The attractiveness of toxic bait is not always accompanied by increased mortality in laboratory colonies of Argentine ants, *Linepithema humile* (Hymenoptera: Formicidae)

N.P. Mothapo & T.C. Wossler*

Centre for Invasion Biology, Department of Botany and Zoology, Stellenbosch University, Private Bag X1, Matieland, 7602 South Africa

The Argentine ant, *Linepithema humile*, is a globally known invasive ant species responsible for widespread biodiversity and economic loss, thus there is a growing need to control and preferably locally eradicate these ants from invaded environments and households. We evaluated the attractiveness and toxicity of six commercial baits containing hydramethylnon, imidacloprid and pyriproxyfen targeting Argentine ants, and differing in bait formulation. Hydramethylnon granular baits were most effective, killing workers (85–100 % worker mortality) and queens (63–75 %) in the laboratory within 24 h. Workers were most attracted to and consumed the most of the imidacloprid gel bait, but, contrary to previous studies, mortality on this bait was low. The pyriproxyfen bait was least effective in killing workers, as was expected, and was the least consumed of all baits. We advocate the use of a fine granular hydramethylnon formulation for the effective control of Argentine ants. The attractiveness and consumption of the gel formulation was encouraging, therefore increased concentrations of the toxicant imidacloprid in gel formulation should be tested for improved bait efficacy. Combinations of toxicants that act on multiple levels, such as the larvicide pyriproxyfen and the respiratory toxicant hydramethylnon, should be tested in order to maximise colony eradication from brood to queens.

Key words: Argentine ants, toxic bait, hydramethylnon, imidacloprid, pyriproxyfen.

INTRODUCTION

Increased urbanisation and habitat degradation has led to the spread of many invasive plants and insects, which thrive in these environments (King & Tschinkel 2008). Among these are the invasive ant species which are some of the most economically damaging pest species of agricultural and urban environments (Rust *et al.* 2004; Daane *et al.* 2008). Invasive ants tend honeydew-producing bugs (Hemiptera), which are directly associated with crop damage in agricultural settings (Silverman & Brightwell 2008; Nyamukondiwa & Addison 2011). The ants are also a nuisance in human dwellings of all types and can serve as vectors for diseases (Edwards & Baker 1981). Due to these negative effects of invasive ants, there is a growing need to control them in both urban and natural environments.

Chemical control of ants using toxic baits and barrier methods is a popular tool in managing pest ants in urban environments (Rust *et al.* 1996). Barrier methods involve the application of a toxicant and repellent preparation, either in liquid or granular form, around the base of trees, vines and man-made structures to prevent entry by ants (Klotz *et al.* 2000a; Silverman & Brightwell 2008; Nyamukondiwa & Addison 2011). However, barrier methods are highly influenced by environmental conditions and are prone to degrade in higher temperatures, and are likely to affect non-target organisms (Klotz *et al*. 2007). Toxic baits on the other hand are made of nutritionally attractive substances such as sugars, fats and protein-based foods which are laced with a toxicant (Hooper-Bui & Rust 2000; Silverman & Brightwell 2008), most commonly hydramethylnon, boric acid, sulfluramid, fipronil, thiamethoxam and imidacloprid (Hooper-Bui & Rust 2000; Silverman & Roulston 2001; Nondillo *et al.* 2014). An advantage to using toxic baits is their target specificity, thus limiting their environmental impact (Klotz & Shorey 2000). Also, once ingested by workers, the toxicant is easily spread throughout the colony *via* worker to worker trophallaxis (Klotz & Reid 1993; Sheets *et al.* 2000, Hu *et al.* 2005; Choe & Rust 2008), maximising the chance of entire colony eradica-

*Author for correspondence. E-mail: wossler@sun.ac.za

ISSN 1021-3589 [Print]; 2224-8854 [Online]
DOI: http://dx.doi.org/10.4001/003.024.0352

tion. On the downside however, bait uptake is strongly influenced by season, formulation and the toxicant used (Hooper-Bui & Rust 2000; Buczkowski & Bennett 2008, Brightwell *et al.* 2010).

