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COMPARISON OF COMMERCIAL BAIT FORMULATIONS FOR EFFICACY AGAINST BAIT AVERSE GERMAN COCKROACHES (*BLATTELLA GERMANICA*) (DICTYOPTERA: BLATTELLIDAE)

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Abstract Commercial gel bait formulations, including two new formulations of indoxacarb (0.6% AI; RG2V3 and DPX411), were evaluated for efficacy against a "bait averse" strain of German cockroaches (Saginaw strain). Bait consumption and subsequent mortality of the Saginaw cockroaches were compared with that of the VPI, susceptible strain. Both cockroach strains were exposed to bait formulations in choice tests where dry dog food was the alternative food resource. Feeding indices were calculated for each bait formulation. Feeding indices were positive for all bait products offered to the VPI susceptible strain. Negative feeding indices were calculated for MaxForce FC (0.01% fipronil), MaxForce (2.15% hydramethylnon) and Avert Formula 3 (0.05% abamectin B1) when these baits were offered to the bait averse Saginaw strain. However, feeding indices for the indoxacarb bait formulations were positive, indicating that the bait averse cockroaches preferred both of the indoxacarb baits to dog food. Mortality due to bait exposure was also significantly different between the susceptible and bait averse strains. In bioassays evaluating the VPI strain, all of the baits produced 100% mortality within the 7 d test period. The indoxacarb RG2V3 formulation produced the most rapid results with 100% mortality recorded within 24h. In bioassays evaluating the bait averse strain, mortality was dependent on the bait formulation offered. After 7d only 5% mortality was recorded for the Saginaw cockroaches exposed to fipronil bait. Hydramethylnon bait produced < 50% mortality during the same period. Mortality in the abamectin and indoxacarb DPX411 bioassays was 75% and 88.8%, respectively, in 7 d. However, the greatest efficacy for control of bait averse cockroaches was observed in bioassays evaluating indoxacarb RG2V3, where 100% mortality was achieved within 3 d.
Key Words Indoxacarb, gel bait, *Blattella germanica*, consumption, mortality

INTRODUCTION

Over the last decade bait formulations, particularly those with gel matrices, have become the preferred method of German cockroach control in homes, schools, and commercial facilities throughout the United States (Reierson, 1995; Harbison et al., 2003). These gel baits offer several advantages when compared with spray formulation insecticides. First, there have been only a few indications of physiological resistance (Wang et al., 2004) in the field to the most widely used active ingredients in the gel formulations (fipronil, hydramethylnon, abamectin, and imidacloprid). Second, these baits can be placed in precise locations where they are available to cockroaches but are inaccessible to people and pets. Perhaps the single greatest advantage of using gel baits is that they have been highly attractive to German cockroaches so that bait consumption and subsequent mortality result in superior control and customer satisfaction (Harbison et al., 2003). However, within the last decade, there have been documented cases of behavioral resistance to bait formulations among field populations of German cockroaches (Silverman and Ross, 1994; Wang et al., 2004).

Behavioral resistance is manifested by cockroaches avoiding a bait formulation that they would previously consume. This avoidance develops as the result of selection for aversion to the food ingredients within the bait matrix rather than to the active ingredients (Ross 1996). Silverman and Bieman (1993) documented the first case of nutrient avoidance in the T-164 strain German cockroaches. These cockroaches were averse to D-glucose, a typically phagostimulatory molecule present in several commercial bait formulations. The cause of the "bait aversion" was traced to a naturally occurring semi-dominant gene (*Glu*) within the cockroach population. As the susceptible cockroaches died, there was an increase in the proportion of cockroaches that exhibited the aversion behavior. Because of the potential of cockroaches to develop this aversion to glucose several manufacturers changed their bait matrices and reduced or eliminated D-glucose in the formulations (Silverman and Bieman, 1999). Although several of the most widely sold commercial bait formulations no longer use glucose as a food attractant, incidents of German cockroach bait aversion have developed again in recent years. In 1999, pest management professionals began to encounter German cockroach populations that

refused to eat gel bait formulations (Harbison et al., 2003; Morrison et al., 2004). Most of these populations were from locations where poor sanitation was a problem and baits had been used for extended periods to keep chronic cockroach infestations to a minimum (Wang et al., 2004). Wang et al., (2004) documented bait aversion behavior in two different cockroach strains from apartment units in Gary, IN and Cincinatti, OH. However, pest management professionals have reported many additional encounters with the bait aversion behavior across the nation (Kramer and Miller, 2004).

