, museums, libraries and archives (Kulma et al. 2021, Sammet et al. 2021). They are an indoor nuisance that can be encountered throughout the infested building (Aak et al. 2019, Aak et al. 2021), and they are considered a major destructive pest when they chew and defecate on irreplaceable and valuable items in historic collections (Brimblecombe et al. 2021, Querner & Sterflinger 2021, Querner et al. 2022a, Querner et al. 2022b, Rukke et al. 2023).

Insecticidal bait with indoxacarb as the active ingredient is effective against *C. longicaudatum* through both primary (ingestion of bait) and secondary poisoning (ingestion of dead conspecifics), and population decimation is achieved through strategic placement of a limited amount of insecticidal bait droplets (Aak et al. 2020a, Aak et al. 2020b, Rukke et al. 2021, Rukke et al. 2023). Its use against *C. longicaudatum* has partly been investigated for field durability, and sustained toxic effects after 6 months of degradation in indoor environments is known (Aak et al. 2020b). The biology of *C. longicaudatum* encompasses a slow development with a lifecycle spanning 2–3 years, mainly nocturnal activity, utilization of cracks, crevices or small hidden harborages, and a high dispersal potential (Aak et al. 2020a, Aak et al. 2021). This implies the possibility of relapsing populations and failing control in the long run. Several experiments conducted in various indoor environments have shown that a time frame of 2–3 months is required to obtain 90% population reduction after application of poisoned bait. Following the first major population reduction a few months of minor activity, mainly by surviving nymphs, should be expected (Aak et al. 2020a, Rukke et al. 2021, Rukke et al. 2023). The uncertainty of residual bait effects and total long-term eradication success may promote repeated applications of bait. This will consequently increase the amount of insecticidal bait at the indoor localities and elevate the risk of human exposure to toxins.

The present study is a direct follow-up study of our previous investigations revealing bait as a safe and efficient indoor control method. It is conducted with the same laboratory methods, but we investigate a much longer period of aging/degradation of the field applicated droplets of bait. We describe the survival of *C. longicaudatum* populations feeding on aged bait and compare the effect to control populations and population feeding on fresh bait. The results are discussed in relation to application strategies, potential excessive use of bait and general safety aspects related to the use of toxins indoors.

Materials and methods

Advion cockroach baits (0.6% indoxacarb; Syngenta, Basel, Switzerland, approved for control of *C. longicaudatum* in Norway (approval # NO-2015-0103)) of different degrading states were investigated for sustained efficacy. To assess the effect of old, hardened and degraded bait two locations described in previous control studies (Aak et al. 2020a, Rukke et al. 2021) were revisited, and some of the bait droplets deposited 30 and 43 months prior to this study were collected. These small droplets of bait, strategically applied alongside and partially underneath skirtings, were collected with tweezers and a scalpel, and stored in a sealed collection vial until the experiments. Additionally, to assess the effect of two different degrading states of tubed bait, a newly purchased bait tube and a used bait tube were used. The used bait tube was from the study 30 months ago and had been stored at room temperature in its original tube. Experimental *C. longicaudatum* were laboratory reared in stock cultures established from more than 40 individuals from each of four localities in the Oslo area in 2016 and 2017. Four different no-choice treatments with *C. longicaudatum* feeding on the different bait droplets without competing food sources were set up (Table 1). The survival of the different treatments was compared to each other, and to control animals without access to food. The setup of the survival experiments were an exact replica of the tests described in (Aak et al. 2020b). In brief, the test arena (14.5 cm x 27.0 cm) supplied 32 individuals (16 adults and 16 juveniles) of *C. longicaudatum* with hiding places, access to water and cotton balls for egg deposition. The arenas were maintained at 24°C and 60% relative humidity. The collected old bait droplets and equivalent droplets of tubed bait were introduced into the respective arenas, and survival of *C. longicaudatum* was checked daily for a total of 18 days. Differences in survival were assessed using Kaplan-Meier survival analyses with a subsequent Holm-Sidak test for multiple comparison. The Holm-Sidak test isolates the group or groups that differ from the others using multiple comparison with Bonferroni correction. Surviving individuals were checked for abnormal behavior (not hiding from light, not responding to touch, or showing erratic movement of limbs) at the end of the experiment.