Optimal toxic baits must contain a formulation that is more attractive than naturally occurring foods in the environment (Stringer *et al.*1964). It cannot be repellent and thus deter feeding (Nelson & Daane 2007), and it must be effective across a range of concentrations with delayed toxic action to enable sufficient time for sharing among colony members (Stringer *et al.* 1964; Klotz & Reid, 1993; Silverman & Roulston 2001; Stevens *et al.* 2002). The formulation in which the bait is presented is an important consideration, *i.e.* gel, liquid or granule as it may affect the handling and uptake of the toxicant (Krushelnycky & Reimer 1998a). Granular baits with large particle size may attract ants that are mass recruiters while deterring solitary foragers that carry individual items back to the nest (Hooper-Bui & Rust 2000). On the other hand, liquid sugar baits may attract ants that prefer sweet food substances (Nyamukondiwa & Addison 2011, 2014). Baits that are tailored to the specific diet of ants are more effective in that they are readily consumed and their application can be effective in controlling the specific ant species (Mathieson *et al.* 2012). In designing the bait, the foraging strategy, ecology and activity patterns of the target ant species need to be known (Rust *et al.* 2000; Nondillo *et al.* 2014).

The invasive Argentine ant, *Linepithema humile* (Mayr, 1868), is a world-renowned economically damaging pest species of urban, agricultural and natural environments (Bond & Slingsby 1984; Suarez *et al.* 2001). Although baits with boric acid, fipronil, hydramethylnon, imidacloprid and thiamethoxan have been widely used in the management of Argentine ants in agricultural systems as well as in laboratory studies (Klotz *et al.* 1998; Hooper-Bui & Rust 2000 2001; Rust *et al.* 2004; Nelson & Daane 2007; Daane *et al.* 2008; Nyamukondiwa 2008; Nyamukondiwa & Addison 2011; Blight *et al.* 2011), the preference of Argentine ants to these various toxic baits is not well known. Even though hydramethylnon, imidacloprid and pyriproxyfen have widely been used in Argentine ant control in other parts of the world, these toxicants have not been tested within the South African context, a genetically distinct population from Argentine ants introduced into other parts of the world (Van Wilgenburg *et al.* 2010; Vogel *et al.* 2010). In addition, although Argentine ants show a strong preference for liquid sugar baits (Klotz *et al.* 2004; Rust *et al.* 2004; Daane *et al.* 2006; Nyamukondiwa 2008), a formulation widely used in South African agricultural systems (Nyamukondiwa 2008; Addison & Samways 2000; Nondillo *et al.* 2014; Nyamukondiwa & Addison 2011, 2014), their attraction to other bait matrices, such as granular and gel baits, is unknown for the southern African population (Forschler & Evans 1994; Krushelnycky & Reimer 1998b). Moreover, the attractiveness of the bait formulation to the ants relative to other typical household food items that the ants may encounter is rarely considered. In this study we compare the mortality of Argentine ant workers and queens to six commercial ant baits containing various concentrations of the delayed-action insecticide hydramethylnon in granular form (Klotz & Reid 1993), the acute action insecticide imidacloprid in granular form (Klotz & Reid 1993), and the insect growth regulator pyriproxyfen in gel form (Table 1) which is not expected to cause rapid mortality of the adult worker caste (Oi *et al.* 2000; Invest & Lucas 2008). The toxic baits differed in their formulation, and therefore the preference by and the consumption of the different formulations by Argentine ant workers was also assessed.

MATERIAL AND METHODS

Ant collection and maintenance

Colonies of Argentine ants were collected from Helderberg Nature Reserve in the Western Cape Province (34.21°S 18.87°E), South Africa. Whole nests consisting of workers, brood and queens were dug out using garden trowels and collected in plastic containers (22 cm × 22 cm × 8 cm) lined with Fluon™ (polytetrafluoro-ethylene, Whitford Plastics Ltd, England) to prevent ant escape. Experimental and control colonies were set up from these stock colonies once brought to the laboratory. Experimental and control colonies were housed in covered plastic containers (22 cm × 22 cm × 8 cm), darkened by covering the sides with black duct tape, along with some soil from the original nesting material to provide ants with a nesting substrate. Each experimental and control colony consisted of exactly 1000 workers, 1 cm^2 of brood and one queen. All colonies were provided with water *ad libitum* and 25–35 % sugar water and kept under the following laboratory conditions:

Table 1. A description of the six toxic baits tested and the active ingredients found in the commercial baits.

