College of Agriculture, Food and Environment
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FINAL REPORT

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Project Title: The perfect meal: Determining how aging effects cockroach gel bait performance

Principal Investigator:

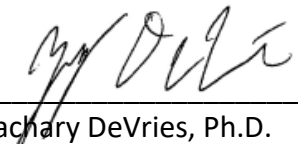
Dr. Zachary DeVries, Ph.D.
Assistant Professor
Department of Entomology, University of Kentucky
S-225 Agricultural Science Center North
1100 S. Limestone
Lexington, KY 40546-0091
Phone: 859-562-2856
Email: zdevries@uky.edu
Web: https://devrieslab.weebly.com/

Study Sponsor:

The Pest Management Foundation
Attn: Dr. Jim Fredericks, Ph.D.
10460 North Street
Fairfax, VA 22030
Phone: 703-887-1087
Email: jfredericks@pestworld.org

Study Personnel:

Zachary DeVries (Assistant Professor, Dept. of Entomology, University of Kentucky), Sudip Gaire (Postdoctoral Research Scholar, Dept. of Entomology, University of Kentucky), and Isabelle Lucero (Graduate Research Assistant, Dept. of Entomology, University of Kentucky)



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Dr. Zachary DeVries, Ph.D.
Assistant Professor of Urban Entomology
University of Kentucky

Date

1. Executive Summary

German cockroaches, *Blattella germanica* (L.), remain one of the most dangerous and difficult urban pest species to manage. This is due in large part to the extensive use of residual sprays containing pyrethroids, to which German cockroaches are highly resistant. Baits, however, remain effective and are capable of eradicating cockroach infestations from structures independent of other management strategies. That said, there are still many questions surrounding baits, particularly related to their efficacy over time after application. To address these questions and improve baiting efficacy, we evaluated bait performance over time, looking specifically at the effects of aging and relative humidity on bait consumption and effectiveness. Our results indicated that most baits remain both palatable and effective for at least 3-6 months after application, although there are some differences among baits. Further, aging at a low humidity had little effect on bait consumption and efficacy, while aging at a high humidity resulted in no consumption of baits due to mold development on the baits. These results should provide pest management professionals (PMPs) with confidence that baits will remain effective long after application, potentially saving them both time and money.

2. Introduction

The German cockroach, *Blattella germanica* (L.), is a worldwide pest found indoors in close association with humans. German cockroaches remain one of the most important urban pest species, largely due to the negative effects they have on human health. Specifically, German cockroaches produce a suite of potent allergens known to induce asthma (Gore and Schal 2007), and they harbor and can transmit several pathogenic microorganisms (Brenner 1995, Schal and DeVries in press). Despite years of research, PMPs still rank German cockroaches as one of the most difficult species to manage (PCT 2016).

The challenges associated with German cockroach control are tightly linked with the high use and low efficacy of residual sprays (Miller and Meek 2004, Nalyanya et al. 2009). Indoor residual sprays labeled for cockroaches are primarily formulated with pyrethroids as the active ingredient. However, German cockroaches from across the world have been repeatedly shown to be highly resistant to pyrethroids (Cochran 1989, Atkinson et al. 1991, Wei et al. 2001, Chai and Lee 2010, Fardisi et al. 2017, Wu and Appel 2017, DeVries et al. 2019b). Knowing that cockroaches are often resistant to residual sprays, PMPs are often forced to turn to alternative control methods.

Cockroach baits are one such strategy which have gained popularity over the past three decades. Baits offer several distinct advantages over residual sprays beyond proven efficacy (Miller and Meek 2004, Sever et al. 2007). First, baits are easy to use and require little to no training to apply correctly. This is not to say bait placement is always optimal, but rather the use of baits is rather intuitive and the label instructions have a tendency of being simple when compared to residual sprays. Baits also only require a small amount of active ingredient (AI) to be placed into the environment, with applications made in targeted areas where cockroaches are present (or are likely to be present). These areas also are generally concealed and inaccessible to children and pets, reducing unnecessary pesticide exposure. When we consider the AIs used in baits, there are more options available for baiting than for residual sprays. At last count, there were at least six different modes of action (MOAs) available for baits, giving PMPs considerably

more options than residual sprays. Furthermore, the AIs used in baits have lower documented levels of resistance when compared to the AIs used in residual sprays (DeVries et al. 2019b). This doesn't mean that baits are always effective, but it does indicate that resistance is less likely to be a problem for baits given the slow development of resistance in areas with bait use (as evident by area-wide surveys; Chai and Lee 2010, Wu and Appel 2017) and the ability to quickly switch to products with alternative MOAs. Lastly, baits can result in secondary kill (Buczkowski et al. 2001). This phenomenon effectively extends the range of baits to all aggregation sites, and has been heavily implemented in baiting success, although never directly shown in the field.