As the bait aversion behavior becomes more prevalent there is an urgent need for new bait formulations that provide all of the advantages of the older formulations, but do not elicit aversion behavior. Bait manufacturers have been re-formulating their bait matrices to replace ingredients that cause the averse behavior, e. g. MaxForce Select (Bayer Environmental Science, Raleigh, NC), while other chemical manufactures have been developing completely new bait products. The DuPont Company (Wilmington, DE) is in the process of developing two new cockroach gel baits that use indoxacarb (0.6%) as the active ingredient. Indoxacarb has already been documented as an effective dust bait and non-repellant toxicant in gel bait formulations at 0.25% AI (Appel, 2003). However, indoxacarb (0.6%) gel bait formulations have never been evaluated for efficacy in controlling bait averse cockroaches.

The research presented here is part of a comprehensive study to determine the efficacy of commercial and experimental bait formulations for controlling bait averse German cockroaches. Bait consumption and mortality were compared in bioassays where susceptible and "bait averse" cockroaches were exposed to different commercial bait formulations including 2 new formulations of 0.6% indoxacarb (DPX411 and RG2V3).

MATERIALS AND METHODS

Cockroaches. Bait consumption and subsequent mortality were compared in two strains of *B. germanica*. The VPI strain is a standard susceptible strain that has been maintained in the laboratory for >30 years and was colonized prior to the widespread use of synthetic insecticides and commercial cockroach baits (Ross and Cochran, 1966). The Saginaw strain was collected in 2002 from a single family rental home in Saginaw, MI. The cockroaches were collected for evaluation after the tenants complained that the baits used by their pest management company were not working (MaxForce FC, MaxForce Hydramethylnon, Siege Gel). Laboratory evaluations at VPI & SU determined that the Saginaw strain German cockroaches did exhibit the bait aversion behavior. Both the susceptible and "bait averse" cockroach strains were maintained in the laboratory at the VPI & SU Department of Entomology, Dodson Urban Pest Management Laboratory (DUPML) in Blacksburg, VA. Cockroaches were reared in plastic storage containers (17.83 L) on a diet of dry dog food. Cockroach containers were held at 25˚ C, ~ 60% RH, and on a photoperiod of 12:12 (L: D) h.

Bait Formulations. Five cockroach gel bait formulations were evaluated for their palatability and subsequent ability to kill bait averse German cockroaches. These baits were MaxForce FC Professional Insect Control Roach Killer Bait Gel (0.01% fipronil; Environmental Science, Montvale, NJ), MaxForce Professional, Insect Control Roach Killer Bait Gel (2.15% hydramethylnon), Avert Cockroach Gel Bait Formula 3 (0.05% abamectin B1; Whitmire MicroGen Research Laboratories, St. Louis, MO), and two experimental formulations of indoxacarb (0.6% active ingredient; DuPont Crop Protection, Wilmington DE).

Bait Consumption Bioassay. Prior to testing, groups of 20 adult male German cockroaches either susceptible or bait averse were transferred without anesthesia into plastic display boxes (6.6 L; arenas) lined at the bottom with heavy Kraft paper. Each arena contained a corrugated cardboard harborage and a vial of water plugged with a moist cotton ball. Cockroaches were allowed to acclimate to the closed arena (lid on) without food for 24 h.