Results and discussion

All the different degrading states of bait tested strongly reduced the survival compared to the control (Kaplan–Meier; only least significant test shown, $\chi^2 = 44.00$, df = 1, $p < 0.001$, Figure 1). The study therefore shows a long-lasting effect of applied bait droplets and indicates that a single application of bait may contribute to the management of *C. longicaudatum* populations for as much as 4 years. Additionally, there was no significant difference between 30 months of degradation of baits in apartments compared to baits stored for the same time at room temperature in its original tube (Kaplan–Meier; $\chi^2 = 0.22$, df = 1, $p = 0.869$). This indicates that the palatability of the dried and hardened bait is also a good and comparable alternative to the soft gel in a no-choice situation. Potential effects from differences in palatability could have been revealed by presenting fresh and hard bait in a choice experiment, but *C. longicaudatum* appears to willingly consume sugary baits in both dry and moist form (Figure 1). Previous studies have observed little to no difference in the progress of the survival curves when freshly applied bait and bait dried for 4 weeks is presented in competition with natural food (Aak et al. 2020b). The direct comparison between apartment-applicated (30 months) and tubed bait (30 months) also indicates that the indoor environment shelters the insecticidal bait from chemical degradation even if physical changes occur when moisture evaporates. In terms of final mortality, the fresh and newly purchased bait yielded an intermediate mortality of 94% compared to both 43 months of apartment degradation (79% mortality) and 30 months of apartment degradation (97 % mortality), and there was no significant difference in survival between the fresh bait and the oldest bait (Kaplan–Meier; $\chi^2 = 4.51$, df = 1, $p = 0.186$) or the bait degraded for 30 months (Kaplan–Meier; $\chi^2 = 0.68$, df = 1, $p = 0.793$).

The minor differences in final mortality between fresh and old bait is mostly of academic interest. The lifecycle of *C. longicaudatum* stretches over 2 years at room-temperature (Lindsay 1940, Aak et al. 2019), and major effects from the control measures occur during the initial 2–3 months after application of insecticidal bait (Aak et al. 2020a, Rukke et al. 2021). An additional consideration of a potentially sustained field efficiency is the more detailed observations of the 12 survivors found among the initial 128 individuals exposed to insecticidal bait in the present study (Table 2). Eleven of these survivors had molted during the 18 days of testing (identifiable by cuticula completely covered by new and dark scales). *Ctenolepisma longicaudatum* cease feeding for several weeks prior to molting (Lindsay 1940), and the survivors of the experiments were therefore incapable of consuming the bait until after molting had

