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Effects of age and environmental conditions on cockroach gel bait performance

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Cockroach baits are one of the most effective tools used for German cockroach (*Blattella germanica* L.) control. This is due in part to the number of cockroach baits on the market with various matrix compositions and active ingredients, aiding in the control of insecticide-resistant cockroach populations through bait rotation. However, it remains unclear how cockroach gel baits perform over time and under different environmental conditions. Therefore, we aged six cockroach gel baits for varying times (24 h, 1 mo, 3 mo, and 6 mo) and at three relative humidities (15%, 40%, and 80%), and tested their performance (consumption and efficacy) against fresh baits. German cockroaches consumed various quantities of bait depending upon both bait product and age, with significant declines in aged bait consumption compared to fresh bait consumption for most products tested. However, all baits aged over 24 h caused greater than 93% mortality in all *B. germanica* (L.) populations. Low relative humidity (15%) did not significantly affect bait consumption or efficacy compared to standard relative humidity (40%), but high humidity (80%) resulted in excessive mold growth (*Mucoraceae*) in all trials, preventing testing of aged baits at high humidity. Our findings show that cockroach gel baits remain palatable and effective up to 6 mo after application at 40% and 15% relative humidity in laboratory conditions. This suggests that even though consumption declines in comparison to fresh baits, aged baits may play a role in cockroach control for an extended time after application.

Keywords: cockroach baiting, control, relative humidity, consumption, efficacy

Introduction

German cockroaches (*Blattella germanica* L.) are the most prolific cockroach species found worldwide and are only known to exist in human-built structures (Lee and Wang 2021, Tang et al. 2024). Besides being an unsightly presence and excreting defensive chemicals that result in unpleasant smells within homes, German cockroaches also negatively affect human health (Schal and DeVries 2021). Over 100 pathogenic organisms have been identified on/in German cockroaches, including bacteria, parasitic worms, fungi, protozoa, and viruses (Roth and Willis 1960, Schal and DeVries 2021). While epidemiological studies linking German cockroaches and diseases are largely lacking, their free movement between unsanitary areas (e.g. sewers, trash, waste) to food sources suggests they are likely responsible for unreported pathogen transmissions. German cockroaches also produce metabolites that can cause serious allergies and asthma (Schal and DeVries 2021). Human sensitivity to cockroach allergens varies among individuals, but prolonged exposure has been linked with increased sensitivity (Rosenstreich et al. 1997, Gruchalla et al. 2005). While the effects of German cockroaches on human health are numerous, a central tenet for mitigating these effects is source reduction (e.g. cockroach eradication) (Arbes et al. 2004, Schal 2011, Rabito et al. 2017).

German cockroaches have been found to be resistant to many classes of insecticides applied in homes (Chai and Lee 2010, Fardisi et al. 2017, Wu and Appel 2017, DeVries et al. 2019b, González-Morales et al. 2022, Lee et al. 2022, Gits et al. 2023). Of these insecticides, those with pyrethroid active ingredients (AIs) remain one of the few insecticide classes available for general use indoors, even though they have been repeatedly shown to be ineffective in managing German cockroach infestations (Miller and Meek 2004, Nalyanya et al. 2009, DeVries et al. 2019a, Gordon et al. 2024).

A key factor in their failure is the high levels of pyrethroid resistance documented in German cockroach populations from around the world due to selection pressure from their extensive indoor use (Cochran 1989, Wei et al. 2001, Chai and Lee 2010, Gondhalekar et al. 2011, Fardisi et al. 2017, Wu and Appel 2017, DeVries et al. 2019b, Gordon et al. 2024). Conversely, insecticidal baits have proven incredibly effective at reducing or eliminating populations (Miller and Meek 2004, Wang and Bennett 2006, Sever et al. 2007, Rabito et al. 2017, DeVries et al. 2019a, Miller and Smith 2020).

Baits are defined as an active ingredient (AI) embedded within a food matrix (Appel and Rust 2021). There are multiple classes of AIs with varying modes of action available in cockroach baits, which allows baits to be rotated and as a result decreases the likelihood of resistance to a single AI developing (Appel and Rust 2021). In addition to their ability to combat resistance, baits have several key advantages over other insecticide formulations, namely their ability to be placed in precise locations, allowing for use in sensitive environments (Appel and Rust 2021), and a lower amount of AI needed to cause mortality than residual sprays (Appel and Abd-Elghafar 1990, Schal 2011). Baits are also capable of causing secondary and even tertiary mortality if unexposed cockroaches consume the corpses or excrement from cockroaches that have already consumed baits (Buczkowski et al. 2001, 2008, Hamilton et al. 2023).

Despite clear advantages over residual broadcast sprays, baits do have some limitations that can impact their efficacy. Improper application of insecticidal baits can cause them not to work, as baits must be placed where cockroaches are aggregating or where they will find them while foraging. Many baits also take time to work (limited "knockdown" effect), take substantial time to apply correctly, and often have higher costs, which could all contribute to low adoption rates specifically in multifamily housing (Miller and Meek 2004, PCT 2022). Furthermore, it is unknown how bait age and the environmental conditions in which baits are placed affect bait performance due to water loss and texture changes. Relative humidity levels and temperature can vary widely among homes depending on season, geographical location, and microclimates within the home, but there is limited knowledge on how these relative humidity levels affect bait performance after application (Nguyen and Dockery 2016). Additionally, there has been very little research done on bait performance with time, with most bait performance papers looking at fresh bait efficacy (Appel 1992, Ajjan and Zhang 1997, Nalyanya et al. 2001, Oz et al. 2010). Given the high upfront costs of bait-centered integrated pest management (IPM) programs compared to those that only use sprays (Miller and Meek 2004), it is critical that we understand the factors that influence gel bait efficacy so we can ensure these practices have maximal efficacy and minimal costs.

To better understand gel bait performance when tested against German cockroaches, we aged six gel baits for up to 6 mo and in three different relative humidities and evaluated palatability and effectiveness (ability to cause mortality) against one insecticide susceptible German cockroach population and two field-collected German cockroach populations with insecticide-resistant traits. We used two-choice consumption assays in all experiments to give the German cockroaches an alternative food source to the insecticidal bait as they would have in the field. The advantages and limitations of gel baits for use in cockroach IPM programs in terms of bait age after application, relative humidity, and against resistant populations are discussed.

Materials and Methods

German Cockroach Populations

All *B. germanica* cockroaches used for this study were reared at the University of Kentucky in plastic jars (3.8L [128 oz], Uline, Pleasant Prairie, Wisconsin, USA) with corrugated cardboard harborages (Uline) in a temperature-controlled room kept at 23 to 25 °C and 30% to 50% relative humidity. The room was maintained on a 12:12 L:D cycle. Each population was provided water and rat chow (Mazuri Rat and Mouse Diet, PMI Nutrition International, St. Louis, Missouri, USA) ad libitum.

Three German cockroach populations were tested: Orlando Normal, CC29, and VS101. Orlando Normal (ON; also known as "American Cyanamid"), is a laboratory population originally collected in 1947 in a Florida apartment. Since collected, this population has been maintained in the laboratory and reared without insecticide selection pressure, resulting in Orlando Normal being known to be susceptible to a wide range of insecticides. Populations CC29 and VS101 were collected from infested apartments in Raleigh, NC, in 2018, and both populations are known to be resistant to both fipronil and pyrethroids (cypermethrin) (González-Morales et al. 2022). Only adult male cockroaches were used for all experiments for standardization and to ensure consumption variations were due to experimental factors, not life stage. Cockroaches were separated after briefly anesthetizing with CO_2.

Baits and Bait Aging

Six cockroach gel baits were used for aging experiments, four were professional-grade and two were consumer-grade (Table 1). Each bait was tested both fresh (no aging) and at various ages after application. Four aging time points at 40% relative humidity (RH) were tested: 24 h, 1 mo, 3 mo, and 6 mo. Each bait was also aged for 6 mo at 15% RH and 80% RH. Those aged at 15% RH were tested on all three populations, but those aged at 80% RH grew a mold covering, and as a result were not tested in aged trials.

Baits were applied to plastic vial caps (23 × 8 mm, Discount Vials, Madison, Wisconsin, USA) and then placed into sealed

Table 1. Cockroach gel baits tested.

Bait name	Active ingredient	Manufacturer	Location	24 h aged bait application quantity
Maxforce FC Magnum	0.05% Fipronil	Envu Environmental Science	Cary, NC, USA	100–150 mg
Advion Cockroach	0.6% Indoxacarb	Syngenta	Basel, Switzerland	250–300 mg
Vendetta Plus	0.05% Abamectin; 0.5% Pyriproxyfen	McLaughlin Gormley King (MGK) Company	Minneapolis, Minnesota, USA	150–200 mg
Alpine (Rotation 2)	0.5% Dinotefuran	BASF SE Corporation	Ludwigshafen, Germany	100 mg
Combat Max	0.01% Fipronil	Henkel Corporation	Stamford, Connecticut, USA	100–200 mg
Raid Roach	0.3% Indoxacarb	S.C. Johnson Company	Racine, Wisconsin, USA	200–300 mg

containers that maintained a set humidity for the desired aging period. Humidities were maintained using saturated salt solutions as follows: 15% RH, Lithium Chloride, $LiCl$ (Sigma-Aldrich, St. Louis, Missouri, USA); 40% RH, Magnesium perchlorate hexahydrate, $Mg(ClO_4)_2 * 6H_2O$ (Sigma-Aldrich); 80% RH, Ammonium sulfate, $(NH_4)_2SO_4$ (Sigma-Aldrich) (Winston and Bates 1960). Humidities were verified using relative humidity and temperature data loggers (HOBO, Onset, Cape Cod, Massachusetts, USA) placed in each container. All baits aged for more than 24 h were applied in 500 mg quantities to the caps, while baits aged for only 24 h were applied in varying quantities (100 to 300 mg) to ensure > 10% consumption of the bait after correcting for water loss (Table 1).

Gel Bait Water Content and Water Retention

To find the dry weight of each bait type tested, all baits were applied in 500 mg quantities (wet weight) to plastic vial caps (23 × 8 mm, Discount Vials) with 5 replicates per bait type, and placed in a drying oven (Isotemp Incubator Model 655D, Fisher Scientific, Waltham, Massachusetts, USA), at 63 ± 3 °C. The baits were weighed to the nearest 1 mg using a digital analytical balance (MIDUO, Beijing, China) every 24 h for 7 d, then twice a week for 4 wk, until the weight did not deviate by more than 2 mg for 2 wk. These data were used to determine the initial water content of each bait. To determine how much water was retained, baits were weighed before and after placing into humidity chambers for each aging period.