No-Choice Tests. Commercial bait formulations (no experimental formulations) were evaluated in no-choice tests to determine if the Saginaw strain was in fact a bait averse strain. After the cockroach acclimation period, an aliquot (~1g) of a single bait formulation was put into a Petri dish (35 x 10 mm) and weighed gravimetrically on a balance. The weight of the dish plus the bait was recorded to the nearest 0.001 g. Individual dishes of bait were then put into each of the cockroach arenas. Lids were placed on the arenas and cockroaches allowed to feed ad libitum for 2 h. After 2 h the baits were removed and reweighed to determine cockroach consumption. Moisture controls were established for each bait formulation tested. These controls were used to correct bait weight change due to moisture gain or loss. The moisture controls consisted of a Petri dish of bait in a closed

arena as described above, except that no cockroaches were put into the arena. Each bait formulation had at least 5 replicates.

Choice Tests. Choice test bioassays were conducted for both commercial and experimental bait formulations. Choice tests were conducted as described for the no-choice consumption bioassays except that a Petri dish of commercial dry dog food pellets (~ 5g; Wells Pet Food Co., Monmouth, IL) was also put into the cockroach arena as an alternative food choice. Baits and dog food were weighed as described above and cockroaches were allowed to forage for 2 h. Moisture controls were established for both the dog food and bait formulations and used to correct consumption data. Each bait formulation had at least 5 replicates.

Bait Efficacy Bioassay. Groups of 20 adult male German cockroaches either susceptible or bait averse were transferred from rearing containers into plastic display boxes as described for the bait consumption bioassays. Cockroaches were allowed to acclimate for 24 h without food prior to testing. After the acclimation period the cockroaches were provided with an aliquot of bait and a competing food resource of dry dog food. Cockroaches were allowed to forage ad libitum and mortality was recorded every 24 h for 7 d. Control bioassays were set up as described above, except that control cockroaches were provided with 2 Petri dishes of dog food and no bait. Each mortality bioassay had at least 5 replicates.

Statistical Analysis. Bait consumption data in each bioassay was corrected for moisture loss (or gain), and the number of mg of bait consumed/cockroach was determined. No-choice consumption data was compared between the susceptible and the bait averse strains using the Student's *t*-test (SAS Institute, 2002). Consumption data from the choice tests was used to calculate a feeding index to determine cockroach response to the different baits: Feeding index = bait consumed (mg) - dog food consumed (mg)/ bait consumed (mg) plus dog food consumed (mg). A positive feeding index indicated a stimulatory feeding response and a negative index indicated a feeding aversion response (Silverman and Bieman, 1993). Cockroach feeding indices were compared between formulations (by strain) using Analysis of Variance (ANOVA). Means were separated using Fisher's Test of Least Significant Difference (SAS Institute, 2001). For all consumption tests, values of $P \leq 0.05$ were used to indicate significance. Mean cockroach mortality was corrected using Abbot's Formula (Abbott, 1926) and compared between the different bait formulations by day. Significant differences were indicated by the failure of the confidence intervals (CI; CI = 2 SE) to overlap.

RESULTS

Bait consumption bioassays. Prior to evaluating cockroach consumption of the toxic bait formulations, both cockroach strains were offered a diet of dry dog food in a no-choice bioassay to determine if there were inherent feeding differences between the strains. Finding no differences between the strains' consumption of dog food (Table 1) we initiated the no-choice bait bioassays. The VPI strain consumed significantly more of the abamectin and fipronil bait formulations than the Saginaw strain (Table 1). These consumption differences indicated that the Saginaw strain did indeed exhibit the gel bait aversion behavior. Interestingly, consumption of the hydramethylnon formulation was not significantly different between the susceptible and the bait averse cockroach strains. The Saginaw strain response to the hydramethylnon bait in these bioassays was unique because preliminary bioassays evaluating the Saginaw cockroaches (Miller and McCoy, unpublished data) determined that there was an aversion to the hydramethylnon formulation.

The VPI strain feeding indices for the commercial and experimental bait formulations are shown in Figure 1. If a positive response indicates a stimulation of feeding behavior, we can conclude that all of the baits tested were stimulatory to the VPI strain, including the two 0.6% indoxacarb formulations. Although all feeding indices were positive, the feeding index calculated for the 0.01% fipronil bait was significantly lower (P = 0.003) than the other baits tested (Figure 1).

