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J.L. Lewis [ID] and B.T. Forschler

Department of Entomology, University of Georgia, Athens, GA, USA

ABSTRACT

Eastern subterranean termite, *Reticulitermes flavipes* (Kollar), workers were exposed for 7 days to one of five chitin synthesis inhibitors (CSIs): diflubenzuron, hexaflumuron, lufenuron, noviflumuron, and novaluron in commercially available bait matrices. Following a 7 day exposure period, termite donors (D) were combined with naïve (not exposed) termite recipients (R) at five D:R ratios (20:0, 15:5, 10:10, 5:15, and 1:19) and mortality determined daily for up to 68 days. Lethal time and percent mortality data suggest efficient transfer at all D:R ratios for all CSIs tested, except diflubenzuron at 1:19. Despite the data indicating transfer of lufenuron in bioassay, this material may not be effective in field use based on behavioral observations that include limited movement by donors. We also report frequency of visible evidence of CSI intoxication, including the previously described "jackknife" pose and an additional physical deformity, we term "curled-body". The implications these data and observations have for laboratory evaluation and field population management using commercial termite baiting systems is discussed.

Introduction

The history of managing social insect pests has long included the use a toxic food matrix – termed a bait – with the intent of using the victim's social behaviors to assist in the control effort (Kofoid 1934; Vinson 1986; Williams et al. 2001; Evans & Iqbal 2014). Termites are social insects of world-wide importance in wildlands as integral members of processes involving the degradation and recycling of carbon whilst in agriculture, urban and human-built habitats are often serious economic pests (Su & Scheffrahn 2000; Jouquet et al. 2001; Rouland-Lefevre 2011; Evans et al. 2013). The commercialization of termite baits has demonstrated the utility of the baiting paradigm against the subterranean termites (Rhinotermitidae), yet there are concerns related to the efficacy of termite baiting, including the end-result of colony elimination, mode of transfer, and speed of action (Evans & Iqbal 2014). There is, however, little doubt that the most successful termite bait toxins involve chitin synthesis inhibitors (CSIs) (Evans & Iqbal 2014).

Chitin, an amino-polysaccharide (poly-β-(1, 4)-N-acetyl-D-glucosamine), is a major structural component of insect cuticle synthesized in ectodermal cells of the trachea, salivary glands, epidermis, foregut, and hindgut (Anderson 1979; Cohen 1987). Benzoylphenyl urea insecticides are considered CSIs that cause death by interfering with cuticle sclerotization

during molting (Cohen 1987). CSIs display arthropod specificity and delayed toxicity and have been examined as an alternative to neurotoxin insecticides (Verloop & Ferrell 1977; Retnakaran & Wright 1987; El Saidy et al. 1989; Su & Scheffrahn 1991; Medina et al. 2003). Slow acting toxicants have limited utility in crop protection but provide advantages when used in a pesticidal baiting program against social insects (Williams & Lofgren 1981; Su et al. 1982; Reierson 1995).

Baits containing CSIs were commercialized for termite control in the late 1990s because these active ingredients (AIs) demonstrated ready consumption within a range of concentrations, and delayed toxicity (Su et al. 1982; Su et al. 1987; French 1994; Su 2003; van den Meiracker et al. 2002; Evans 2010). The CSI AIs used in USEPA registered termite baiting systems include diflubenzuron 0.25% AI (Whitmire Micro-Gen, St. Louis, MO), hexaflumuron 0.50% (Dow AgroSciences, Indianapolis, IN), noviflumuron 0.50% (Dow AgroSciences, Indianapolis, IN), lufenuron 0.15% (Syngenta Corporation, Greensboro, NC), and novaluron 0.5% (BASF Corporation, Reaserch Triangle Park, NC). These CSIs are analogs differing in halogen substitution or side-chain modification on the phenyl ring and it is assumed that they have the same mode of action, impact on termite behavior, transfer efficiency, and dose response (Figure 1).
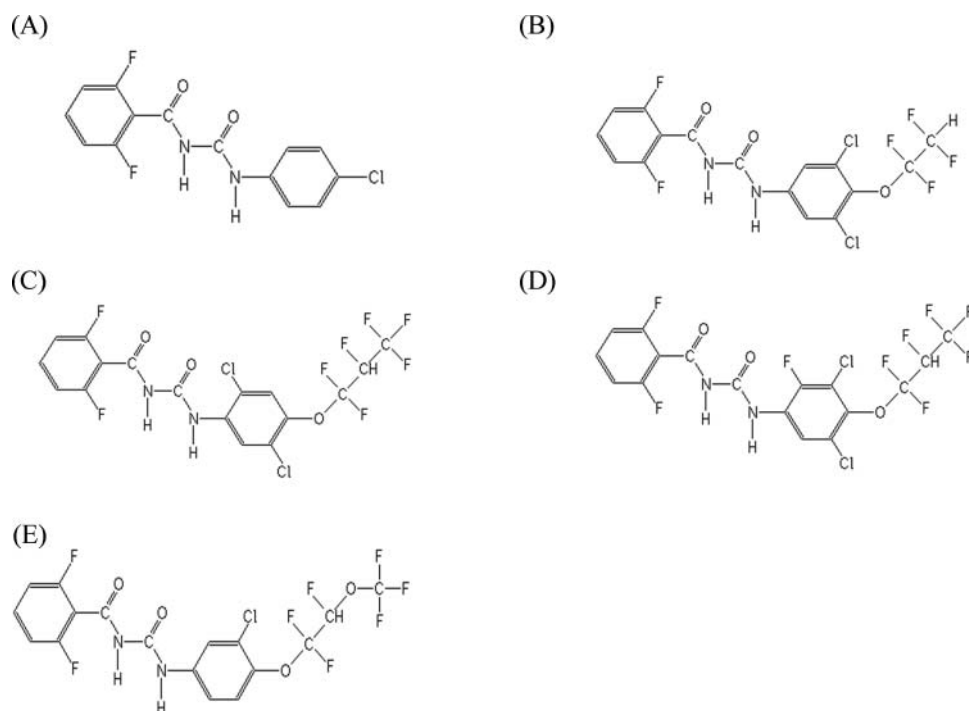
Figure 1. Chemical structure of benzoylphenyl urea insect growth regulators. (A) Diflubenzuron. (B) Hexaflumuron. (C) Lufenuron. (D) Noviflumuron. (E) Novaluron.