Aged Bait Experimental Design

Aged bait experiments were conducted in rectangular plastic containers (5.6 [width] × 14 [length] × 8.4 [height] cm; Prep Naturals, Philadelphia, Pennsylvania, USA) and contained one corrugated cardboard harborage tent (6.7 cm × 8.6 cm, Uline), two small test tubes (1.2 × 1.5 cm, Fisher Scientific, Waltham, Massachusetts, USA) with water and cotton balls (Dynarex Corporation, Orangeburg, NY, USA), a plastic vial cap (23 × 8 mm, Discount Vials) holding rat chow (500 mg; Mazuri Rat and Mouse Diet) and a plastic vial cap (23 × 8 mm, Discount Vials) holding gel bait (Fig. 1).

For each aging experiment, 20 adult male German cockroaches were sorted into experimental arenas and then allowed to acclimate for 24 h. To prevent cockroaches from escaping, the walls of each arena were greased with mineral oil (Cumberland Swan/Vi-Jon, Inc., Smyrna, TN, USA). After acclimating for 24 h, food (rat chow) was removed, but water was left in the arena. The cockroaches were then deprived of food for 24 h. Simultaneously, the aged baits were removed from their containers and allowed to acclimate to the temperature and humidity of the room for 24 h. After the 24 h food deprivation and bait acclimation period ended, the baits were weighed to the nearest 1 mg using a digital analytical balance (MIDUO) and added to the arenas. No bait was added to the negative control, and in total, mortality for no-bait controls were less than 3%. Rat chow (equal weight to the bait) was also acclimated for 24 h and added in parallel with the bait in two-choice assays. Once the caps of bait and rat chow were added, weights of bait along with mortality were recorded daily for 7 d, or until full mortality of every replication was observed. Mortality was defined as the inability of cockroaches to right themselves and make coordinated movements away from a probe. Each treatment was replicated 3 to 5 times. In addition, three to five environmental controls with no cockroaches were run to account for the natural weight change of the baits due to water loss/gain. We used the following equation to adjust measured consumption for environmental water loss or gain:

Fig. 1. Bait consumption arena containing one carboard harborage, two test tubes with water and cotton balls, one cap containing rat chow, and one cap containing cockroach gel bait.

$$\text{Bait Consumption} = (\text{Bait}_{T0} * (EC_{T1}/ EC_{T0})) - \text{Bait}_{T1}$$

Bait_{T0} = Bait weight at time 0
Bait_{T1} = Bait weight at time 1
EC_{T0} = Environmental control (no cockroaches) bait weight at time 0
EC_{T1} = Environmental control (no cockroaches) bait weight at time 1

Fresh Bait Experimental Design

Due to rapid water loss in baits during the first 24 h following application, consumption was difficult to measure accurately after 24 h using environmental controls to account for water loss. Therefore, fresh bait consumption was determined by running dose–response assays, which consisted of multiple replicates identical to the aged bait experiments but with different amounts of bait. The amount of bait applied varied based on the product being tested, with five replicates performed for each bait quantity, and a minimum of three bait quantities used for each experiment, with quantities falling above and below the maximum quantity of bait that each population could consume in 24 h. These experiments were scored as either a 0 (no bait remaining) or a 1 (with bait remaining) after 24 h. Mortality was assessed daily for 7 d, with Day 7 mortality reported for the lowest bait application amount that resulted in bait remaining for all replicates.

Data Analysis

A 1-way Kruskal–Wallis test followed by a pairwise Wilcoxon test was used to compare all percent water content and percent water retention values between bait types. All fresh bait consumption quantities were determined using Logistic regression followed by a post-hoc inverse prediction, with bait consumption capacity predicted as the amount of bait that would lead to 50% of trials having no bait remaining after 24 h, resulting in a predicted fresh value. In all 40% RH consumption assays, a One Sample *T*-test was used to compare all values to the predicted fresh value (each test was performed independently). A 1-way ANOVA followed by a post-hoc Tukey Kramer HSD test was used to compare all values aged for 24 h and older amongst each other. A *t*-test was used to compare consumption results for baits aged for 6 mo at 15% RH to those aged for 6 mo at 40% RH. Kaplan–Meier survival analysis was used to analyze the impact of bait age on survival of cockroaches. Within each bait-population group, all fresh baits and baits aged at 40% RH were compared using the log-rank test. Lack of variation (i.e. 100% mortality by Day 1) sometimes drove singularity issues, and thus bait-population tests with these issues were not analyzed. Kaplan–Meier survival analysis with the log-rank test was also used to analyze the impact of humidity (15% vs 40%) on baits aged for 6 mo.

Results

Bait Water Content and Loss

All gel baits were found to contain over 40% water when first applied, with the percent water content differing significantly with bait type (Table 2; $x^2 = 27.00$, df = 5, $P < 0.001$). Post-hoc comparisons revealed Advion Cockroach to have a significantly higher water content than all other bait types, followed by Raid Roach, which had significantly higher water content than Alpine (Rotation 2), Maxforce FC Magnum, and Combat Max bait (Table 2). In general, Maxforce FC Magnum and Combat Max retained the most water over time, while Advion Cockroach and Raid Roach bait lost the most water over time. Overall, we found bait type to have a significant effect on percent water retention at all aging time points (24 h: $x^2 = 29.12$, df = 5, $P < 0.001$; 1 mo: $x^2 = 87.07$, df = 5, $P < 0.001$; 3 mo: $x^2 = 78.85$, df = 5, $P < 0.001$; 6 mo at 40% RH: $x^2 = 53.35$, df = 5, $P < 0.001$; 6 mo at 15% RH: $x^2 = 79.60$, df = 5, $P < 0.001$; Table 2).

Predicted Fresh Bait Consumption

Predicted values for bait consumption varied widely among baits and populations tested (Table 3). Predicted total bait consumption ranged from 31.1 mg (Maxforce FC Magnum, ON) to 125.0 mg (Advion Cockroach, CC29) (Table 3).

Consumption and Efficacy of Maxforce FC Magnum Bait Aged at 40% Relative Humidity

We found the Orlando Normal population consumed significantly less Maxforce FC Magnum bait at 24 h ($t_4 = 5.19$, $P = 0.007$), 1 mo ($t_4 = 3.27$, $P = 0.03$), and 6 mo ($t_4 = 6.64$, $P = 0.003$), than predicted fresh bait consumption, but not at 3 mo ($t_4 = 0.29$, $P = 0.78$) (Fig. 2A). When comparing bait consumption among aging times, we found bait age to have no significant effect on bait consumption (Fig. 2A; $F_{3,16} = 3.07$, $P = 0.06$). Seven days after the bait was introduced, fresh and aged bait caused 100% mortality for all replicates tested on the Orlando Normal population (Table 4). Survivorship was not evaluated due to a lack of variability in aging time points (most reached 100% mortality within one day).

We found the CC29 population consumed significantly less Maxforce FC Magnum bait at 3 months ($t_2 = 5.91$, $P = 0.03$) than predicted fresh bait consumption, but not at 24 h ($t_2 = 0.39$, $P = 0.74$), 1 mo ($t_2 = 0.02$, $P = 0.99$), or 6 mo ($t_2 = 0.38$, $P = 0.74$) (Fig. 2B). When comparing bait consumption among aging times, we found bait age to have no significant effect on bait consumption (Fig. 2B; $F_{3,8} = 0.21$, $P = 0.89$). Survivorship was significantly different among aged baits ($\chi^2 = 65.1$, df = 4, $P < 0.001$), with fresh baits having significantly greater survival than aged baits (Table 4). Seven days after the bait was introduced, mortality was greater than 87% for all bait ages tested (Table 4).

We found the VS101 population to not consume a significantly different amount of Maxforce FC Magnum bait at 24 h ($t_2 = 0.62$, $P = 0.62$), 1 mo ($t_2 = 0.10$, $P = 0.93$), 3 mo ($t_2 = 0.89$, $P = 0.47$), or 6 mo ($t_2 = 2.39$, $P = 0.14$) when compared with predicted fresh bait consumption (Fig. 2C). When comparing bait consumption among aging times, we found bait age to have no significant effect on bait consumption (Fig. 2C; $F_{3,8} = 1.09$, $P = 0.41$). Survivorship was significantly different among aged baits ($\chi^2 = 24.2$, df = 4, $P < 0.001$), with fresh baits having significantly greater survival than baits aged for 3 or 6 mo (Table 4). Seven days after the bait was introduced, mortality reached 100% for all bait ages tested (Table 4).

Consumption and Efficacy of Advion Cockroach Bait Aged at 40% Relative Humidity

We found the Orlando Normal population consumed significantly less Advion Cockroach bait at 24 h ($t_4 = 31.12$, $P < 0.001$), 1 mo ($t_4 = 28.04$, $P < 0.001$), 3 mo ($t_4 = 12.22$, $P < 0.001$), and 6 mo ($t_4 = 30.89$, $P < 0.001$) than predicted fresh bait consumption (Fig. 3A). When comparing bait consumption among aging times, we found bait age to have a significant effect on bait consumption (Fig. 3A; $F_{3,16} = 25.36$, $P < 0.001$). Post-hoc comparisons revealed the Orlando Normal population consumed significantly more bait aged for 3 mo than bait aged for 24 h, 1 mo, and 6 mo, although there was no difference among bait consumed at all other time points (Fig. 3A). Survivorship was significantly different among aged

Table 2. Percent water content and percent water retention of gel baits.

Bait name	Percent water content[1]	Percent water retention				
		24 h[1]	1 mo	3 mo	6 mo	6 mo 15% RH
Maxforce FC Magnum	43.0 ± 0.7[a]	55.2 ± 2.1[b]	19.8 ± 0.4[c]	20.6 ± 0.7[c]	13.5 ± 0.7[c]	11.7 ± 0.4[c]
Advion Cockroach	57.1 ± 0.3[d]	38.1 ± 0.8[a]	9.7 ± 0.3[a]	11.4 ± 0.4[a]	5.9 ± 2.0[a]	6.7 ± 0.5[a]
Vendetta Plus	52.7 ± 0.4[bc]	52.8 ± 1.2[b]	17.0 ± 0.5[c]	15.2 ± 0.6[b]	11.1 ± 0.6[b]	10.0 ± 0.2[b]
Alpine (Rotation 2)	51.8 ± 0.2[b]	51.6 ± 1.6[b]	13.4 ± 0.2[b]	14.5 ± 0.5[b]	10.3 ± 0.5[b]	8.0 ± 0.2[a]
Combat Max	42.6 ± 0.1[a]	49.9 ± 0.9[b]	20.0 ± 0.3[c]	20.5 ± 0.3[c]	13.6 ± 0.6[c]	9.8 ± 0.2[b]
Raid Roach	53.9 ± 0.1[c]	37.1 ± 1.8[a]	12.0 ± 0.1[d]	7.5 ± 0.7[d]	5.4 ± 1.1[a]	3.7 ± 0.6[d]

[1]Different superscript lowercase letters indicate significant differences in percent water content and percent water retention among baits at each aging time point measured (vertical columns; Kruskal-Wallis test, followed by pairwise Wilcoxon test, $P < 0.05$).

baits (χ^2 = 16.3, df = 4, P = 0.003), although post-hoc testing revealed no significant pairwise differences (Table 4). Seven days after the bait was introduced, mortality reached 100% for all bait ages tested (Table 4).