The feeding indices calculated for the Saginaw strain indicated that the abamectin, hydramethylnon, and fipronil baits all induced the feeding aversion response (Figure 2). A negative feeding index was calculated for all three commercial baits tested, however, the indices for the MaxForce products were significantly lower than that of the abamectin bait. In contrast, the feeding indices calculated for the two 0.6% indoxacarb bait formulations were positive for the bait averse cockroach strain. These results indicated that although the Saginaw strain was averse to the other commercial gel bait formulations, the 0.6% indoxacarb formulations actually stimulated the feeding response. Both 0.6% indoxacarb formulations had positive feeding indices but

Table 1. Consumption (2 h) comparison of commercial gel bait formulations offered to VPI susceptible and Saginaw "bait averse" strains of German cockroaches.

Cockroach Strain	Bait Formulation	Consumption mg/cockroach ± SEM	n	*t* - Value	*P* - Value*
VPI	0.05% abamectin B1	5.77 ± 0.40	10	-9.88	<0.0001
Saginaw	0.05% abamectin B1	0.05 ± 0.03	5		
VPI	0.01% fipronil	2.67 ± 0.13	9	-5.09	0.0002
Saginaw	0.01% fipronil	0.65 ± 0.46	6		
VPI	2.15% hydramethylnon	1.88 ± 0.38	5	-0.20	0.847
Saginaw	2.15% hydramethylnon	1.73 ± 0.64	5		
VPI	dog food	1.19 ± 0.24	12	-0.92	0.37
Saginaw	dog food	0.89 ± 0.24	7		

* Values of P ≤ 0. 05 indicate significance (SAS Institute 2001)

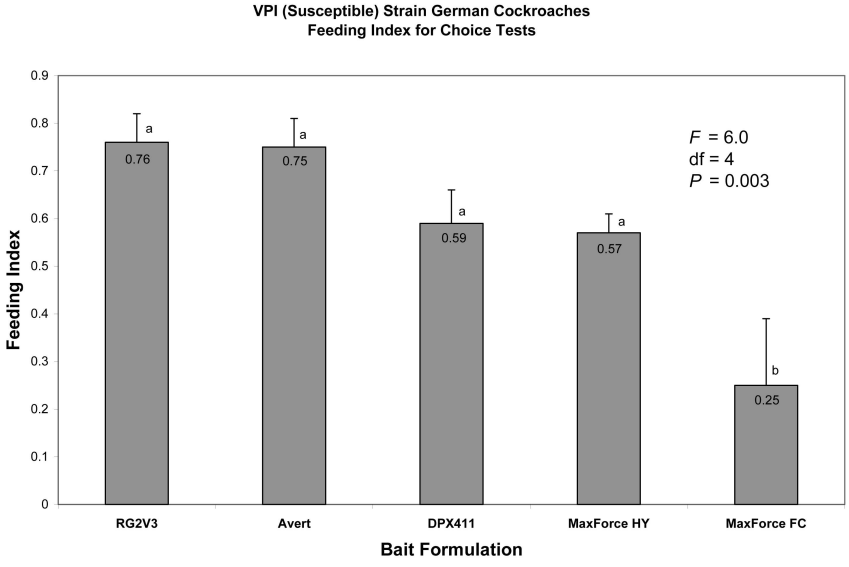

Figure 1. Feeding indices (mean ± SEM) for bait formulations offered to the VPI susceptible strain cockroaches. Feeding indices followed by the same letter are not significantly different; Fisher's Test of Least Significant Difference; $P \geq 0.05$.

the feeding index for the RG2V3 formulation was significantly higher ($P < 0.001$) than that of the DPX411 formulation (Figure 2).