We used laboratory bioassays to record mortality associated with each of five CSI-containing termiticidal baits using the eastern subterranean termite, *Reticulitermes flavipes* (Kollar). Efficiency of CSI transfer was measured using five donor-to-recipient (D:R) ratios along with notation of cannibalism and other physical deformities. The null hypothesis was that all CSI AIs would provide the same profile of mortality and be equally efficacious in transfer between nestmates.

Material and methods

Insects and chemicals

Five populations of *R. flavipes* (Kollar) were collected from field sites, separated by at least 100 m, from Whitehall Forest, Clarke County, Georgia. Termites were identified to species using published keys to the soldier caste (Scheffrahn and Su 1994). Termites were collected using moistened corrugated cardboard and placed into a plastic container (26.99 × 19.37 × 9.52 cm) with weathered pine wood slats (approx. 12.5 × 2.54 × 0.2 cm) in complete darkness inside an environmental chamber (27 °C, ≥90% relative humidity (RH)) until use in bioassay (Forschler and Townsend 1996). Termites used as donors were obtained by placing 300 workers (4th instar or older) in a plastic Petri dish (100 × 25 mm diameter) with 20 grams of sand moistened with 3.2 ml of distilled water for 7 days with a known weight (6.5 ± 1.5 g) of the appropriate commercially available bait matrix (proprietary α-cellulose formulations) containing one of the following CSI treatments: no CSI (control),

diflubenzuron 0.25% (Whitmire Micro-Gen, St. Louis, MO), hexaflumuron 0.50% (Dow AgroSciences, Indianapolis, IN), noviflumuron 0.50% (Dow AgroSciences, Indianapolis, IN), and 0.5% novaluron (BASF Corporation, Research Triangle Park, NC). The lufenuron (Syngenta Corporation, Greensboro, NC) treatments were presented on 2 ± 0.2 g corrugated cardboard at 0.15% AI as this is the bait matrix of that commercial product.

Toxicant transfer

Donor termites were placed in a plastic Petri dish (65 × 15 mm diameter) containing filter paper (Whatman #1, 55 mm diameter) moistened with distilled water. Nestmate workers from the same population (recipient termites) were added to provide various D:R ratios for a total of 20 workers per Petri dish. Recipient termites (R) were treated as described for the donor termites (D) except R were exposed to non-treated α-cellulose tablets and marked with DecoColor Paint Marker (Uchida American DecoColor Paint marker) to differentiate them from D. Several D:R ratios were tested: 20:0, 15:5, 10:10, 5:15, and 1:19. Each D:R was replicated at least 5 times from each termite population.

Data collection

All dead and moribund termites were removed daily and the condition (whole or missing body parts) of each cadaver was noted. We set a limit of <20% mortality in the corresponding control as the benchmark

Figure 2. Physical deformities observed in termites exposed to CSIs. (A) Jackknife pose. (B) Curled body.

for including a replicate in the data analysis. Information on the presence of termites found in the "jackknife" position (Su & Scheffrahn 1993) characterized by the head and last abdominal segments being in close proximity because the thorax and first abdominal segments were raised (Figure 2(A)). Termites in the jackknife pose also displayed visibly wrinkled cuticle near the tip of the abdomen (Figure 2(A)). We also observed termites that arched in the opposite direction that we term the "body curl" position (Figure 2(B)). The body curl was not characterized by any deformity of the cuticle. The body curl position was observed only in moribund termites because in death these termites "relaxed" into a normal straight position.