Cockroach baits are generally assumed to remain effective long after application, although few studies have evaluated this. Both Nalyanya et al. (2001) and Appel (2003) found different baits remained attractive and effective up to seven days, but efficacy beyond this time remains unknown. Because many accounts are maintained on quarterly service agreements, it is important that these products provide efficacy for periods longer than 1 week. In addition, if baits remain effective for extended periods of time they could also be used as part of a proactive management strategy in locations with a high probability of becoming infested (e.g. low-income multi-family housing) (Zha et al. 2018, Wang et al. 2019). Furthermore, aging is not only a factor of time but of environmental conditions. When applied to locations with cockroaches, baits are often placed in humid environments (e.g., behind the fridge, under the sink). While designed with preservatives to improve stability, it remains unclear how well baits perform after aging in humid environments.

Clearly, baits offer numerous benefits over residual sprays, most important being their proven efficacy in the field (Sever et al. 2007, DeVries et al. 2019a, Miller and Smith 2020). That said, their ability to remain effective over time is unknown. Therefore, we evaluated the effects of aging on bait consumption and effectiveness.

3. Project Goals and Study Objectives

The overall goal of this project was to improve bait efficacy by better understand how aging and humidity impact bait performance. Specifically, we aimed to define how long baits are effective after placement and thus how often baiting is required to ensure continuous effective cockroach control. To this end, this study incorporated the following objectives:

1. Determine the effects of aging on gel bait consumption and effectiveness.
2. Determine the effects of aging in environments with different relative humidity on gel bait consumption and effectiveness.

4. Materials and Methods

German cockroach populations and rearing: Three German cockroach populations (one susceptible and two recently apartment collected) were used for this project. The insecticide-susceptible population (Orlando Normal) has been maintained in the laboratory for more than 70 years and is known to be susceptible to all insecticides. The apartment collected populations were originally collected in 2018 (VS101) and 2019 (CC29) from two separate low-income apartment complexes in Raleigh, NC, and have been maintained in the laboratory since collection. German cockroach populations were reared under standard laboratory conditions

(temperature: 25°C, 40% relative humidity (RH), and 12:12 h (L:D)). Only adult males were used for all experiments.

Bait products and aging: Four gel bait products commonly used by pest management professionals were used for all experiments: Vendetta Plus (AIs: abamectin B1 0.05%, pyriproxyfen 0.50%; MGK, Minneapolis, MN), Maxforce FC Magnum (AI: fipronil 0.05%; Envu, Cary, NC), Advion (AI: Indoxacarb 0.6%; Syngenta, Basel, Switzerland), and Alpine Rotation 2 (AI: Dinotefuran 0.5%; BASF, Ludwigshafen, Germany). These products were applied into plastic dishes at approximately 0.5g/dish, then aged at 25°C and various humidities (15%, 40%, 80% RH) for the following times: 24 hours (fresh), 1 month, 3 months, and 6 months. Lower amounts were used for the fresh (24h) aging experiments to ensure accurate measurements with the high level of water loss. It should be noted that not all time-humidity combinations were tested for each population (indicated in the figures).

Bait consumptions and effectiveness: Consumption and efficacy were evaluated using two-choice bioassays (Fig. 1). The arena measured as follows: l = 20cm, w = 15cm, h = 10cm. The sidewalls of the containers were greased with mineral oil to prevent escape. One side of the arena contained both water and harborage and the other side of the container contained rat chow and the treatment (bait of varying ages). Adult male German cockroaches (n = 20 per arena) were introduced into the arena and allowed 48h to acclimate: 24h with food and water, followed by 24h with only water (no food). This was done to standardize feeding motivation among all cockroaches tested. Running in parallel, baits were allowed 24h to acclimate to minimize fluctuations in water content. After acclimation, aged bait treatments were introduced into the arena. Controls with no bait (only rat chow) were also run. Both the aged bait and rat chow were weighed prior to roach introduction, then daily for 7 d after the experimental start date. An equal number of environmental controls were also run to account for water loss/gain of the baits and rat chow. Mortality was recorded daily for 7 d, with dead cockroaches removed from the arena. In total, five replications were completed for each experimental unit (bait product and age). These assays were performed using all cockroach populations. All weights were corrected using the environmental control data. These ensured we properly calculated the amount of each product consumed by the cockroaches.