Bait Efficacy Bioassay. German cockroach mortality reflected the feeding indices calculated for the bait consumption data. The VPI susceptible strain which had positive feeding indices for all baits tested were very susceptible to all bait formulations, with 100% mortality in all bioassays at the end of 7 d (Figure. 3). The indoxacarb RG2V3 formulation was the fastest acting bait, producing 100% mortality within 24 h. Both the indoxacarb DPX411 and the fipronil formulations produced 100% mortality within 48 h. The abamectin and hydramethynlnon formulations produced 100% mortality by days 6 and 7, respectively (Figure 3).

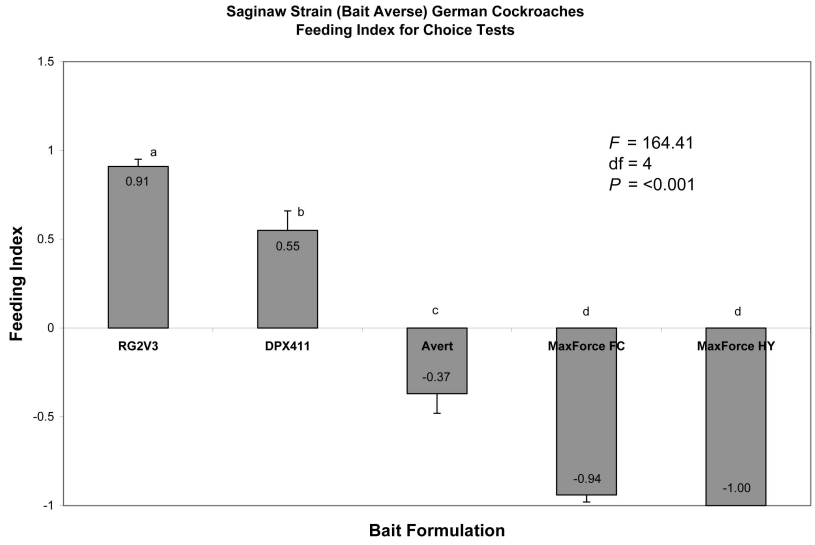

Figure 2. Feeding indices (mean ± SEM) for bait formulations offered to the Saginaw bait averse strain cockroaches. Feeding indices followed by the different letters are significantly different; Fisher's Test of Least Significant Difference; $P \leq 0.05$.

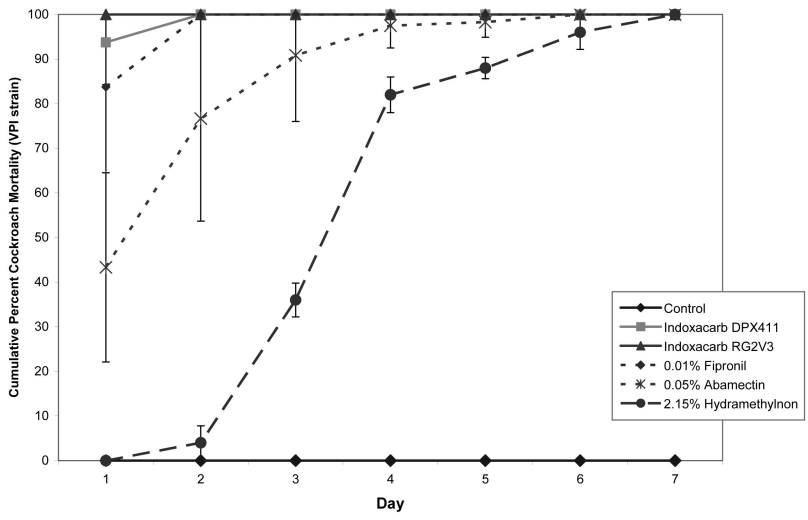

Figure 3. Cumulative daily mortality (mean ± confidence intervals (CI)) for VPI susceptible strain cockroaches fed commercial and experimental bait formulations. Significant differences in mortality are identified by failure of CI values to overlap; $P \leq 0.05$.