Statistical analysis

Mortality data were adjusted using Abbott's formula (Abbot 1925) and compared using SAS-JMP (version 7.0) statistical software (2007 SAS Institute, Inc., Cary, NC) by treatment, ratio, and time. Termite mortality was evaluated with general linear model (GLM) analysis of variance and Tukey–Kramer honestly significant difference test for multiple mean comparisons ($\alpha = 0.05$). Mortality data were also subjected to probit regression to obtain lethal time (LT) estimates. If the confidence interval (CI) ($\alpha = 0.05$) of the LT values did not overlap, they were considered significantly different.

Results

A comparison of LT estimate differences based on CI overlap provided similar LT_{90} values for all CSIs tested with the first two D:Rs (20:0 and 15:5) (Table 1). The 10:10 D:R indicated no difference between the LT_{90} estimates for the novaluron, hexaflumuron, noviflumuron, and lufenuron treatments, that lufenuron and diflubenzuron were not different but diflubenzuron was different than the other three (Table 1). However, diflubenzuron clearly separates statistically from all the other CSIs at the 5:15 and 1:19 D:Rs with longer LT_{90} estimates (Table 1). Lufenuron at the 1:19 D:R also statistically separates from the remaining chemistries (Table 1).

The LT_{50} data indicate a similar trend when comparing CSI treatments (Table 1). The LT_{50} data examined within treatments implies that noviflumuron is transferred most efficiently as signified by the lack of statistical difference across all D:R ratios. The difference in LT_{50} values for the hexaflumuron and novaluron D:R comparisons were statistically the same despite ranging between 4 and 5 days (Table 1). The lufenuron LT_{50} data display a clear statistical trend toward less transfer as the number of donors decrease as well as diflubenzuron – especially at the two lower D:R ratios (Table 1).

The percent mortality data, by treatment, from the 20:0 ratio provided the benchmark for efficacy assuming all exposed termites consumed the respective toxicant during the 7 days they were confined with the treated matrix (Figure 3(A)). All the CSI treatments at the 20:0 D:R and 27 °C, provided sufficient mortality (>97%) by day 68 to conclude the bioassay (Figure 3 (A); $F = 589.60$; df $= 11$; $p < 0.0001$). The mortality data in the remaining D:Rs ($F = 272.62$, $F = 221.88$, $F = 76.79$, $F = 73.82$, respectively) demonstrate the effective transfer of CSIs in this confined bioassay system (Figure 3(B)–3(E); df $= 11$; $p < 0.0001$). Hexaflumuron, noviflumuron, and novaluron provided equivalent mortality at days 42 (df $= 20$; $F = 35.65$) and 68 (df $= 15$; $F = 29.48$) (Figure 3(A)–3(E); $p < 0.0001$). Lufenuron provided higher mortality on days 7 ($F = 5.41$) and 21 ($F = 6.02$) in the 20:0 D:R treatment indicating a quicker affect compared to the other CSIs that was not realized in the lower D:Rs (Figure 3; df $= 11$; $p < 0.0001$). Lufenuron mortality was statistically similar to the three previously mentioned CSIs at days 21 and 42 for all D:Rs except 1:19 (df $= 11$; $F = 73.82$; $p < 0.0001$) indicating a reduction in transfer at low donor ratios (Figure 3(E)). The diflubenzuron treatment produced statistically lower mortality by day 68 in the 10:10 and 5:10 D:Rs (df $= 7$; $F = 20.21$; $p < 0.0001$) and at day 42 in the three lowest D:Rs – 10:10, 5:10, and 1:19 (df $= 9$; $F = 12.67$; $p < 0.0001$), indicating reduced transfer compared to the other CSIs (Figure 3).

Table 1. LT estimates (LT$_{50}$ and LT$_{90}$) ($\pm$95% CI) by treatment, CSIs and D:R ratios along with corresponding regression slopes for *Reticulitermes flavipes*.