Fig. 1 – Feeding arena for consumptions and effectiveness bioassays.

Data Analysis: The total amount of bait consumed after 24h was compared among groups using analysis of variance (ANOVA; SAS 9.4, SAS Institute, Cary, NC). Mortality was analyzed by calculating the average percent mortality after 7 days then comparing among groups using analysis of variance (ANOVA; SAS 9.4). Significant differences among bait ages were determined

using the Tukey-Kramer multiple comparison test. A Student's T-test was used to compare bait consumption at 6 months between 15% and 40% RH for each product tested.

5. Results

Effects of aging on gel bait consumption and efficacy: Gel bait age had a significant effect on consumption for all German cockroach populations tested (Table 1, Fig. 2-4). However, despite some significant effects, German cockroaches still consumed gel bait after 3 month (apartment-collected) or 6 month (laboratory insecticide-susceptible population) of aging. It should also be noted that only one substantial (>50%) decline in consumption was observed (ON, Alpine Rotation 2; 24h hour = 27.2mg[±1.9], 6 month = 5.4mg[±0.7]).

Table 1. Results from ANOVAs performed on bait consumption for all bait aging experiments for each population

Population	Bait	*df*	*F*	*P*
ON	Advion	3,16	25.36	<0.001
	Alpine Rotation 2	3,16	57.51	<0.001
	Maxforce FC Magnum	3,16	3.07	0.058
	Vendetta Plus	3,16	3.83	0.031
CC29	Advion	1,8	0.49	0.506
	Alpine Rotation 2	1,8	278.76	<0.001
	Maxforce FC Magnum	1,8	3.44	0.101
	Vendetta Plus	1,8	1.68	0.231
VS101	Advion	1,8	4.88	0.058
	Alpine Rotation 2	1,8	3.27	0.108
	Maxforce FC Magnum	1,8	0.12	0.737
	Vendetta Plus	1,8	2.22	0.175

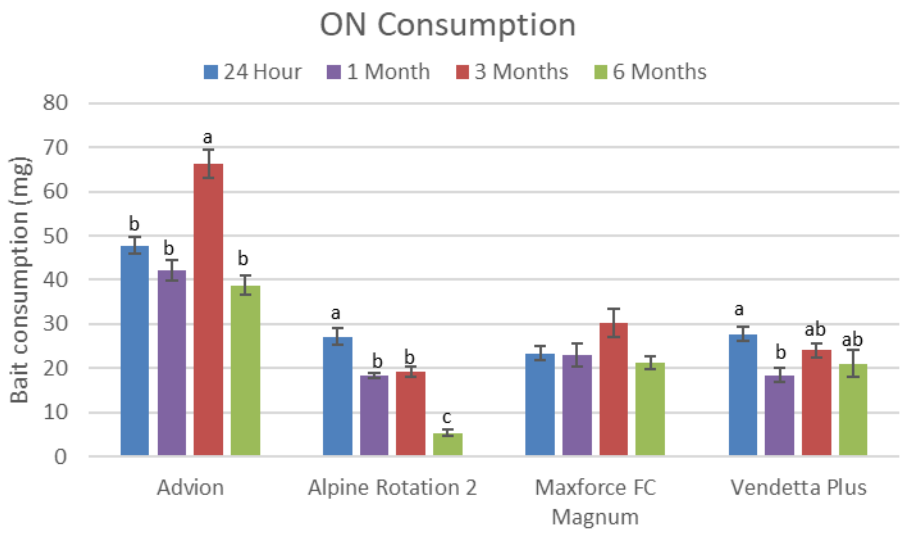

Fig. 2 Average one-day bait consumption (±SEM) by insecticide susceptible German cockroach population ON. Bars denoted with different letters indicated significant differences in consumption at different bait ages within that product (ANOVA followed by Tukey Test; $P < 0.05$).