As expected from the bait consumption results, mortality for the bait averse Saginaw strain varied with the bait formulation offered. Mortality for bait averse cockroaches offered fipronil bait was very low with only 5% dead after 7 d (Figure 4). Mortality in the hydramethylnon bait bioassays was significantly greater than that in the fipronil bioassays with 46% mortality after 7 d. The abamectin bait performed significantly better than either of the MaxForce products with 75% mortality after 7 d. The most effective and fastest acting baits were the 0.6% indoxacarb formulations. Mortality in the indoxacarb DPX411 bioassays reached 88.8% by day 7 and the indoxacarb RG2V3 formulation reached 100% by day 3 (Figure 4).

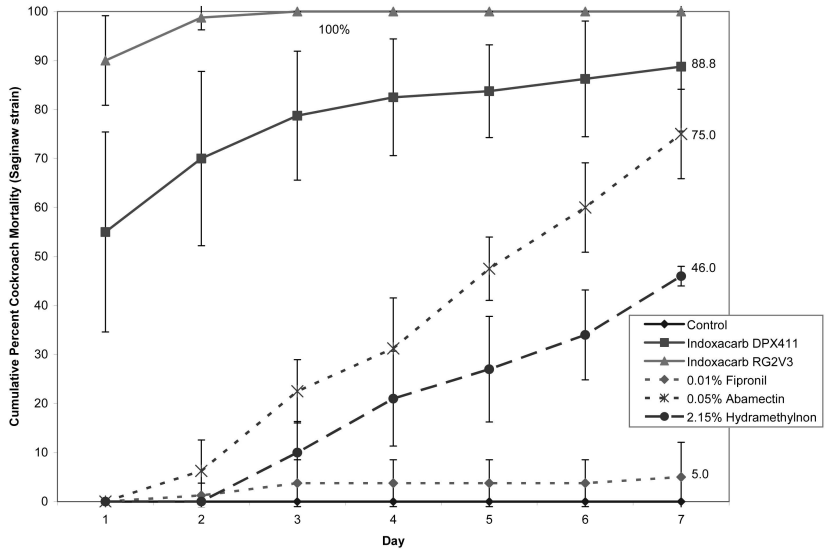

Figure 4. Cumulative daily mortality (mean ± confidence intervals (CI)) for Saginaw bait averse strain cockroaches fed commercial and experimental bait formulations. Significant differences in mortality are identified by failure of the CI values to overlap; $P \leq 0.05$.

DISCUSSION

Silverman and Ross (1994) documented behavioral resistance to D-glucose in 1993, and predicted that resistance to the components in bait matrices might become a significant factor in the long term effectiveness of baits. Time and the shift in German cockroach management practices to widespread bait use have supported this hypothesis (Kramer and Miller, 2004). While the frequency of behaviorally resistant cockroaches is typically low in field populations, the number of these individuals has increased greatly over several generations due to bait induced selection (Braness, 2004). Thus, we are finding populations of bait averse cockroaches in many different geographic locations across the nation (Kramer and Miller, 2004).

Similar to the Cincy (Ohio) and Dori (Indiana) cockroach strains evaluated by Wang et al. (2004), the Saginaw strain (Michigan) exhibited "aversion" behavior in response to gel bait formulations containing different active ingredients. Therefore, we were able to conclude that the Saginaw strain was indeed bait averse, although they did not respond to all commercial gel bait formulations the same way.

The response of the Saginaw strain to the hydramethylnon bait in the no-choice tests was unexpected. In preliminary evaluations (not reported), as in the choice tests conducted in this study, Saginaw consumption of the hydramethylnon bait was always significantly less than that of the VPI strain. The fact that consumption was the same between the bait averse and susceptible strains in our no-choice tests suggested that a change in the genetic composition of the bait averse population may be occurring. On closer examination of the no-choice data, we observed that in two of the five replications there was almost no feeding (average 0.19 mg/cockroach) on the hydramethylnon bait formulation. Yet in 3 of the replications, consumption was relatively high (average 2.7 mg/cockroach). Because these cockroaches have been in colony for > 1 year with no exposure to any commercial bait matrices it is possible that the aversion behavior within the population is becoming less prevalent. However, because of the negative feeding index calculated for the hydramethylnon bait in the choice tests (strongly indicating an aversion response), further investigation will be needed to truly determine if the aversion behavior is decreasing or if these no-choice results are anomalous.