Trmt[a]	D:R[b]	LT$_{50}$ (95% CI) [c,d]		LT$_{90}$ (95% CI) [c,d]		Slope $\pm$ SE
CON		253 (218 − 304)		456 (390 − 552)		0.20 $\pm$ 0.02
NVL	20:00	24 (22 − 25)	A	42 (40 − 45)	A	2.16 $\pm$ 0.13
	15:05	24 (22 − 25)	A	43 (40 − 48)	A	2.04 $\pm$ 0.17
	10:10	23 (22 − 24)	A	42 (40 − 44)	A	2.11 $\pm$ 0.10
	5:15	27 (26 − 29)	B	46 (43 − 50)	A	2.13 $\pm$ 0.13
	1:19	25 (24 − 27)	AB	44 (41 − 46)	A	2.21 $\pm$ 0.12
DFB	20:00	24 (22 − 26)	A	43 (40 − 46)	A	2.13 $\pm$ 0.16
	15:05	27 (25 − 28)	A	45 (42 − 48)	A	2.20 $\pm$ 0.12
	10:10	28 (27 − 29)	A	48 (46 − 50)	A	1.99 $\pm$ 0.08
	5:15	54 (47 − 68)	B	97 (80 − 129)	B	0.94 $\pm$ 0.15
	1:19	67 (59 − 78)	B	121 (104 − 148)	B	0.73 $\pm$ 0.08
HEX	20:00	23 (22 − 24)	A	40 (39 − 42)	A	2.28 $\pm$ 0.11
	15:05	22 (21 − 23)	A	39 (38 − 41)	A	2.33 $\pm$ 0.10
	10:10	26 (25 − 27)	B	43 (41 − 45)	AB	2.31 $\pm$ 0.09
	5:15	26 (25 − 27)	B	43 (41 − 46)	AB	2.29 $\pm$ 0.11
	1:19	27 (26 − 28)	B	45 (43 − 47)	B	2.28 $\pm$ 0.08
NFM	20:00	25 (23 − 27)	A	43 (40 − 46)	A	2.23 $\pm$ 0.15
	15:05	25 (23 − 27)	A	44 (41 − 48)	A	2.07 $\pm$ 0.14
	10:10	26 (25 − 27)	A	44 (42 − 45)	A	2.25 $\pm$ 0.08
	5:15	29 (27 − 31)	A	48 (44 − 53)	A	2.09 $\pm$ 0.16
	1:19	29 (27 − 30)	A	47 (44 − 50)	A	2.18 $\pm$ 0.12
LUF	20:00	17 (13 − 19)	A	39 (36 − 44)	A	1.76 $\pm$ 0.19
	15:05	21 (19 − 23)	A	39 (37 − 42)	A	2.18 $\pm$ 0.16
	10:10	25 (24 − 26)	B	44 (42 − 47)	A	2.10 $\pm$ 0.10
	5:15	26 (24 − 27)	B	44 (41 − 47)	A	2.21 $\pm$ 0.14
	1:19	32 (31 − 34)	C	53 (50 − 57)	B	1.91 $\pm$ 0.11

Note: [a]Trmt = treatments were exposed to termites for 7 days and include: CON = control (α cellulose), NVl = novaluron, DFB = diflubenzuron, HEX = hexaflumuron, NFM = noviflumuron, LUF = lufenuron. Mortality was counted daily for 42 days after initial exposure.
[b]D:R = donor-to-recipient ratio.
[c]LT = lethal time by day for 50% or 90% mortality.
[d]CI = confidence intervals followed by the same upper case letter, within a treatment, indicate that CI overlap and LT values for those D:R ratios are considered similar.

Observations from cadaver removal

D:R ratio was not an important variable in the observed condition/treatment of the dead (df = 47, 308; F = 1.08; p = 0.3603) and, therefore, data were pooled by CSI treatments. The daily procedure of removing dead termites from bioassay showed that the CSI treatments had a significant effect on observed acts of cannibalism, with most of the cadavers being intact (65%–75%; N = 60) (Figure 4). The number of dead removed from the controls, by contrast, provided a much lower percentage of intact cadavers (24 $\pm$ 32%; N = 60/CSI; df = 5, 47; F = 52.67; p < 0.0001) (Figure 4.). Body parts most likely to be missing in the control included the abdomen (13 $\pm$ 18%; F = 13.58), head (18 $\pm$ 15%; F = 18.03), or thorax (9 $\pm$ 10%; F = 14.53) and were at least two times greater than that seen in CSI treatments (Figure 4; df = 5, 47; p < 0.0001). Controls also were more likely to have legs (11 $\pm$ 11%; F = 5.14; p = 0.0002) and antennae (17 $\pm$ 15%; F = 6.19; p < 0.0001) missing, compared to all CSIs treatments that averaged 6 $\pm$ 5% and 9 $\pm$ 7%, respectively (Figure 4; df = 5, 47). Burial of cadavers was (3 $\pm$ 6%; N = 360) uncommon and did not differ between treatments (Figure 4; df = 5, 47; F = 1.30; p = 0.1004). The likelihood of individuals being unaccounted for and presumed completely cannibalized were rare averaging 3% (N = 360) or less over 68 days (Figure 4; df = 5, 47; F = 2.78; p = 0.0179) for both controls and treatments.