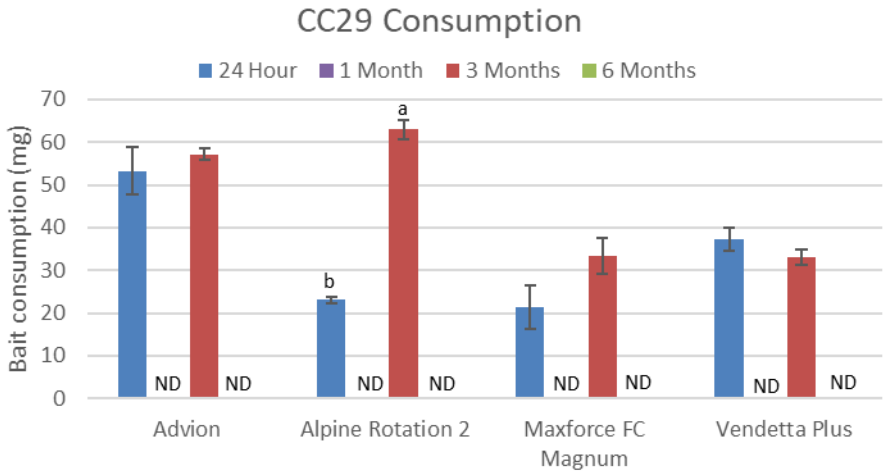

Fig. 3 - Average one-day bait consumption (±SEM) by field-collected German cockroach population CC29. Bars denoted with different letters indicated significant differences in consumption at different bait ages within that product (ANOVA followed by Tukey Test; $P < 0.05$). Bars marked at "ND" indicated no data has been collected.

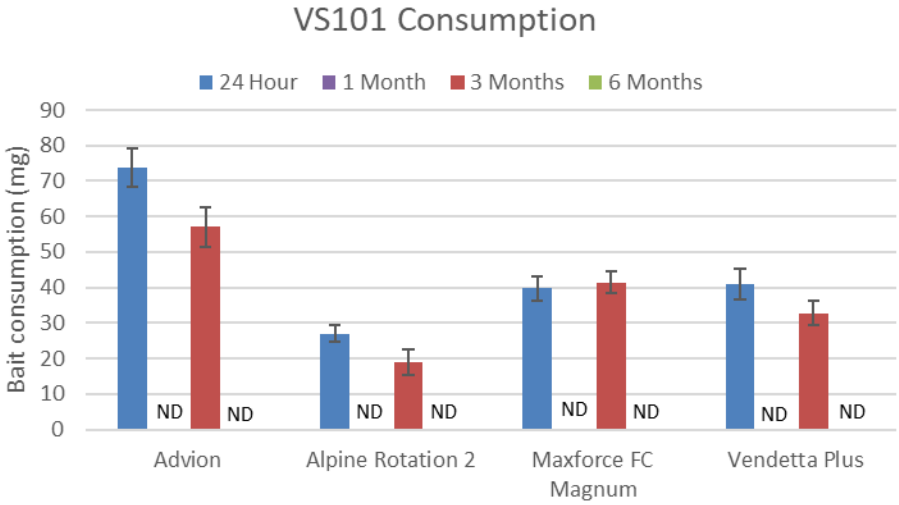

Fig. 4 - Average one-day bait consumption (±SEM) by field-collected German cockroach population VS101. No significant differences were detected in consumption among any of the different bait ages within products tested (ANOVA, $P > 0.05$). Bars marked at "ND" indicated no data has been collected.

Despite the minor effect that gel bait age had on some product consumption, efficacy remained high for all bait ages (>85% mortality for all populations; Fig. 5-7,). Further, there was only one significant age-associated mortality decline observed (CC29, Vendetta Plus; 24h hour = 97.0[±2.0], 3 month = 85.0%[±2.7]; $F_{1,8} = 12.52$, $P = 0.008$; Table 2).