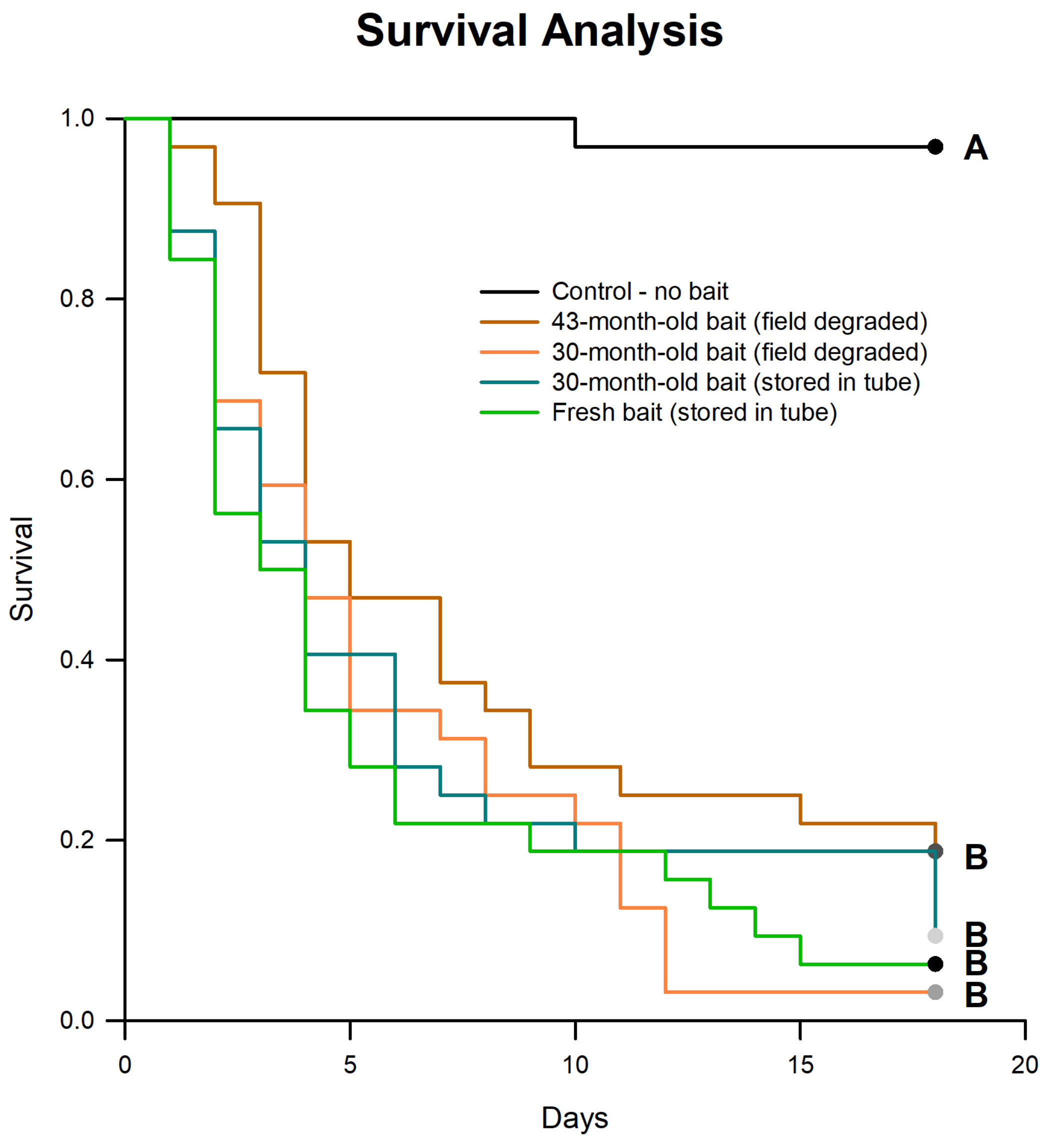

FIGURE 1. Survival of *Ctenolepisma longicaudatum* (Escherich, 1905) in population boxes with 16 adults and 16 juveniles after exposure to different types of Advion cockroach poisoned baits. Different capital letters (A and B) next to lines indicate significant differences in survival between the treatments (Kaplan-Meier survival analyses, Holm-Sidak test, $p < 0.05$).

occurred. Several instances of molting during the experiment were observed, and 9 of the 11 freshly molted individuals were acting abnormally at the end of the experiment (Table 2). The abnormal behavior is likely an effect of consumption of insecticidal bait after molting because none of the control animals showed this behavior. This means that as much as 98% of the individuals tested were negatively influenced by the bait and probably would have died if given additional time in the experiments. Also, many individuals were observed feeding on their dead conspecifics. This confounds the results slightly as secondary poisoning is an important aspect of the bait

TABLE 1. Type of Advion cockroach bait presented to *Ctenolepisma longicaudatum* populations of 32 individuals.