We observed two physical deformities displayed by moribund termites exposed to CSIs termed "jackknife" (Su & Scheffrahn 1993) and "body curl" (Figure 2). Within treatments, the occurrence of either jackknife (F = 1.0832; p = 0.3603) or body curl (F = 1.3732; p = 0.2559) was not different between D:R ratios (Figure 5). The jackknife pose was seen in termites exposed to all CSIs, with the exception of novaluron, and was most common in the diflubenzuron treatment (Figure 5; F = 17.7874; p < 0.0001). Diflubenzuron provided comparable frequencies of both jackknife and body curl 38 $\pm$ 15% and 39 $\pm$ 18%, respectively (F = 0.0181; p = 0.8936). All other CSIs provided a higher frequency of body curl (>60%) with hexaflumuron providing the highest incident (Figure 5; F = 60.1281; p < 0.0001).

Discussion

Maintaining healthy termites in bioassay is essential when comparing experimental results (Lenz & Williams 1980) and we established a threshold of <19% mortality in the control group before including a replicate in our data analysis. Maintaining termites in small plastic containers for months is a difficult process as indicated by publications, such as Vahabzadeh et al. (2007) that reported 70% control mortality at day 45. Four subterranean termite/CSI studies published had <20% mortality in the controls (Karr et al. 2004;
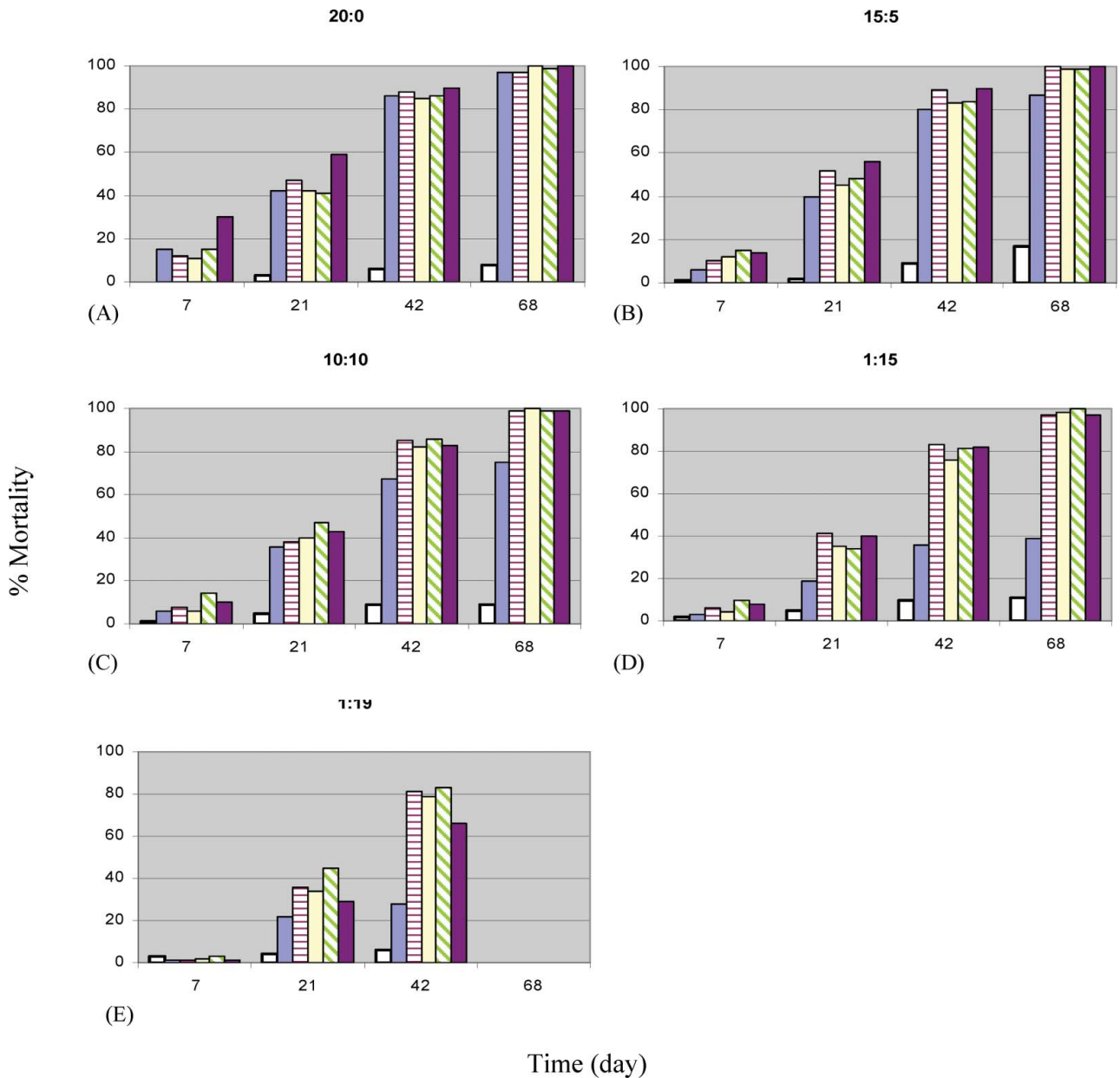
Figure 3. Termite mortality over time (7, 21, 42, and 68 day) exposed to CSIs by D:R ratios as follows: (A) 20:0, (B) 15:5, (C) 10:10, (D) 5:15, and (E) 1:19.
Note: CSI treatments included C = control, D = diflubenzuron, H = hexaflumuron, N = noviflumuron, E = novaluron, and L = lufenuron. Ratio of donors exposed to CSI to recipients.