We found the CC29 population consumed significantly less Advion Cockroach bait at 24 h (t_2 = 21.72, P = 0.002), 1 mo (t_2 = 107.52, P < 0.001), 3 mo (t_2 = 15.41, P = 0.004), and 6 mo (t_2 = 23.65, P = 0.002) than predicted fresh bait consumption (Fig. 3B). When comparing bait consumption among aging times, we found bait age to have a significant effect on bait consumption (Fig. 3B; $F_{3,8}$ = 5.94, P = 0.02). Post-hoc comparisons revealed the CC29 population consumed significantly more bait aged for 24 h than bait aged for 3 mo and 6 mo, although there was no difference among bait consumed at all other time points (Fig. 3B). Survivorship was not significantly different among aged baits (χ^2 = 7.7, df = 4, P = 0.10). Seven days after the bait was introduced, mortality was greater than 95% for all bait ages tested (Table 4).

We found the VS101 population consumed significantly less Advion Cockroach bait at 24 h (t_2 = 7.53, P = 0.02), 1 mo (t_2 = 5.49, P = 0.03), 3 mo (t_2 = 8.49, P = 0.01), and 6 mo (t_2 = 14.92, P = 0.004) than predicted fresh bait consumption (Fig. 3C). When comparing bait consumption among aging times, we found bait age to have a significant effect on bait consumption (Fig. 3C; $F_{3,8}$ = 7.01, P = 0.01). Post-hoc comparisons revealed the VS101 population consumed significantly more bait aged for 1 mo than bait aged for 3 mo, and 6 mo, although there was no difference among bait consumed at all other time points (Fig. 3C). Survivorship was not significantly different among aged baits (χ^2 = 7.0, df = 4, P = 0.13). Seven days after the bait was introduced, mortality reached 100% for all bait ages tested (Table 4).

Consumption and Efficacy of Vendetta Plus Bait Aged at 40% Relative Humidity

We found the Orlando Normal population consumed significantly less Vendetta Plus bait at 24 h (t_4 = 31.20, P < 0.001), 1 mo (t_4 = 40.74, P < 0.001), 3 mo (t_4 = 35.62, P < 0.001), and 6 mo (t_4 = 19.93, P < 0.001), than predicted fresh bait consumption (Fig. 4A). When comparing bait consumption among aging times, we found bait age to have a significant effect on bait consumption (Fig. 4A; $F_{3,16}$ = 3.83, P = 0.03). Post-hoc comparisons revealed the Orlando Normal population consumed significantly more bait aged for 24 h than bait aged for 1 mo, although there was no difference among bait consumed at all other time points (Fig. 4A). Survivorship was significantly different among aged baits (χ^2 = 104.5, df = 4, P < 0.001), with fresh baits having the shortest survival time and baits aged 24 h having the longest survival time (Table 4). Seven days after the bait was introduced, mortality reached 100% for all bait ages tested (Table 4).

We found the CC29 population consumed significantly less Vendetta Plus bait at 1 mo (t_2 = 9.53, P = 0.01) and 6 mo (t_2 = 7.42, P = 0.02), than predicted fresh bait consumption, but not at 24 h (t_2 = 2.06, P = 0.18) or 3 mo (t_2 = 1.87, P = 0.20) (Fig. 4B). When comparing bait consumption among aging times, we found bait age to have no significant effect on bait consumption (Fig. 4B; $F_{3,8}$ = 3.55, P = 0.07). Survivorship was significantly different among aged baits (χ^2 = 81.1, df = 4, P < 0.001), with fresh baits having the shortest survival time and baits aged one month having the longest survival time (Table 4). Seven days after the bait was introduced, mortality reached 100% for all bait ages tested (Table 4).

Table 3. Fresh bait predicted consumption for 20 adult male cockroaches.

Bait name	Population	Predicted fresh value (mg)	Chi-square	Probability	DF	95% confidence interval	X-intercept(± standard error)	Slope (± standard error)
Maxforce FC Magnum	Orlando Normal	31.1	5.9	0.015	1	(22.8, -)	−7.465 ± 3.476	0.240 ± 0.116
	CC29	35.0	28.7	<0.001	1	(25.2, 44.4)	−5.923 ± 2.082	0.169 ± 0.057
	VS101	52.9	10.8	0.001	1	(37.2, 66.2)	−9.451 ± 3.976	0.179 ± 0.074
Advion Cockroach	Orlando Normal	105.5	23.0	<0.001	1	(95.7, 118.8)	−14.321 ± 4.917	0.136 ± 0.047
	CC29	125.0	23.4	<0.001	1	(114.1, 136.1)	−39.247 ± 16.606	0.314 ± 0.133
	VS101	117.7	8.0	0.005	1	(110.4, 125.0)	−26.000 ± 13.468	0.221 ± 0.116
Vendetta Plus	Orlando Normal	81.1	12.5	<0.001	1	(68.3, 100.9)	−18.749 ± 8.162	0.231 ± 0.101
	CC29	62.1	13.9	<0.001	1	(43.7, 72.7)	−7.590 ± 3.000	0.122 ± 0.045
	VS101	83.7	4.1	0.044	1	(74.5, 92.9)	−12.355 ± 6.853	0.147 ± 0.083
Alpine(Rotation 2)	Orlando Normal	60.8	22.4	<0.001	1	(49.1, 70.9)	−7.884 ± 2.655	0.130 ± 0.042
	CC29	59.8	19.2	<0.001	1	(−101.6, 221.2)	−121.454 ± 46884.095	2.031 ± 781.401
	VS101	65.0	17.5	<0.001	1	(52.7, 77.3)	−20.351 ± 8.730	0.313 ± 0.134
Combat Max	Orlando Normal	89.3	17.3	<0.001	1	(79.4, 104.1)	−15.328 ± 5.911	0.171 ± 0.067
	CC29	96.3	4.1	0.044	1	(87.1, 105.5)	−14.212 ± 8.207	0.147 ± 0.083
	VS101	109.4	17.0	<0.001	1	(−894.7, 1113.4)	−246.931 ± 196259.840	2.257 ± 1784.180
Raid Roach	Orlando Normal	109.0	28.7	<0.001	1	(109.0, 130.8)	−28.164 ± 11.715	0.236 ± 0.098
	CC29	88.9	16.2	<0.001	1	(72.4, 100.0)	−20.640 ± 8.976	0.232 ± 0.100
	VS101	95.0	9.2	0.003	1	(76.0, 114.0)	−7.154 ± 2.785	0.075 ± 0.029

The equation for the logistic regression curve is as follows:.

$$y = \frac{e^{(b_0 + b_1 x)}}{1 + e^{(b_0 + b_1 x)}}$$

b_0 = x-intercept.
b_1 = slope.
x = predicted fresh value.
y = predicted probability = 0.50.

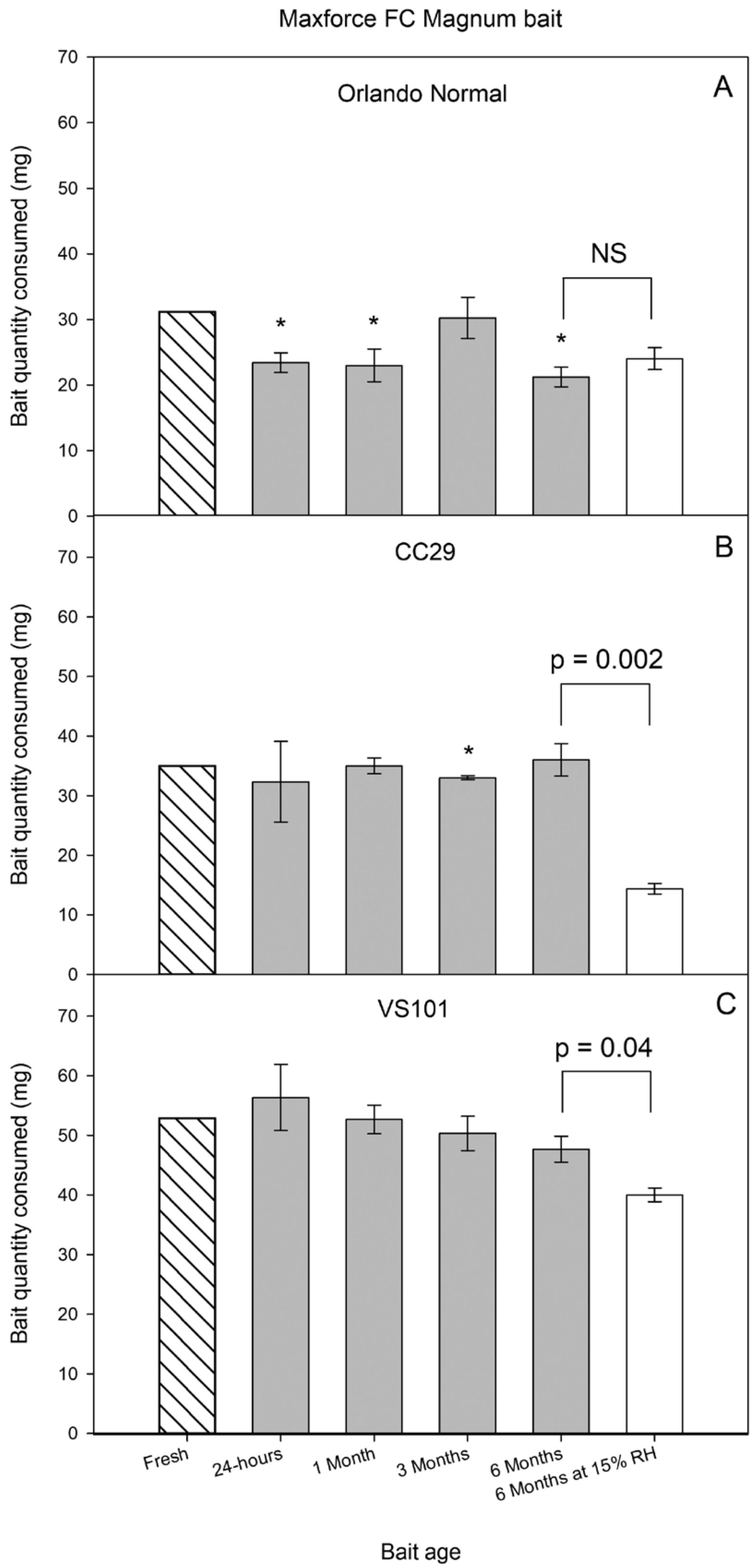

Fig. 2. Maxforce FC Magnum gel bait consumption (24 h) for the following populations: (A) Orlando Normal, (B) CC29, (C) VS101. Error bars represent the standard error of the mean (SEM). Asterisks above the bar graphs indicate consumption of aged bait at 40% relative humidity which is significantly different than the predicted fresh bait consumption value ($P < 0.05$). Brackets above bar graphs indicate a difference (*t*-test, $P < 0.05$), or lack of difference (*t*-test, NS = not significant, $P > 0.05$), between baits aged 6 mo at 40% and 15% relative humidity.