Bait supply	Degradation time	Consistency
Private home	43 months	Hard
Apartment building	30 months	Hard
Old bait tube	30 months	Soft
New bait tube	< 1 month	Soft

TABLE 2. Survival of *Ctenolepisma longicaudatum* (Escherich, 1905) exposed to different types of Advion cockroach poisoned baits, and the moulting status and behaviour of the survivors at the end of the experiment on day 18. The data in this table is pooled across treatments (n = number of individuals) because there was no statistical difference in the mortality caused by the different treatments.

	n	%	Total
Dead	116	91%	
Survivors	12	9%	128
	Intact scales	**Lost scales**	**%**
Survivors	11	1	
Abnormal behaviour*	9	0	75%
Healthy	2	1	25%

* Not hiding from light, not responding to touch, or showing erratic movement of limbs.

effect (Aak et al. 2020b). If secondary poisoning occurred also after as much as 43 months of field degradation this should be considered a benefit for the practical application and the long-term control effects. Secondary poisoning might rejuvenate the bait after years of desiccation, because dead individuals may represent a food source of better nutritional quality and palatability than the hardened and dried bait.

The findings are important for the practical use of bait against *C. longicaudatum* because this shows that multiple applications may be redundant. A single bait application will strongly reduce the number of man-hours needed to decimate or eradicate a local population. In some cases, the bait may have been completely consumed or removed through cleaning routines. Although secondary poisoning then should supply continued effect inside hidden harborages with dead individuals (Aak et al. 2020b), repeated application can be advocated in these cases. If baits are placed in protected cracks and crevices, the locations *C. longicaudatum* use for hiding during daytime, a single application should suffice due to the identified sustained effect of the bait.

Different insecticidal baits are in general considered a low-risk method for use in indoor environments (Dhang 2014, Wang et al. 2019) because control is obtained with minor amounts of baits containing even less of the active ingredient (typically in the range from 0.1-3%) that is directly consumed by the pest species. Even with the low risk of a bait formulation, pesticides should, in general, be avoided as much as possible in an indoor environment (Bonnefoy et al. 2008, Dhang 2014, Rani et al. 2021). Fortunately, only diminutive amounts (bait: 1 g/100 m^2, active ingredient: 0.006 g/100 m^2) of widely distributed small bait droplets (10 mg) are needed for efficient control of *C. longicaudatum* (Aak et al. 2020a, Rukke et al. 2021, Rukke et al. 2023). The strategy used is therefore quite different compared to bait strategies used against cockroaches and ants, where larger amounts often are needed, and placement of bait is more localized and directed towards aggregations or ant trails (Oi et al. 2000, Ko et al. 2016, Rabito et al. 2017). Additionally, infestations of cockroaches, ants, and silverfish may overlap or co-exist and consequently require different application volumes and strategies for full effect in multi-species infestations. The producers of bait often recommend repeated applications of bait when used against other pest species, and the experience with the control of *C. longicaudatum* in Norway indicates that some pest control technicians do not easily trust the effects of small amounts of bait and therefore may apply too large amounts repeatedly (personal observations by Aak and Rukke). This study clearly shows the sustained toxic effect of the applied bait and should direct pest control technicians towards a single application even if the effect of the insecticidal bait may manifest slowly and minor activity may persist in the building for some time (Aak et al. 2020a, Rukke et al. 2021, Rukke et al. 2023).

Recently there has been an increase in infestations of *C. longicaudatum* in several European and Asian countries (Goddard et al. 2016, Kulma et al. 2018, Schoelitsz et al. 2019, Thomsen et al. 2019, Kulma et al. 2021, Sammet et al. 2021, Zhang et al. 2021, Bednár et al. 2024), and the two additional silverfish species *Ctenolepisma calvum* and *Ctenolepisma lineatum* have also been shown to thrive indoors (Molero-Baltanas et al. 2012, Hage et al. 2020, Aak et al. 2021, Kulma et al. 2022, Querner et al. 2022b, Shimada et al. 2022, Watanabe et al. 2023, Bednár et al. 2024). This silverfish nuisance can be expected to maintain its indoor populations in the years to come, and to safeguard the indoor environment, only necessary amounts of pesticides in as protected places as possible must be applied. In combination with previous control studies (Aak et al. 2020a, Aak et al. 2020b, Rukke et al. 2021, Rukke et al. 2023), this study highlights a very limited requirement of insecticidal bait to combat *C. longicaudatum* populations. Compared to traditional indoor pesticide applications, the knowledge generated in this study can contribute to a societal benefit by reduction of exposure to harmful pesticidal substances in the indoor environment.

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