King et al. 2005; van den Meiracker et al. 2005; Gautam & Henderson 2014). Our findings (Figure 3(A)) correspond well with van den Meiracker et al. (2005) (25 °C and 30 °C; continuous exposure), King et al. (2005) (21 °C; 14 day exposure), and Karr et al. (2004) (26 °C; 7 day exposure) that reported at least 80% mortality in their treatments, while Gautam and Henderson (2014) (27 °C; continuous exposure) had lower mortality at day 42 for two of three CSIs they examined.

Exposure to benzolurea CSIs results in a variety of symptoms in insects, including disruption of molting and egg hatch (Merzendorfer 2013). Factors that can influence frequency of molting and, therefore, time to CSI mortality would include temperature. Subterranean termite workers can take 50–125 days between molts with temperatures ranging from 30 °C to 18 °C (Weesner 1956; Buchli 1958; van den Meiracker et al. 2002; Swoboda & Miller 2005; Raina et al. 2008). When investigating the impact of a slow acting compound, it is essential to conduct a study at a temperature that will permit observation of mortality in a timely manner (van den Meiracker et al. 2002). Despite the fact that the annual soil temperature of the Georgia Piedmont in the southeastern USA average 22 °C at a depth of 20 cm (GeorgiaWeather.net 2008), we chose, for comparative analysis, a higher temperature (27 °C)
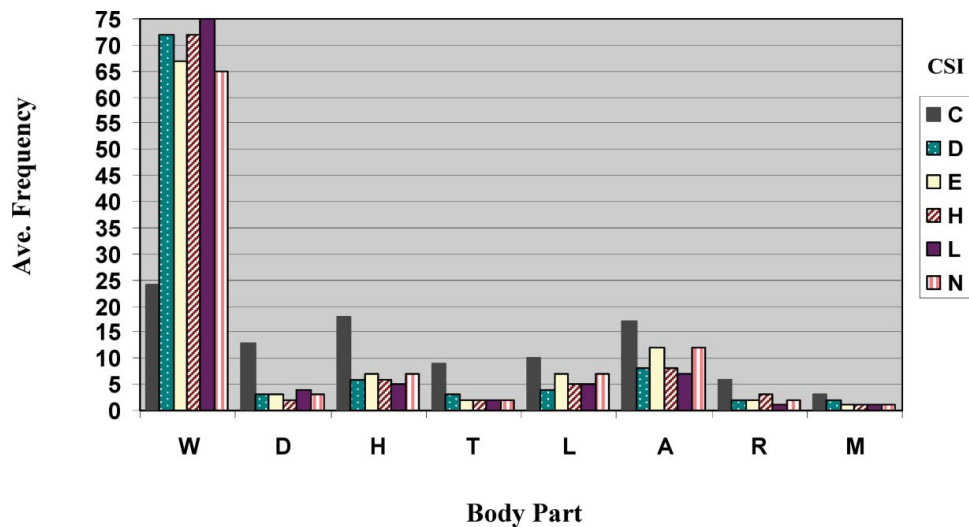
Figure 4. Evidence of cannibalism observed during daily post-exposure examination of Petri dishes combining all D:R ratios over the 68-day experimental period. Treatments included: C = control, D = diflubenzuron, H = hexaflumuron, N = noviflumuron, E = novaluron, and L = lufenuron. Observation of the dead and signs of cannibalistic acts (body part missing): W = whole body intact; D = abdomen; H = head; T = thorax; L = legs; A = antennae; R = body buried; and M = whole body missing. (colour version available online)

in order to observe mortality in a time frame that would allow for recording both lethal affects and appropriate control survivorship. The time to mortality, therefore, in field use of these baits can be expected to be longer.

CSIs impact not only the molting process, but also physiology (peritrophic matrix, fat body, trachea, oocytes, and midgut) and biochemical (DNA synthesis) processes (Retnakaran et al. 1985; Zimmermann & Peters 1987; Nakagawa & Matsumura 1994; Morales-Ramos et al. 2006; Merzendorfer 2013). We observed termites that displayed the jackknife pose, a condition attributed to aborted molting (Su & Scheffrahn 1993; Getty et al. 2000). The jackknife pose was recorded in all CSI treatments at low frequency (≤5%) and were

comparable to the findings reported by Su and Scheffrahn (1993, 1996), although the jackknife pose was common (35%) in our diflubenzuron replicates (Figure 5). Most of the dead or moribund termites in our test did not display obvious signs of "molting inhibition", yet we recorded what we termed the curled-body pose (Figure 5). This display in a moribund state has not been previously described and we cannot account for a physiological explanation. An explanation for the curled-body posture, prior to death, should be pursued in future research as it may indicate a cause of mortality separate from the molting process.