Table 4. Mortality data and mean survival time for all bait-population-age combinations tested.

Bait	Population	Age	Percent Mortality (7d)	Mean Survival Time (d)[1,2]
Maxforce FC Magnum	ON	fresh	100	1.05 ± 0.03
		24 h	100	1.00 ± 0.00
		1 mo	100	1.00 ± 0.00
		3 mo	100	1.00 ± 0.00
		6 mo 40%	100	1.00 ± 0.00
		6 mo 15%	100	1.00 ± 0.00
	CC29	Fresh	87	2.51 ± 0.23[a]
		24 h	97	1.13 ± 0.06[b]
		1 mo	100	1.13 ± 0.10[b]
		3 mo	100	1.02 ± 0.02[b]
		6 mo 40%	100	1.00 ± 0.00[b]
		6 mo 15%	97	1.65 ± 0.19*
	VS101	Fresh	100	1.23 ± 0.05[a]
		24 h	100	1.07 ± 0.03[ab]
		1 mo	100	1.03 ± 0.02[ab]
		3 mo	100	1.02 ± 0.02[bc]
		6 mo 40%	100	1.02 ± 0.02[bc]
		6 mo 15%	100	1.12 ± 0.08
Advion Cockroach	ON	Fresh	100	1.08 ± 0.04
		24 h	100	1.00 ± 0.00
		1 mo	100	1.00 ± 0.00
	ON	3 mo	100	1.01 ± 0.01
		6 mo 40%	100	1.12 ± 0.06
		6 mo 15%	99	1.00 ± 0.00
	CC29	Fresh	98	1.67 ± 0.14
		24 h	100	1.38 ± 0.07
		1 mo	97	1.78 ± 0.19
		3 mo	97	1.65 ± 0.14
		6 mo 40%	95	2.08 ± 0.22
		6 mo 15%	98	1.82 ± 0.17
	VS101	Fresh	100	1.18 ± 0.04
		24 h	100	1.13 ± 0.05
		1 mo	100	1.22 ± 0.10
		3 mo	100	1.05 ± 0.04
		6 mo 40%	100	1.07 ± 0.03
		6 mo 15%	100	1.12 ± 0.05
Vendetta Plus	ON	Fresh	100	1.37 ± 0.07[d]
		24 h	100	2.87 ± 0.11[a]
		1 mo	100	2.18 ± 0.13[bc]
		3 mo	100	1.82 ± 0.09[c]
		6 mo 40%	100	2.43 ± 0.11[b]
		6 mo 15%	100	1.79 ± 0.09*
	CC29	Fresh	100	1.50 ± 0.10[c]
		24 h	100	2.22 ± 0.15[b]
		1 mo	100	3.20 ± 0.19[a]
		3 mo	100	2.70 ± 0.14[ab]
		6 mo 40%	100	2.68 ± 0.18[ab]
		6 mo 15%	100	2.73 ± 0.19
	VS101	Fresh	100	1.48 ± 0.10[c]
		24 h	100	2.77 ± 0.15[a]
		1 mo	100	2.70 ± 0.19[a]
		3 mo	100	1.93 ± 0.15[bc]
		6 mo 40%	100	2.38 ± 0.22[ab]
		6 mo 15%	100	1.32 ± 0.10*

Table 4. Continued

Bait	Population	Age	Percent Mortality (7d)	Mean Survival Time (d)[1,2]
Alpine (Rotation 2)	ON	Fresh	100	1.05 ± 0.03
		24 h	100	1.00 ± 0.00
		1 mo	100	1.02 ± 0.01
		3 mo	100	1.09 ± 0.04
		6 mo 40%	100	1.12 ± 0.04
		6 mo 15%	100	1.06 ± 0.03
	CC29	Fresh	99	1.33 ± 0.09
		24 h	100	1.08 ± 0.04
		1 mo	100	1.18 ± 0.07
		3 mo	100	1.38 ± 0.13
		6 mo 40%	100	1.43 ± 0.15
		6 mo 15%	100	1.15 ± 0.07
	VS101	Fresh	100	1.11 ± 0.06
		24 h	100	1.03 ± 0.02
		1 mo	100	1.00 ± 0.00
		3 mo	100	1.17 ± 0.09
		6 mo 40%	100	1.07 ± 0.03
		6 mo 15%	100	1.07 ± 0.03
	ON	Fresh	100	1.00 ± 0.00
		24 h	100	1.00 ± 0.00
		1 mo	100	1.00 ± 0.00
		3 mo	100	1.04 ± 0.03
		6 mo 40%	100	1.06 ± 0.03
		6 mo 15%	100	1.00 ± 0.00*
	CC29	Fresh	96	1.48 ± 0.11
		24 h	100	1.25 ± 0.06
Combat Max		1 mo	100	1.42 ± 0.06
		3 mo	100	1.38 ± 0.08
		6 mo 40%	100	1.48 ± 0.08
		6 mo 15%	100	1.43 ± 0.07
	VS101	Fresh	100	1.07 ± 0.04
		24 h	100	1.23 ± 0.06
		1 mo	100	1.18 ± 0.05
		3 mo	100	1.07 ± 0.03
		6 mo 40%	100	1.08 ± 0.04
		6 mo 15%	100	1.23 ± 0.06*
Raid Roach	ON	Fresh	100	1.00 ± 0.00
		24 h	100	1.00 ± 0.00
		1 mo	100	1.00 ± 0.00
		3 mo	100	1.02 ± 0.01
		6 mo 40%	100	1.03 ± 0.03
		6 mo 15%	100	1.00 ± 0.00
	CC29	Fresh	97	1.53 ± 0.09
		24 h	93	1.57 ± 0.19
		1 mo	97	1.55 ± 0.16
		3 mo	98	1.33 ± 0.08
		6 mo 40%	97	1.63 ± 0.18
		6 mo 15%	97	1.72 ± 0.15
	VS101	Fresh	100	1.26 ± 0.06
		24 h	100	1.35 ± 0.11
		1 mo	100	1.30 ± 0.11
		3 mo	100	1.27 ± 0.06
		6 mo 40%	100	1.25 ± 0.08
		6 mo 15%	100	1.15 ± 0.05

[1]Different lowercase letters indicate significant differences in mean survival time among different aged baits within each bait-population group based on Kaplan–Meier survival analysis followed by a log-rank test.
[2]Mean survival times marked with and * indicate significant differences in mean survival time between baits aged for 6 months at 15% and 40% relative humidity within each bait-population group based on Kaplan–Meier survival analysis followed by a log-rank test.

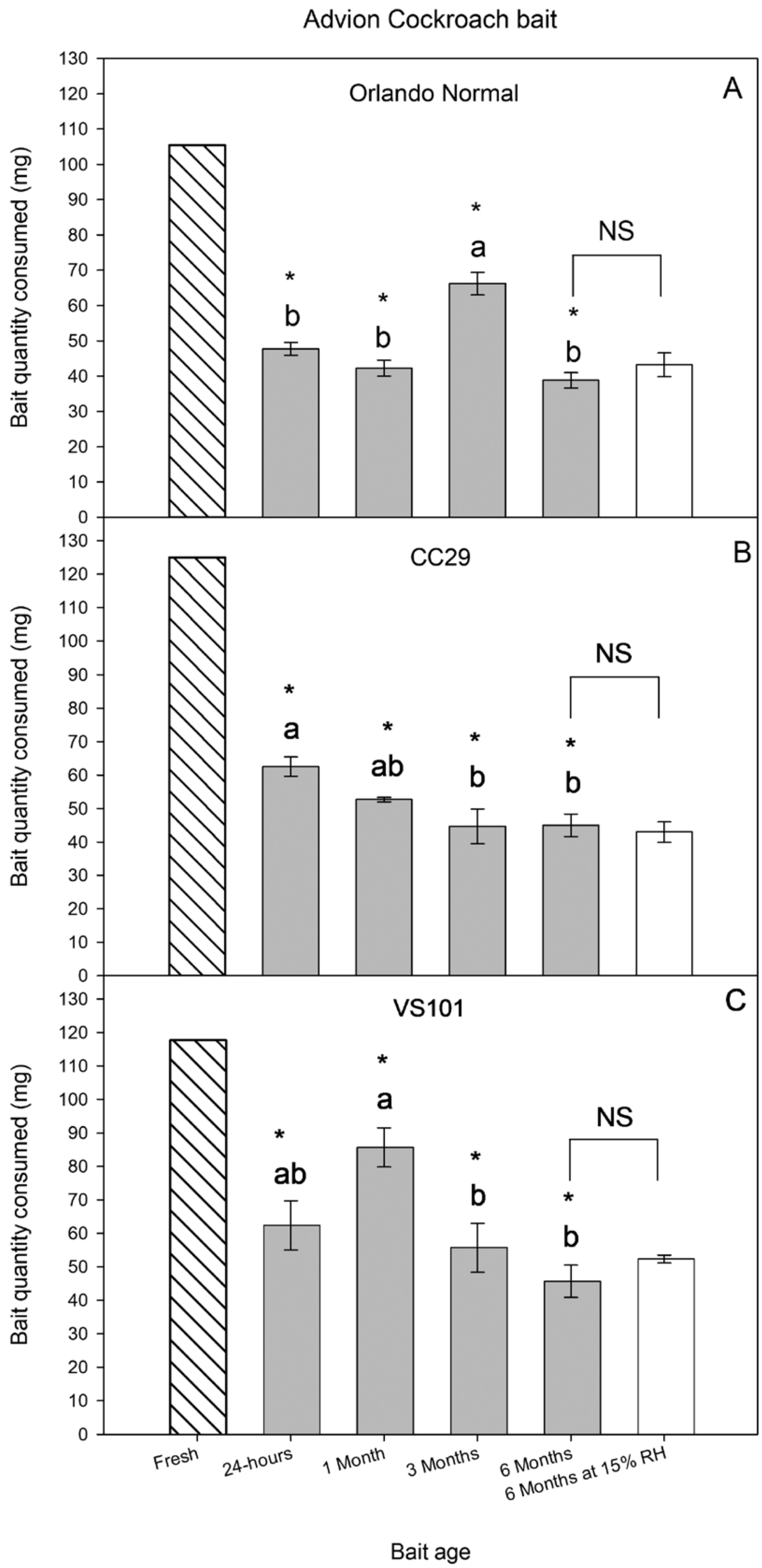

Fig. 3. Advion Cockroach gel bait consumption (24 h) for the following populations: (A) Orlando Normal, (B) CC29, (C) VS101. Error bars represent the standard error of the mean (SEM). Asterisks above the bar graphs indicate consumption of aged bait at 40% relative humidity which is significantly different than the predicted fresh bait consumption value ($P < 0.05$). Different lowercase letters above bar graphs indicate significant differences among consumption of aged bait at 40% relative humidity (ANOVA, followed by independent *t*-test, $P < 0.05$). Brackets above bar graphs indicate a difference (*t*-test, $P < 0.05$), or lack of difference (*t*-test, NS = not significant, $P > 0.05$), between baits aged 6 mo at 40% and 15% relative humidity.