The results of the choice-test bioassays indicated that the feeding indices and subsequent mortality were the two most important indicators of cockroach strain differences in response to gel bait formulations. The bait consumption results in our study were similar to those published by Wang et al. (2004) although the baits were evaluated differently. Wang et al. (2004) found that the bait averse German cockroaches (Cincy strain) consumed significantly less of Avert and MaxForce FC bait matrices (no active ingredient) than the susceptible Jwax strain in choice tests. Wang et al. (2004) therefore concluded that the aversion was caused by the inert ingredients in the bait matrix and not the active ingredient. In our study, the Saginaw strain was also averse to the MaxForce and Avert products although these products were willingly consumed by the VPI strain.

The Saginaw strain was not averse to the experimental formulations of indoxacarb (0.6%). Feeding indices indicated that both of the indoxacarb formulations were phagostimulatory for Saginaw and VPI strain cockroaches. From these data we conclude that the indoxacarb formulations either do not contain the ingredients responsible for inducing the aversion behavior or that the effects of these ingredients are suppressed by other ingredients in the bait matrices.

Because cockroach mortality is the result of toxic bait consumption, bait aversion behavior can significantly reduce the efficacy of commercial bait formulations. The response of the VPI cockroaches to the fipronil formulation compared with that of the Saginaw cockroaches exemplifies how a bait can be extremely effective for controlling a population of cockroaches that are willing to consume it, yet completely ineffective in controlling another population that will not. However, this study documents that new bait products (e.g. 0.6% indoxacarb) that have been formulated to reduce or eliminate those ingredients that induce the bait aversion response can be very effective for controlling both susceptible and bait averse cockroach strains.

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REFERENCES CITED

Abbott, W.S. 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265-267.

Appel, A.G. 2003. Laboratory and field performance of an indoxacarb bait against German cockroaches (Dictyoptera: Blattellidae). J. Econ. Entomol. 96: 863-870.

Braness, G.A. 2004. Insecticides and pesticide safety. In, Mallis, A.: Handbook of Pest Control. GIE Media Inc, Cleveland, OH. pp. 1098-1163.

Kramer, R.D. and D.M. Miller. 2004. Bet on the roaches. Pest Control Technology. 32: 54, 56, 58-60.

Morrison, G., J. Barile and T.E. Macom. 2004. Roaches take the bait again. Pest Control. Technol. 32: 62, 64, 66.

Ross, M.H. 1996. Behavioral modifications and their implications for cockroach resistance to toxic baits. In, K. B. Wildey (ed.), Proceedings of the International 2nd Conference on Insect Pest s in the Urban Environment, Edinburgh, U.K. BPC Wheatons, Exeter, UK, pp. 393-399.

Ross, M.H. and D.G. Cochran. 1966. Genetic variation in the German cockroach. I. Additional genetic data and the establishment of tentative linkage groups. J. Hered. 57: 825-834.

SAS Institute. 2001. SAS/STAT User's guide, version 8.1. SAS Institute, Cary, NC.

Silverman, J. and D.N. Bieman. 1993. Glucose aversion in the German cockroach, *Blattella germanica*. J. Insect. Physiol. 39: 925 933.

Silverman, J. and D.N. Bieman. 1999. High fructose containing insecticide compositions and methods using same. United States Patent: 5,906,983 [Online]. Available: http://patft.uspto.gov/netahtml/search-bool.html.

Silverman, J. and M.H. Ross. 1994. Behavioral resistance of field-collected German cockroaches (Blattodea: Blattellidae) to baits containing glucose. Environ. Entomol. 23: 425-430.

Wang, C., M.E. Scharf and G.W. Bennett. 2004. Behavioral and physiological resistance of the German cockroach to gel baits (Blattodea: Blattellidae). J. Econ. Entomol. 97: 2067-2072.