Bait used for elimination of subterranean termite colonies can be efficacious under one of two scenarios. The first is that every termite in a targeted population
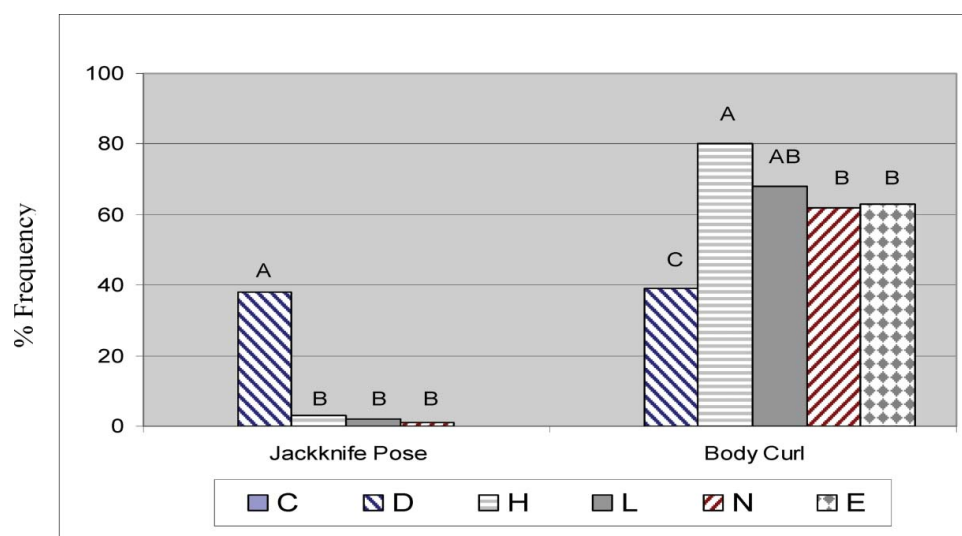
Figure 5. Frequency of dead or moribund termites exhibiting physical deformities following exposure to CSIs over a 68-day period. CSI exposures include nonactive C = control, D = diflubenzuron, H = hexaflumuron, L = lufenuron, N = noviflumuron, and E = experiment CSI. Physical deformities were jackknife pose and body curl. Different letters indicate significant differences ($p < 0.05$) between bait treatments because these deformities were not observed in the controls. (colour version available online)

visits the toxicant-laden bait and feeds. This "all-must-visit" scheme could be successful if termites moved between feeding locations on a "regular" (perhaps daily) basis. The second scenario requires movement of the AI by bait-fed termites to nestmates that do not contact treated bait. There are no field data to collaborate either of the aforementioned scenarios and most of the literature assumes the second scheme (French 1991; Evans & Iqbal 2014). The movement scenario depends on distribution of AI to termites that never feed on the toxicant-laden bait and by extension the most efficacious bait would be one that is, somehow, relocated from the bait station.

Termiticidal agents introduced into a termite population can be distributed through eusocial behaviors, such as grooming, food sharing, and cannibalism (Randall & Doody 1934; Beard 1974; Su & Scheffrahn 1996; Peppuy et al. 1998; Ibrahim et al. 2003; Hu 2005; Haagsma & Rust 2005). Movement of a food-borne AI within a subterranean termite population is a matter of conjecture although recent observational data do provide some insights. Food sharing, that is traditionally lumped under the term "trophallaxis" (Sleigh 2002), involves two–five different mechanisms of transfer (Whitman & Forschler 2007). Stomodeal and proctodeal exchanges are the most commonly considered food sharing options employed in interpreting termite bait-toxin transfer data (Sheets et al. 2000; Karr et al. 2004; Haagsma & Rust 2005; King et al. 2005). Stomodeal trophallaxis (or autofeeding stomodeal) is a recipient driven process involving donors that are chewing something that could have been obtained by one of the three ways: from a recently completed stomodeal or proctodeal exchange and food self-procured by the donor (autofeeding cellulose) (Whitman & Forschler 2007). The model of recipient-driven exchange obviates stomodeal transfer of bait toxins because termites have not been observed chewing food as they move from location to location. According to Whitman (2007), exchange of regurgitated food is a rare event. We, therefore, propose the most likely route of CSI trophallaxic transfer is a proctodeal donation some distance from the toxicant-bait feeding site. The meal taken at a toxin-containing bait station must be provided, after movement away from the station, within the timeframe of "clearance" or movement of food through the alimentary tract. Clearance or "half-life" has been described using radiolabeled CSIs as the time an AI remains in the termite body (Sheets et al. 2000). Most of the literature report that CSI "transfer" (and we are assuming the proctodeal route as determined by the experimental design of feeding donors in one arena and placing them with recipients in a separate arena) in termites peaks between 8 hours and 2 days (Sheets et al. 2000; Karr et al. 2004; Haagsma & Rust 2005; Spomer & Kamble 2006). That 48-hour time frame for movement of a food bolus through the alimentary tract of a subterranean termite matches previous observational data using stained α-cellulose (Forschler 1996) in addition to experiments of radiolabeled food transfer (Suárez & Thorne 2000).