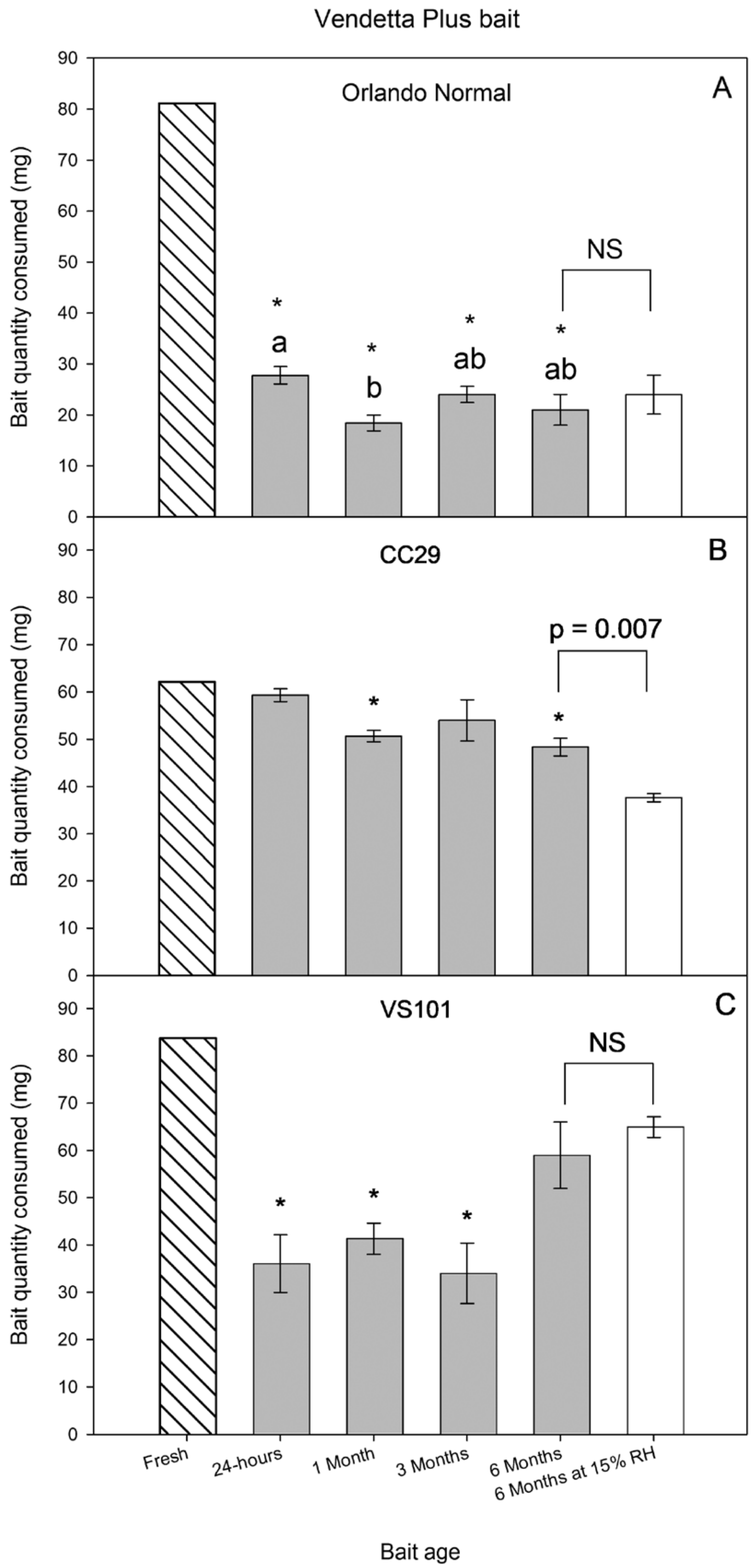

Fig. 4. Vendetta Plus gel bait consumption (24 h) for the following populations: (A) Orlando Normal, (B) CC29, (C) VS101. Error bars represent the standard error of the mean (SEM). Asterisks above the bar graphs indicate consumption of aged bait at 40% relative humidity which is significantly different than the predicted fresh bait consumption value ($P < 0.05$). Different lowercase letters above bar graphs indicate significant differences among consumption of aged bait at 40% relative humidity (ANOVA, followed by independent *t*-test, $P < 0.05$). Brackets above bar graphs indicate a difference (*t*-test, $P < 0.05$), or lack of difference (*t*-test, NS = not significant, $P > 0.05$), between baits aged 6 mo at 40% and 15% relative humidity.

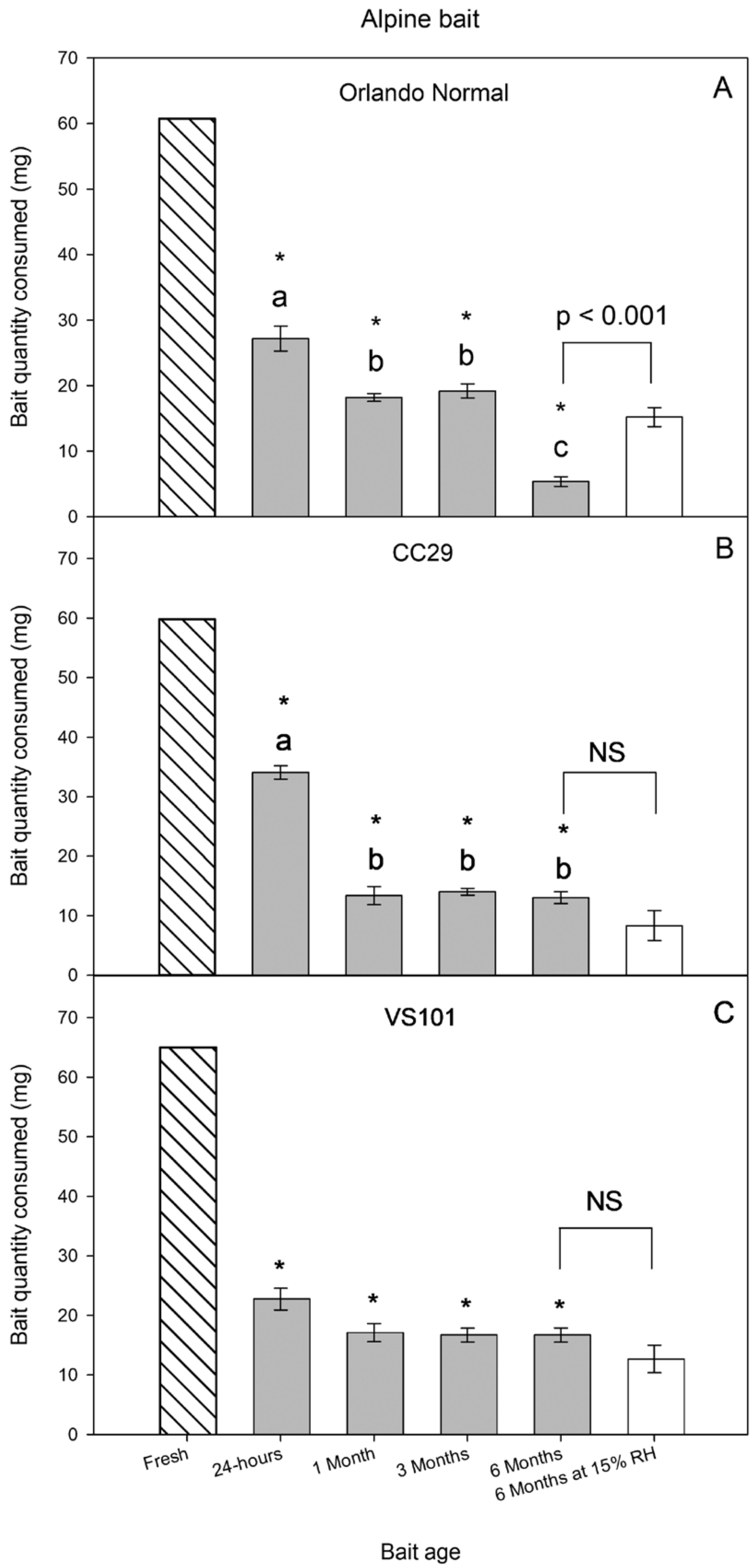

Fig. 5. Alpine (Rotation 2) gel bait consumption (24 h) for the following populations: (A) Orlando Normal, (B) CC29, (C) VS101. Error bars represent the standard error of the mean (SEM). Asterisks above the bar graphs indicate consumption of aged bait at 40% relative humidity which is significantly different than the predicted fresh bait consumption value ($P < 0.05$). Different lowercase letters above bar graphs indicate significant differences among consumption of aged bait at 40% relative humidity (ANOVA, followed by independent *t*-test, $P < 0.05$). Brackets above bar graphs indicate a difference (*t*-test, $P < 0.05$), or lack of difference (*t*-test, NS = not significant, $P > 0.05$), between baits aged 6 mo at 40% and 15% relative humidity.

We found the VS101 population consumed significantly less Vendetta Plus bait at 24 h (t_2 = 7.82, P = 0.02), 1 mo (t_2 = 12.91, P = 0.006), and 3 mo (t_2 = 7.82, P = 0.02) than predicted fresh bait consumption, but not at 6 mo (t_2 = 3.54, P = 0.07) (Fig. 4C). When comparing bait consumption among aging times, we found bait age to have no significant effect on bait consumption (Fig. 4C; $F_{3,8}$ = 3.75, P = 0.06). Survivorship was significantly different among aged baits (χ^2 = 50.8, df = 4, P < 0.001), with fresh baits having the shortest survival time and baits aged 24 h and 1 mo having the longest survival times (Table 4). Seven days after the bait was introduced, mortality reached 100% for all bait ages tested (Table 4).

Consumption and efficacy of Alpine (Rotation 2) Bait Aged at 40% Relative Humidity

We found the Orlando Normal population consumed significantly less Alpine (Rotation 2) bait at 24 h (t_4 = 17.64, P < 0.001), 1 mo (t_4 = 73.15, P < 0.001), 3 mo (t_4 = 38.98, P < 0.001), and 6 mo (t_4 = 74.00, P < 0.001) than predicted fresh bait consumption (Fig. 5A). When comparing bait consumption among aging times, we found bait age to have a significant effect on bait consumption (Fig. 5A; $F_{3,16}$ = 57.51, P < 0.001). Post-hoc comparisons revealed the Orlando Normal population consumed significantly more bait aged for 24 h than bait aged for 1 mo, 3 mo, and 6 mo, as well as consumed significantly more bait aged for 1 mo and 3 mo than bait aged for 6 mo, although there was no difference among bait consumed at all other time points (Fig. 5A). Survivorship was significantly different among aged baits (χ^2 = 13.0, df = 4, P = 0.01), although post-hoc testing revealed no significant pairwise differences (Table 4). Seven days after the bait was introduced, mortality reached 100% for all bait ages tested (Table 4).