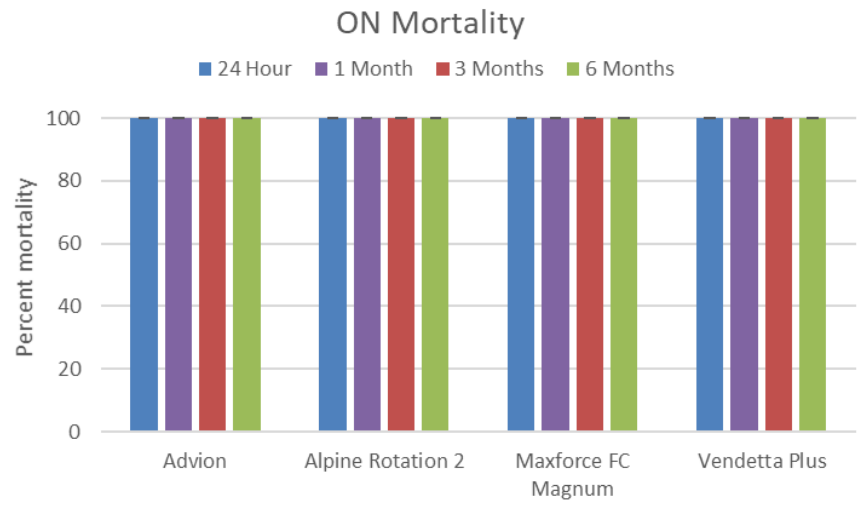

Fig. 5 - Average percent mortality (±SEM) among insecticide-susceptible German cockroach population ON. No significant differences were detected in mortality among any of the different bait ages within products tested (ANOVA, $P > 0.05$).

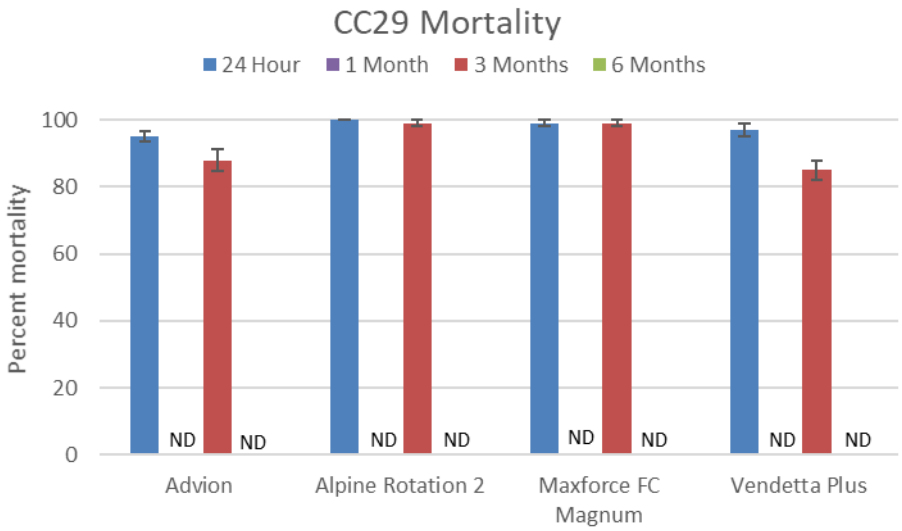

Fig. 6 - Average percent mortality (±SEM) among field-collected German cockroach population CC29. Bars denoted with different letters indicated significant differences in consumption at different bait ages within that product (ANOVA followed by Tukey Test; P < 0.05). Bars marked at "ND" indicated no data has been collected.