If we exclude stomodeal trophallaxis, there remains other ways for a termite to move the toxic from a baiting site. Delivery can be accomplished by dermal contact and by grooming if the AI is processed to the exterior of the cuticle or the cuticle is contaminated by contact with the bait or tainted feces. Grooming has been described as the most consistent behavior observed in worker–worker interactions in various studies (Iwata et al. 1989; Rosengaus & Traniello 1993; Maistrello & Sbrenna 1996; Whitman 2006). If a bait-borne CSI is excreted on the cuticle, it could very likely be efficiently transferred as demonstrated by the trap-treat-and-release work of Myles (1996). The cuticle/grooming route of CSI transfer should be examined in more detail, although Haagsma and Rust (2005) and Sheets et al. (2000) provide data indicating an insufficient amount of radiolabeled CSI is provided to donors by contact with CSI-exposed termites or feces. An additional route, not tested in our experimental design, would involve movement of the bait matrix as a construction material by termites that visit a bait station. It has been noted that termites will use food (including bait matrices) as a substrate for tunnel/gallery construction (Forschler 1996; Duncan 1997; Whitman & Forschler 2007). The use of a toxic bait in gallery construction/maintenance is a topic beyond the scope of this work but it must be noted as a potential mechanism for "transfer" of a termite bait toxicant beyond the confines of a bait station.

Behavioral responses must also be considered when evaluating the efficacy of any termite bait (Su et al. 1982; Haverty et al. 1989; Forschler & Jenkins 2000). Termites that consume toxicant-laced food must be elicited for a food donation a process that could be compromised by donors that display signs of intoxication and are subsequently avoided. In addition, if termites are unable to move from the CSI feeding site, transfer is negated. The mortality results from our bioassay (Table 1) and the work of Lewis and Power (2006), Vahabzadeh et al. (2007), and Gautam and Henderson (2014) indicates that feeding on lufenuron-treated cardboard bait provides transfer comparable to other CSIs in the confines of a laboratory arena. Yet, we observed lufenuron donors displayed a characteristic stance with antennae held straight forward in a "V" orientation when viewed from above. This unusual pose may signify a behaviorally compromised termite because these same donors did not respond by increased movement, as in the controls or other CSI treatments we tested, when lifting the Petri dish lid. We would, therefore, posit that transfer of lufenuron in the field would be compromised because movement of potential donors is reduced. In addition, our data show termites exposed to

CSIs were less likely to be cannibalized (Figure 4). Haagsma and Rust (2005) found transfer of hexaflumuron by cannibalism was efficient only in groups containing donors that were starved, however; further research is needed to elucidate the impact of cannibalism on toxicant transfer. Whitman and Forschler (2007) suggested that observation of the jackknife pose in bioassay is indicative of recognition of unhealthy dead and our data support this contention because the jackknife and body curl was not observed in the controls and cannibalism was lower in the CSI treatments (Figure 4). Observations of nest mate involvement in the termite molting process have been observed yet the role in bait toxicant transfer that consumption, by nest mate helpers, of CSI-contaminated exuvia has not been investigated (Traniello et al. 2002; Whitman & Forschler 2007; Xing et al. 2013).

In conclusion, despite the fact that the dose required to achieve mortality has not been determined for any CSI (Sheets 2000), the commercial bait formulations we tested transferred in an efficient manner within the confines of a Petri dish and suggests a low (ng AI per termite) dose/mortality relationship. Efficiency of bait toxicant transfer is herein described as >90% mortality in 19 not-exposed nestmates confined with one termite that had access to a CSI bait for 7 days. The actual mode(s) of transfer remains unresolved but sufficient evidence is provided to suggest a major route is proctodeal donation. The reduced efficiency of diflubenzuron at the lowest D:Rs tested may be a result of the lower concentration of that CSI bait (0.25%) compared to the three baits with a 0.5% concentration. The lowest concentration of CSI in the baits we tested was lufenuron (0.15%) and it provided equivalent mortality, using the LT_{90} metric (Table 1), with all but the lowest D:R (1:19), yet the behavioral observations suggest that this CSI will not be effectively transferred under field conditions. This is supported by observations of the compromised movement of lufenuron donors and the assumption that movement away from the bait site is a critical component of termite bait field efficacy.

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Disclosure statement

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ORCID

J.L. Lewis ⬤ http://orcid.org/0000-0002-9474-6932

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