We found the CC29 population consumed significantly less Alpine (Rotation 2) bait at 24 h (t_2 = 22.57, P = 0.002), 1 mo (t_2 = 30.47, P = 0.001), 3 mo (t_2 = 79.33, P < 0.001), and 6 mo (t_2 = 46.80, P = 0.001) than predicted fresh bait consumption (Fig. 5B). When comparing bait consumption among aging times, we found bait age to have a significant effect on bait consumption (Fig. 5B; $F_{3,8}$ = 85.90, P < 0.001). Post-hoc comparisons revealed the CC29 population consumed significantly more bait aged for 24 h than bait aged for 1 mo, 3 mo, and 6 mo, although there was no difference among bait consumed at all other time points (Fig. 5B). Survivorship was not significantly different among aged baits (χ^2 = 8.0, df = 4, P = 0.09). Seven days after the bait was introduced, mortality was greater than 99% for all bait ages tested (Table 4).

We found the VS101 population consumed significantly less Alpine (Rotation 2) bait at 24 h (t_2 = 23.09, P = 0.002), 1 mo (t_2 = 31.34, P = 0.001), 3 mo (t_2 = 41.58, P = 0.001), and 6 mo (t_2 = 40.80, P = 0.001) than predicted fresh bait consumption (Fig. 5C). When comparing bait consumption among aging times, we found bait age to have a significant effect on bait consumption (Fig. 5C; $F_{3,8}$ = 4.15, P = 0.048). However, post-hoc comparisons revealed no significant difference in consumption between baits aged for 24 h, 1 mo, 3 mo, and 6 mo (Fig. 5C). Survivorship was not significantly different among aged baits (χ^2 = 5.8, df = 4, P = 0.22). Seven days after the bait was introduced, mortality reached 100% for all bait ages tested (Table 4).

Consumption and Efficacy of Combat Max Bait Aged at 40% Relative Humidity

We found the Orlando Normal population consumed significantly less Combat Max bait at 24 h (t_4 = 14.75, P < 0.001), 1 mo (t_4 = 22.10, P < 0.001), 3 mo (t_4 = 21.48, P < 0.001), and 6 mo (t_4 = 19.68, P < 0.001) than predicted fresh bait consumption (Fig. 6A). When comparing bait consumption among aging times, we found bait age to have a significant effect on bait consumption (Fig. 6A; $F_{3,16}$ = 3.61, P = 0.04). Post-hoc comparisons revealed the Orlando Normal population consumed significantly more bait aged for 6 mo than bait aged for 1 mo, although there was no difference among bait consumed at all other time points (Fig. 6A). Survivorship was significantly different among aged baits (χ^2 = 10.9, df = 4, P = 0.03), although post-hoc testing revealed no significant pairwise differences (Table 4). Seven days after the bait was introduced, mortality reached 100% for all bait ages tested (Table 4).

We found the CC29 population consumed significantly less Combat Max bait at 24 h (t_2 = 6.20, P = 0.03), 1 mo (t_2 = 31.42, P = 0.001), 3 mo (t_2 = 7.06, P = 0.02), and 6 mo (t_2 = 21.86, P = 0.002) than predicted fresh bait consumption (Fig. 6B). When comparing bait consumption among aging times, we found bait age to have no significant effect on bait consumption (Fig. 6B; $F_{3,8}$ = 1.19, P = 0.37). Survivorship was not significantly different among aged baits (χ^2 = 4.6, df = 4, P = 0.33). Seven days after the bait was introduced, mortality was greater than 96% for all bait ages tested (Table 4).

We found the VS101 population consumed significantly less Combat Max bait at 1 mo (t_2 = 9.06, P = 0.01) and 6 mo (t_2 = 13.83, P = 0.005) than predicted fresh bait consumption, but not at 24 h (t_2 = 3.592, P = 0.07) or 3 mo (t_2 = 1.81, P = 0.21) (Fig. 6C). When comparing bait consumption among aging times, we found bait age to have no significant effect on bait consumption (Fig. 6C; $F_{3,8}$ = 2.21, P = 0.16). Survivorship was significantly different among aged baits (χ^2 = 13.7, df = 4, P = 0.008), although post-hoc testing revealed no significant pairwise differences (Table 4). Seven days after the bait was introduced, mortality reached 100% for all bait ages tested (Table 4).

Consumption and Efficacy of Raid Roach Bait Aged at 40% Relative Humidity

We found the Orlando Normal population consumed significantly less Raid Roach bait at 24 h (t_4 = 76.02, P < 0.001), 1 mo (t_4 = 31.09, P < 0.001), 3 mo (t_4 = 7.78, P = 0.001), and 6 mo (t_4 = 29.90, P < 0.001) than predicted fresh bait consumption (Fig. 7A). When comparing bait consumption among aging times, we found bait age to have a significant effect on bait consumption (Fig. 7A; $F_{3,16}$ = 12.68, P < 0.001). Post-hoc comparisons revealed the Orlando Normal population consumed significantly more bait aged for 3 mo than bait aged for 24 h, 1 mo, and 6 mo, although there was no difference among bait consumed at all other time points (Fig. 7A). Survivorship was not significantly different among aged baits (χ^2 = 4.6, df = 4, P = 0.33). Seven days after the bait was introduced, mortality reached 100% for all bait ages tested (Table 4).

We found the CC29 population consumed significantly less Raid Roach bait at 24 h (t_2 = 22.04, P = 0.002), 1 mo (t_2 = 22.52, P = 0.002), 3 mo (t_2 = 13.79, P = 0.005), and 6 mo (t_2 = 14.69, P = 0.005) than predicted fresh bait consumption (Fig. 7B). When comparing bait consumption among aging times, we found bait age to have a significant effect on bait consumption (Fig. 7B; $F_{3,8}$ = 7.87, P = 0.009). Post-hoc comparisons revealed the CC29 population consumed significantly more bait aged for 24 h than bait aged for 6 mo, although there was no difference among bait consumed at all other time points (Fig. 7B). Survivorship was not significantly different among aged baits (χ^2 = 1.1, df = 4, P = 0.89). Seven days after the bait was introduced, mortality was greater than 96% for all bait ages tested (Table 4).

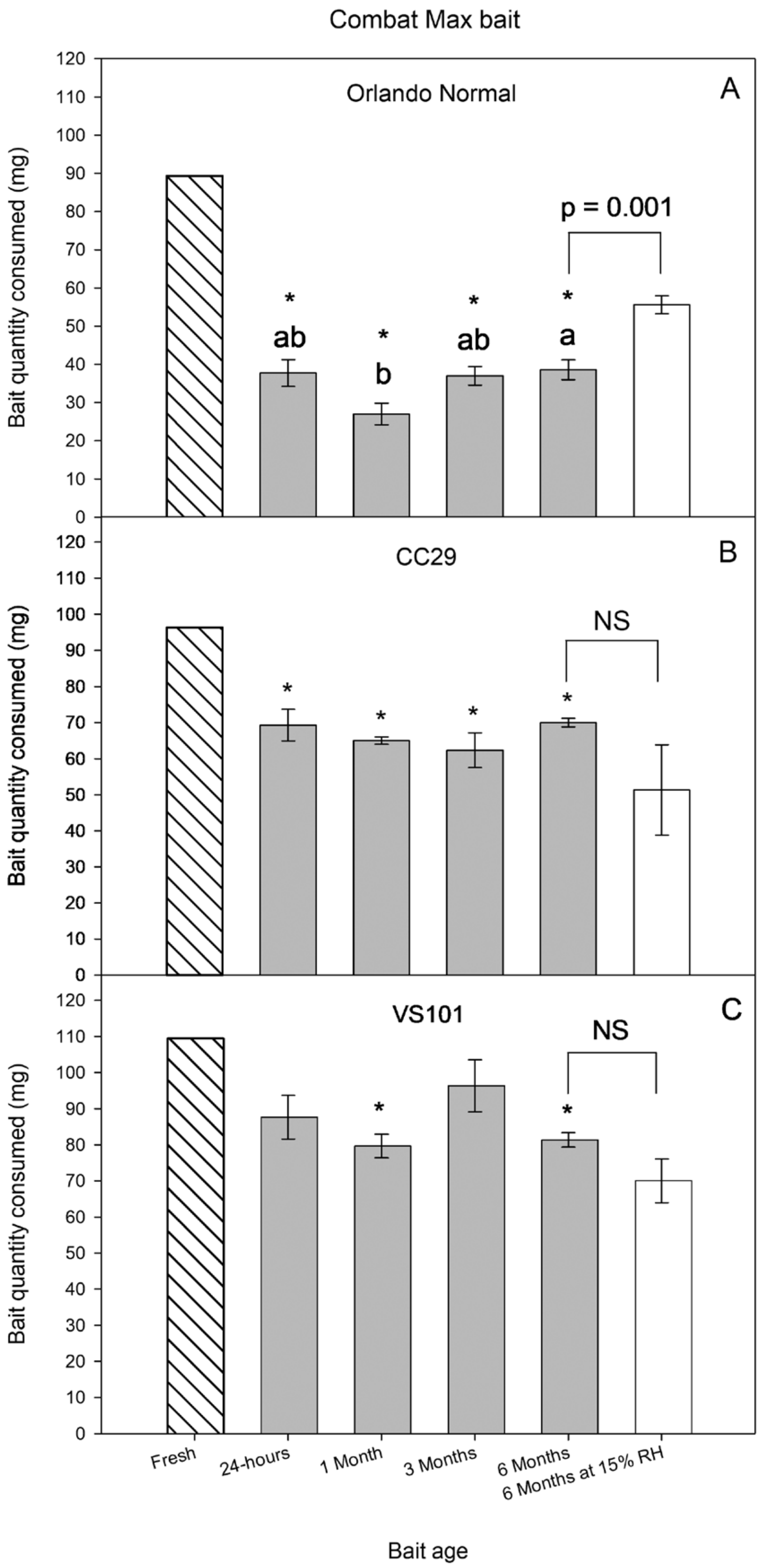

Fig. 6. Combat Max gel bait consumption (24 h) for the following populations: (A) Orlando Normal, (B) CC29, (C) VS101. Error bars represent the standard error of the mean (SEM). Asterisks above the bar graphs indicate consumption of aged bait at 40% relative humidity which is significantly different than the predicted fresh bait consumption value ($P < 0.05$). Different lowercase letters above bar graphs indicate significant differences among consumption of aged bait at 40% relative humidity (ANOVA, followed by independent *t*-test, $P < 0.05$). Brackets above bar graphs indicate a difference (*t*-test, $P < 0.05$), or lack of difference (*t*-test, NS = not significant, $P > 0.05$), between baits aged 6 mo at 40% and 15% relative humidity.