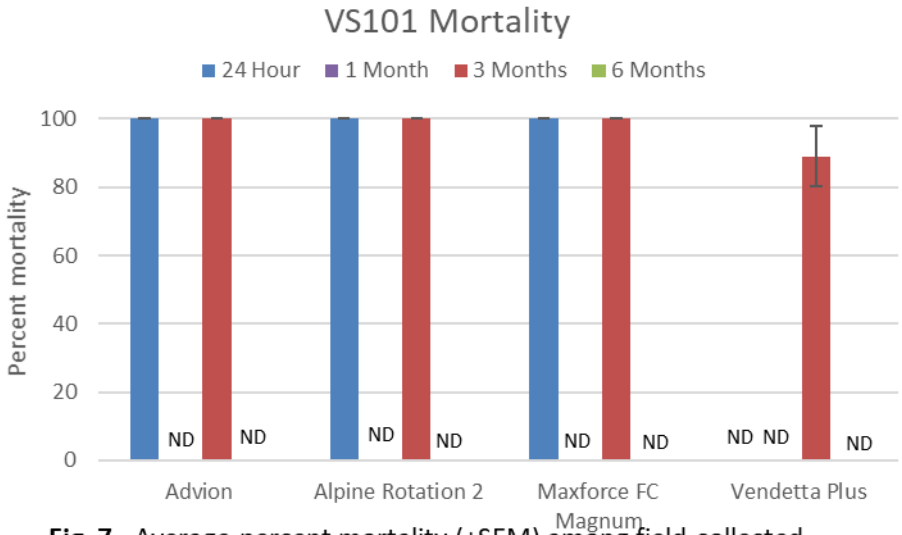

Fig. 7 - Average percent mortality (±SEM) among field-collected German cockroach population VS101. No significant differences were detected in mortality among any of the different bait ages within products tested (ANOVA, *P* > 0.05). Bars marked at "ND" indicated no data has been collected.

Table 2. Results from ANOVAs performed on percent mortality for all bait aging experiments for each population. Cells filled with a "-" indicate no variation in mortality (all reached 100% mortality). Boxes with "ND" indicated data has not been collected yet.

Population	Bait	*df*	*F*	*P*
ON	Advion	3,16	-	-
	Alpine Rotation 2	3,16	-	-
	Maxforce FC Magnum	3,16	-	-
	Vendetta Plus	3,16	-	-
CC29	Advion	1,8	3.50	0.098
	Alpine Rotation 2	1,8	1.00	0.347
	Maxforce FC Magnum	1,8	-	-
	Vendetta Plus	1,8	12.52	0.008
VS101	Advion	1,8	-	-
	Alpine Rotation 2	1,8	-	-
	Maxforce FC Magnum	1,8	-	-
	Vendetta Plus	ND	ND	ND

Effects of aging at extreme humidities on gel bait consumption and efficacy: When gel baits were aged for 6 months at low (15%) and moderate (40%) RH there were only minimal differences in bait consumption (Table 3, Fig. 8). Percent mortality was 100% for all experimental replicates, with no differences between 15% and 40% RH (Fig. 9).

Table 3. Effects of humidity on bait consumption. Results from T-tests comparing bait consumption at 15% or 40% relative humidity after 6 months of aging for all bait products tested.

Population	Bait	*df*	*T*	*P*
ON	Advion	9	1.21	0.303
	Alpine Rotation 2	9	36.39	<0.001
	Maxforce FC Magnum	9	1.59	0.243
	Vendetta Plus	9	0.38	0.555

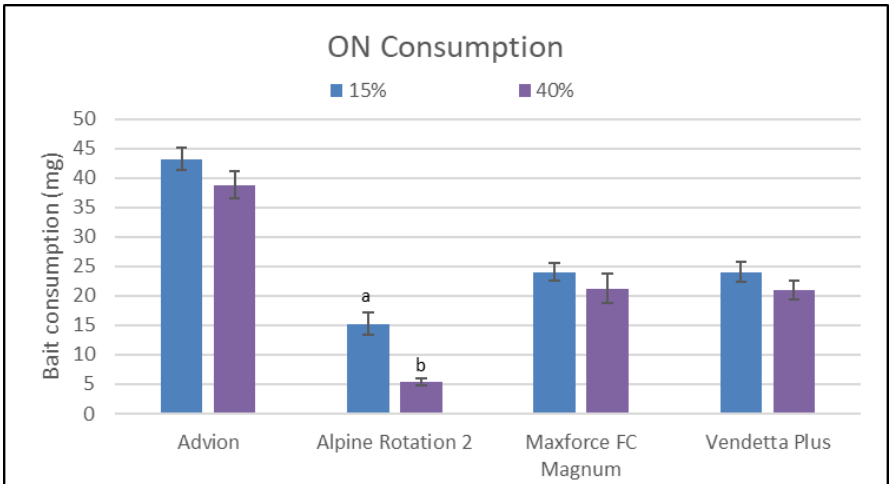

Fig. 8 - Consumption (±SEM) of baits aged at low (15%) and moderate (40%) relative humidity by insecticide susceptible German cockroach population ON . Bars denoted with different letters indicated significant differences in consumption at different bait ages within that product (ANOVA followed by Tukey Test; $P < 0.05$).