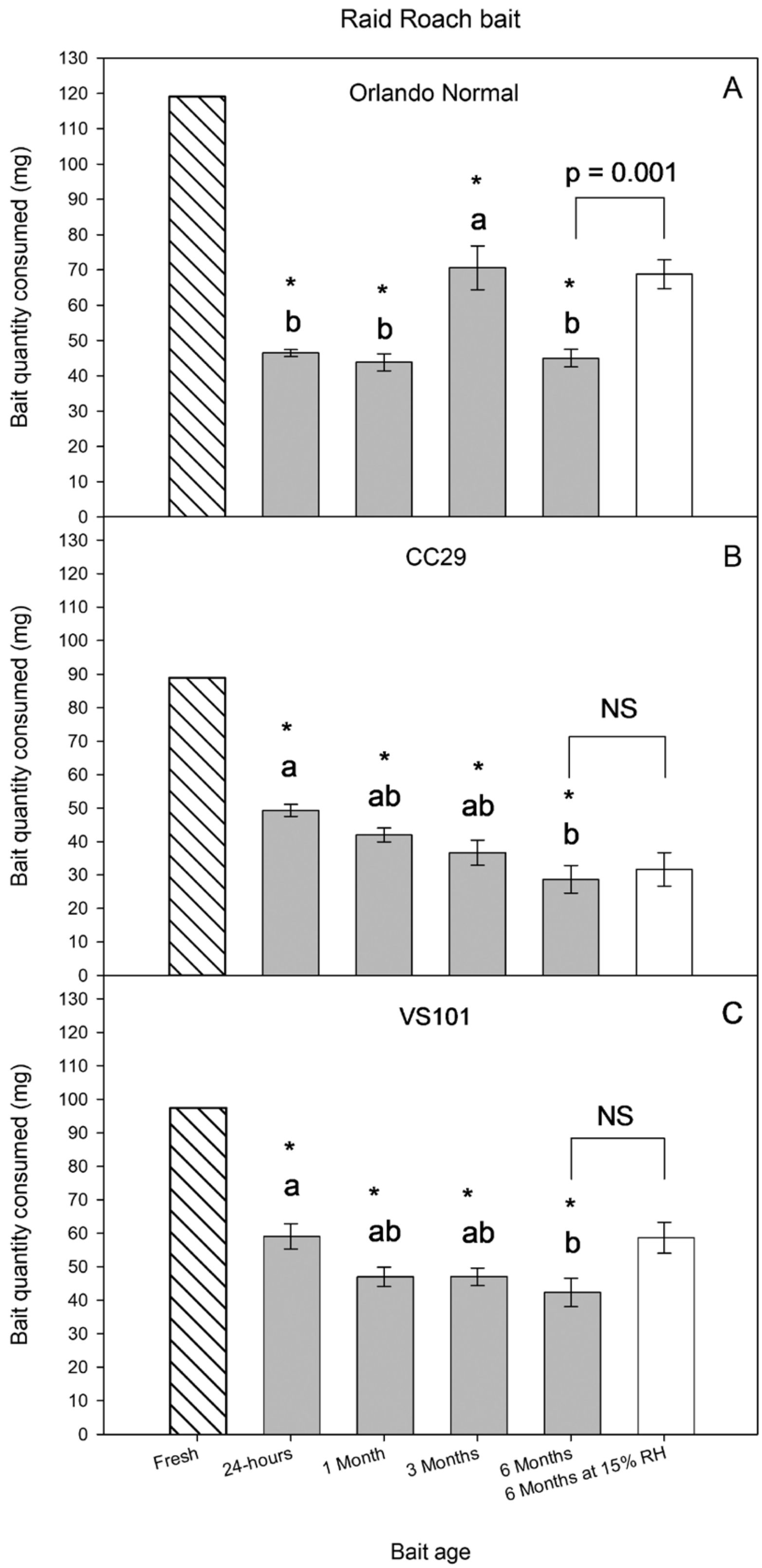

Fig. 7. Raid Roach gel bait consumption (24 h) for the following populations: (A) Orlando Normal, (B) CC29, (C) VS101. Error bars represent the standard error of the mean (SEM). Asterisks above the bar graphs indicate consumption of aged bait at 40% relative humidity which is significantly different than the predicted fresh bait consumption value ($P < 0.05$). Different lowercase letters above bar graphs indicate significant differences among consumption of aged bait at 40% relative humidity (ANOVA, followed by independent *t*-test, $P < 0.05$). Brackets above bar graphs indicate a difference (*t*-test, $P < 0.05$), or lack of difference (*t*-test, NS = not significant, $P > 0.05$), between baits aged 6 mo at 40% and 15% relative humidity.

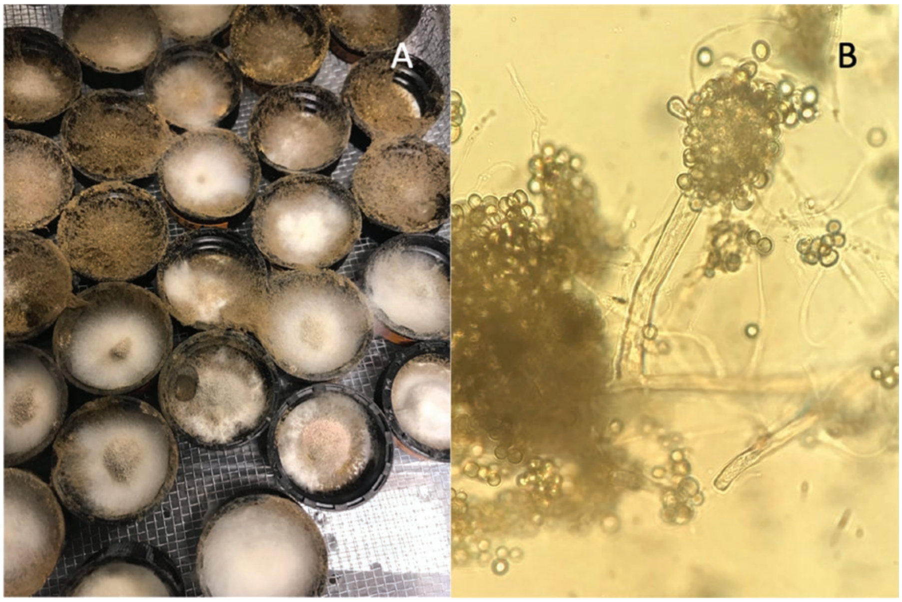

Fig. 8. A) Mold covering of baits aged for 3 to 6 mo at 80% RH, B) Photo of fungi spores (*Mucoraceae*) taken from moldy baits at 400× magnification.

We found the VS101 population consumed significantly less Raid Roach bait at 24 h (t_2 = 10.15, P = 0.01), 1 mo (t_2 = 17.68, P = 0.003), 3 mo (t_2 = 19.36, P = 0.003), and 6 mo (t_2 = 12.94, P = 0.006) than predicted fresh bait consumption (Fig. 7C). When comparing bait consumption among aging times, we found bait age to have a significant effect on bait consumption (Fig. 7C; $F_{3,8}$ = 4.32, P = 0.04). Post-hoc comparisons revealed the VS101 population consumed significantly more bait aged for 24 h than bait aged for 6 mo, although there was no difference among bait consumed at all other time points (Fig. 7C). Survivorship was not significantly different among aged baits (χ^2 = 1.1, df = 4, P = 0.89). Seven days after the bait was introduced, mortality reached 100% for all bait ages tested (Table 4).

Six-month Bait Consumption and Efficacy Comparison Between Baits Aged at 40% and 15% Relative Humidity

When comparing Maxforce FC Magnum bait consumption for baits aged 6 mo at 15% and 40% RH, we found a significant difference for both CC29 (Fig. 2B; t_4 = 7.55, P = 0.002; 15% less than 40%) and VS101 (Fig. 2C; t_4 = 3.10, P = 0.04; 15% less than 40%), but not for ON (Fig. 2A; t_8 = 1.26, P = 0.24). Survivorship was significantly affected by humidity for population CC29 (χ^2 = 14.6, df = 1, P < 0.001), but not for populations ON (both 100% by day 1) or VS101 (χ^2 = 1.1, df = 1, P = 0.30). For all populations, 6 mo 15% RH bait assay mortality was greater than 97% after 7 d (Table 4).

When comparing Advion Cockroach bait consumption for baits aged 6 mo at 15% and 40% RH, we found no significant difference for ON (Fig. 3A; t_8 = 1.10, P = 0.30), CC29 (Fig. 3B; t_4 = 0.44, P = 0.68), and VS101 (Fig. 3C; t_4 = 1.33, P = 0.25). Survivorship was not significantly affected by humidity for ON (χ^2 = 1.3, df = 1, P = 0.26), CC29 (χ^2 = 0.79 df = 1, P = 0.37), or VS101 (χ^2 = 0.36, df = 1, P = 0.55). For all populations, 6 mo 15% RH bait assay mortality was greater than 98% after 7 d (Table 4).

When comparing Vendetta Plus bait consumption for baits aged 6 mo at 15% and 40% RH, we found a significant difference for CC29 (Fig. 4B; t_4 = 5.18, P = 0.007; 15% less than 40%), but not for ON (Fig. 4A; t_8 = 0.62, P = 0.56) or VS101 (Fig. 4C; t_4 = 0.81, P = 0.46). Survivorship was significantly affected by humidity for populations ON (χ^2 = 19.8, df = 1, P < 0.001) and VS101 (χ^2 = 19.3, df = 1, P < 0.001), but not for population CC29 (χ^2 = 0.2, df = 1, P = 0.67). For all populations, 6 mo 15% RH bait assay mortality was 100% after 7 d (Table 4).

When comparing Alpine (Rotation 2) bait consumption for baits aged 6 mo at 15% and 40% RH, we found a significant difference for ON (Fig. 5A; t_8 = 6.03, P < 0.001; 15% greater than 40%), but not for CC29 (Fig. 5B; t_4 = 1.72, P = 0.16) or VS101 (Fig. 5C; t_4 = 1.54, P = 0.20). Survivorship was not significantly affected by humidity for ON (χ^2 = 1.6, df = 1, P = 0.20), CC29 (χ^2 = 2.7, df = 1, P = 0.10), or VS101 (both 100% by day one). For all populations, 6 mo 15% RH bait assay mortality was 100% after 7 d (Table 4).

When comparing Combat Max bait consumption for baits aged 6 mo at 15% and 40% RH, we found a significant difference for ON (Fig. 6A; t_8 = 4.87, P = 0.001; 15% greater than 40%), but not for CC29 (Fig. 6B; t_4 = 1.49, P = 0.21) or VS101 (Fig. 6C; t_4 = 1.77, P = 0.15). Survivorship was significantly affected by humidity for populations VS101 (χ^2 = 4.5, df = 1, P = 0.04) and ON (χ^2 = 5.2, df = 1, P = 0.02), but not for population CC29 (χ^2 = 0.26, df = 1, P = 0.61). For all populations, 6 mo 15% RH bait assay mortality was 100% after 7 d (Table 4).

When comparing Raid Roach bait consumption for baits aged 6 mo at 15% and 40% RH, we found a significant difference for ON (Fig. 7A; t_8 = 4.95, P = 0.001; 15% greater than 40%), but not for CC29 (Fig. 7B; t_4 = 0.46, P = 0.67) or VS101 (Fig. 7C; t_4 = 2.60, P = 0.06). Survivorship was not significantly affected by humidity for ON (χ^2 = 1.0, df = 1, P < 0.32), CC29 (χ^2 = 0.7, df = 1, P = 0.40), or VS101 (χ^2 = 1.2, df = 1, P = 0.28). For all populations, 6 mo 15% RH bait assay mortality was greater than 97% after 7 d (Table 4).

Baits Aged at 80% Relative Humidity Mold Growth

Bait aged for over 2 mo at 80% relative humidity developed mold growth on the surface (Fig. 8A). The fungi that constituted the mold was identified to be part of the family *Mucoraceae*, characterized by having the thallus not segmented or ramified (Fig. 8B).

Discussion

Cockroach gel baits differ in their matrix and consistency depending on product and manufacturer, but all six of the cockroach gel baits tested had greater than 40% water content when first applied (Table 2). On average, cockroach gel baits lost between 44.8% and 62.9% of their total water content within the first 24 h after application, resulting in a large water content difference between fresh and aged gel baits. Previous studies support our findings of gel baits losing over 50% of their water content in the first 24 h after application (Appel 1992). The water loss within the first 24 h and subsequently over time caused a change in the visual and physical texture of each gel bait. Baits aged for 6 mo or fully dried had a hard form (personal observation).

The significant reduction in water content of each bait within the first 24 h correlates with the significant decline in consumption of the bait from fresh bait to 24 h aged bait in 14/18 of the total consumption results separated by population and bait type (3 populations, 6 baits, 18 unique treatment combinations). German cockroaches rely on food, water, and shelter for survival, but water is generally regarded as the most limiting factor for survival (Willis and Lewis 1957, Appel et al. 1983, Bertholf 1983, Appel 2021). The water content of each bait at the time of introduction could have a significant effect on the quantity of bait consumed, with higher fresh bait consumption possibly being due to German cockroaches sensing water within the bait, not just the nutritional value or taste of the bait itself. The water content of the baits might also affect consumption through palatability based on bait consistency (Appel 1992). As all the baits age, their consistency changes and they begin to harden, which could cause consumption to be more difficult as the cockroaches would have to scrape and break away pieces. Therefore, the fresh bait being in a form closer to a liquid than a solid could make it easier for the cockroaches to consume.

The effect water has on bait palatability and consumption may also account for the differences in consumption among aged baits. When excluding fresh bait and just looking at the changes in consumption of the baits we aged, there are two distinct trends in bait consumption with different products: (i) a decline in consumption with bait age, and (ii) bait age having little to no effect on consumption quantity. The first trend is seen in Advion Cockroach bait consumption by CC29 (Fig. 3B) and VS101 (Fig. 3C), Alpine (Rotation 2) bait consumption by Orlando Normal (Fig. 5A) and CC29 (Fig. 5B), and Raid Roach bait consumption by CC29 (Fig. 7B) and VS101 (Fig. 7C). As previously noted, gel baits lose the majority of their water content within the first 24 h after application, but after 24 h, water continues to evaporate from the baits at a very slow rate (Table 2). Water loss could affect bait palatability, resulting in a negative correlation between water loss and bait consumption. If this is the case, it isn't surprising that different baits do not show the same trend, as the matrix of each bait not only affects the consistency the bait has over time but also affects the rate of water loss. However, it is interesting that baits show different consumption trends depending on the population. This could be due to the physiological and behavioral differences among populations. Future work should investigate consumption trends using additional field populations, mixed-stage, and mixed-sex German cockroaches to see if consumption preferences change with both bait type and bait age.

The second trend of bait age having little to no effect on consumption quantity is seen in Maxforce FC Magnum bait consumption by all three populations (Fig. 2), Advion Cockroach bait consumption by Orlando Normal (Fig. 3A), Vendetta Plus bait consumption by all three populations (Fig. 4), Alpine (Rotation 2) bait by VS101 (Fig. 5C), Combat Max bait consumption by all three populations (Fig. 6), and Raid Roach bait by Orlando Normal (Fig. 7A). This trend may lie in the bait matrixes and active ingredients. Maxforce FC Magnum roach killer bait gel has a more gelatinous and glossier matrix than the other five baits tested, which have a paste-like consistency. The consistency of Maxforce FC Magnum gel bait could play a role in its higher percent water retention past 1 month of age (Table 2) and be a possible cause of the trend of consistent consumption with age. However, its consumption pattern could also be due to the active ingredient, Fipronil, present in both Maxforce FC Magnum and Combat Max bait, which causes quick mortality, or illness, in the cockroach populations we tested. Furthermore, the arena size used in this study placed roaches in close proximity to the baits themselves, which could play a part in aged bait consumption quantities. In a field setting, the distance in which baits are applied from cockroach harborages, as well as the availability of alternative food sources, could affect consumption quantities and resulting mortality. Additional experiments are needed to investigate the interactions between the distance of bait placement from harborage and the resulting consumption quantities and mortality.

Although mortality varied by bait type and German cockroach population, mortality remained above 93% for all aged baits from 24 h to 6 mo of age (Table 4). These results show that even if cockroaches consume less aged bait than fresh bait, they still consume enough bait to cause mortality. One possible explanation for this could be that the active ingredient concentration in aged bait increases as the water concentration decreases over time, resulting in a larger dose of AI in the smaller quantities consumed of aged bait. Future work should look to investigate this, as well as if degradation of the AI is occurring as baits age. Overall, our results show cockroach gel baits do not lose their efficacy with age when tested under laboratory conditions.

Previous work on cockroach bait aging focused primarily on attractiveness over short periods of time. Nalyanya et al. (2001) found some baits aged for 7 d retained their attractiveness when tested in y-tube olfactometers. Further, they also found baits aged for 7 d to be attractive in field trials based on increased trap catch in baited jars compared to un-baited controls (Nalyanya et al. 2001), thus supporting our findings that baits remain palatable and effective with age, although only shown in their study for a much shorter period of time (7 d).

The extended aging time points we tested were selected to provide direction for the pest control industry regarding the timing between gel bait reapplication and visits. Historically, pest control operates on a systematic schedule (monthly, quarterly, biannually, etc.) to check progress and reapply products if needed. Our findings that baits remain palatable and effective for up to 6 mo (barring total consumption) suggest that reapplication may not always be necessary, but rather PMPs should focus on applying bait in new locations or using baits with different AIs or formulations to combat resistance and bait aversion (Silverman and Bieman 1993, Durier and Rivault 2003, Miller and Smith 2020, DeVries 2023).

To assess product performance over time in different environments, we chose to age all baits for 6 mo at both 15% and 80% relative humidity. In arid climates, a house may have a very low humidity level (15% RH). On the other extreme, 80% RH represents a very humid microclimate that could be found within a home in a warmer or more humid climate (Nguyen and Dockery 2016). When comparing baits aged for 6 mo at 40% relative humidity to baits aged for 6 mo at 15% relative humidity, we found most baits were not affected by aging at a low relative humidity (12 out of 18 were not significantly different when compared with their 6-mo 40% relative humidity values), and there were no discernable patterns among the populations tested (Figs 2–7). Although there were some differences in consumption due to relative humidity, both 6 mo aged bait at 40% and 15% relative humidity caused greater than 95% mortality for all baits and all cockroach populations (Table 4). This contradicts the assumption that exposure to low relative humidity over time would affect gel bait performance by speeding up water loss or causing greater water loss overall, and instead shows that applying gel baits in an arid climate does not cause them to lose their efficacy.

We also investigated how aging baits at a high relative humidity affected bait performance. For this experiment, we chose to age baits for up to 6 mo at 80% relative humidity. When the aging chambers were checked 2 mo after bait placement, a heavy growth of mold was observed on all baits (Fig. 8A). This was unexpected, as gel baits contain stabilizers to increase their use and shelf life. The fungi that constituted the mold was identified to be part of the family *Mucoraceae*, which is commonly found on decaying organic matter (Fig. 8B) (Walker 1913). This is a common mold, so the likelihood of it growing in a humid microclimate on a gel bait is high (Lennartsson et al. 2014). However, the mold covering spreading to all the baits in the chamber could be an artifact of the proximity of placement in the aging chamber. Therefore, further tests are needed to determine if some baits hold up better in humid environments than others. We also observed cockroaches willing to consume bait with mold growth, but only in areas not covered by mold, or where mold was physically wiped away (IML, personal observation). These findings suggest cockroach gel baits may not be suitable for humid microclimates, such as areas where water may be pooling or conditions are consistently damp. Further tests are needed to determine if this problem can be solved by adjusting the initial water content in the product or the stabilizers.

Although we observed several trends in bait consumption with age, the largest being the significant decline in consumption from fresh to 24 h aged bait, all cockroach populations consumed enough fresh and aged bait at all bait ages to cause greater than 87% mortality (Table 4). This is notable as other studies, like Lee et al. (2022), have shown that field populations with fipronil and pyrethroid-resistant traits showed reduced mortality when exposed to baits. However, the resistance levels for each field-collected population can differ depending upon the location collected and the pesticide exposure each population experienced. Our results support the findings by Gordon et al. (2025), who found much lower survivability of field populations treated with fipronil baits than Lee et al. (2022). Overall, our findings show that baits will maintain their efficacy and palatability for at least 6 mo after application. That said, it should be noted that the longevity of baits outside of the lab was not tested in the current study. Using cockroach gel baits as a management tool has been found to result in successful control of German cockroaches, but one of the main hindrances is the price of application and the time needed to apply gel baits properly (Miller and Meek 2004, Wang and Bennett 2006). Reapplying gel baits less often could help to reduce the overall cost associated with gel baits, as well as save PMPs time, improving the sustainability of this management tool. Furthermore, due to the extended period of efficacy and palatability we found in cockroach gel baits, it is possible they can be applied proactively to address new German cockroach infestations in at-risk areas before they can be established.

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Author contributions

Isabelle Lucero (Conceptualization [equal], Formal analysis [lead], Investigation [lead], Writing – original draft [lead], Writing – review & editing [equal]), Sudip Gaire (Conceptualization [equal], Funding acquisition [equal], Investigation [supporting], Writing – review & editing [equal]), and Zachary DeVries (Conceptualization [equal], Formal analysis [supporting], Funding acquisition [equal], Writing – review & editing [equal])

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