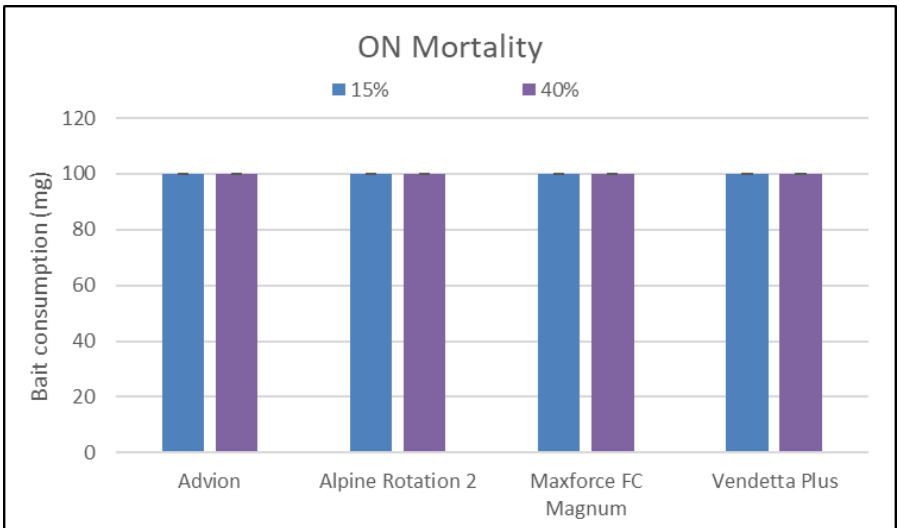

Fig. 9 - Average percent mortality (±SEM) among insecticide-susceptible German cockroach population ON. No significant differences were detected in mortality among any of the different bait ages within products tested (ANOVA, $P > 0.05$).

Interestingly, when baits were aged at 80% RH, we were unable to test them at 6 months due to mold growth (Fig. 10). We were able to identify the mold as a sugar mold in the family Mucoraceae.

Fig. 10 – Mold growth on various cockroach gel baits aged at 80% RH.

6. Discussion

All German cockroach populations tested continued to consume gel baits for at least 3-6 months after they were applied (depending on the population tested). Despite some differences in consumption of gel baits of different ages, Gel baits generally remained palatable across all ages tested. Further, gel baits caused high mortality (>85%) at all ages tested, regardless of any differences in consumption. Together, these results indicate that previously applied gel baits will continue to work long past routine service windows (3 months or less). In addition, this suggest that gel baits can potentially be applied proactively as a preventative measure in environments that are at higher risk for developing infestations. This would provide PMPs with an additional stategy to use when combatting German cockroach problems and possibly allow them to stay ahead of problems when maintaining accounts that have been cleaned out.

Interestingly, low humidity had minimal effect on gel bait consumption and no effects on efficacy (mortality). This means that cockroach gel baits can be assumed to hold up in dry/arid environments, thus providing PMPs with confidence when applying gel baits in these situations. However, in high humidity environments, all gel baits began to develop mold growths. This means that gel baits cannot be expected to hold up in high humidity environments for extended periods of time, and should be avoided in these locations. However, it remains unclear how quickly mold develops in these locations.

Despite clear evidence that gel baits remain both palatable and effective for extended time after application, it remains unclear how German cockroach attraction to gel baits is affected by aging. Attraction is a critical portion of bait efficacy, as the cockroaches have to find the bait for it to work. Future work should evaluate baits to see at what ages they elicit behavioral responses in still air arenas that simulated real-world environments.

Overall, this work provides critical information on how different gel baits will function over time in various environments against both insecticide-susceptible and apartment-collected German cockroaches. These results should provide PMPs with confidence that baits will remain effective long after application, potentially saving them both time and money and opening doors to possible new management strategies in high-pressure environments.

7. Continuing Work and Future Studies

This work is still ongoing, with several 6-month experiments for the apartment-collected German cockroaches in progress. We plan to complete this work in 2023, and subsequently incorporate the update results into both a MS student's thesis (Isabelle Lucero), trade-magazine article, and a peer-reviewed publication. In addition, we are currently performing behavioral assays to see how gel bait age influence cockroach attraction/orientation towards the bait. We plan to complete this work in 2023 and incorporate it into a publication in 2024. Finally, we would like to evaluate bait aging time points longer than 6 months, pending available time and resources.

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