Bait code	Bait name	Appearance	Granule size	Active ingredient	Manufacturer/ distributor
A	Maxforce Ant bait	Granular Yellow-brown Irregular shape Odourless	0.9–3.5 mm	Hydramethylnon 10 g/kg	Bayer, South Africa
B	Maxforce insect bait	Granular Dark brown Irregular shape Fishy odour	0.05–1.0 mm	Hydramethylnon 10 g/kg	Bayer, U.S.A.
C	Efekto No ant	Granular Yellow Irregular shape Fishy odour	0.5–3.95 mm	Pyriproxyfen 5 g/kg	Efekto, South Africa
D	Nip-it Ant control bait	Granular Light brown Cylindrical pellets Fishy/chemical odour	0.9–4.3 mm	Hydramethylnon 10 g/kg	ProTek, South Africa
E	Maxforce garden ant killer	Granular Light pink Irregular pellets Odourless	0.5–4.15 mm	Imidacloprid 0.5 g/kg	Bayer, Europe
F	Maxforce Quantum	Gel Transparent gel Odourless	–	Imidacloprid 0.3 g/kg	Bayer, South Africa

12L:12D cycle at temperatures ranging from 20 °C to 25 °C. Colonies were starved for 72 h prior to experimentation to ensure equal levels of hunger as done in similar studies (Hooper-Bui & Rust 2001; Mathieson *et al.* 2012). The experimental and control colonies were housed separately to prevent control colonies becoming contaminated with the toxicants.

Experimental setup

All experiments were conducted under controlled laboratory conditions. Each bait was offered to eight independent laboratory nests over a 12-week period (n = 6 bait types × 8 replicates) randomising the toxicants across the arenas throughout the study period. Plastic arenas (120 × 60 × 15 cm) layered with plaster of Paris to provide a walking surface for ants and lined with Fluon™ (fluoropolymer dispersion, Whitford Plastics Ltd, England) to prevent ant escape were used to conduct the experiments. The experimental (treatment) colonies were setup with the nest placed at the one end of the arena, while the toxic bait was placed on the opposite end of the arena. A food control (tuna in vegetable oil or syrup, see Nyamukondiwa (2008) showing Argentine ant bait preferences in field conditions) was introduced alongside the toxic bait to compare the attractiveness of the commercial bait to that of a rewarding food supply. Six baits, A = Maxforce Ant bait; B = Maxforce insect bait; C = Efekto No ant; D = Nip-it Ant control bait; E = Maxforce garden ant killer; F = Maxforce Quantum (Table 1) were tested (commissioned by Bayer Africa) for attractiveness and efficacy. Even though bait C, pyriproxyfen, is a larvicide it has been shown to kill adult workers albeit more slowly (Oi *et al.* 2000). Prior to each experiment, 0.5 g of each bait type was weighed on a microbalance (Model X110 with a weighing range of 0.001–100 g, RADWAG, Poland) and placed on a glass coverslip. The corresponding food controls were weighed out for each of the baits: 0.5 g of tuna in vegetable oil for the granular baits and 0.5 g of syrup for the gel bait.

Different food controls were used so that the controls matched the corresponding solid or liquid toxicant formulations. An additional 0.5 g of tuna were weighed out for each experiment and used as a control to calculate desiccation rates. Syrup baits did not lose water; therefore, it was not necessary to evaluate desiccation rates. Observations commenced as soon as nest containers were opened and connected to the arena *via* cardboard bridges and persisted for 8 days. Control colonies (n = 8), housed separately, were maintained under the same conditions as the experimental colonies and provided with tuna with no toxicant in the arena and used to control for everyday mortality rates in the treatment colonies. We used two measures of ant foraging success, recruitment of nest mates to the toxic bait and corresponding food control, as well as consumption of both (Davidson 1997), to compare ant preference to the commercial bait and another food item. We measured recruitment intensity by counting ants present on the cover slip and in contact with the food every 10 min for 90 min, thereafter every 2 h until 17:00 (experiments started at 09:00), followed by observations every 2 h from 09:00–17:00 for the 8 days. At every 2-h reading, all dead ants were collected, counted (mortality rate) and placed in plastic cuvettes with ethanol for storage. No ants were counted overnight. At the end of each experiment, after 8 days, the remaining food and bait were weighed to determine consumption or retrieval rates. All nests were opened and all workers and queens within each nest were counted. During preliminary experiments, food controls were replaced daily; however, ants stopped feeding after day 1 having ingested the toxicant, and consequently food was not replaced in further experiments.

Statistical analyses

Recruitment intensity. The time taken to discover the toxic baits and corresponding food controls did not differ significantly for any of the six baits (paired samples *t*-test, ns). Recruitment intensity for each of the toxic baits was averaged across the eight experiments per time interval for the 6 h (after which foraging rates dropped off rapidly), and recruitment curves (showing mean and standard error) were generated. Recruitment effort over time (recruitment pattern) of workers to the toxic bait and corresponding food control was compared, as well as the recruitment intensity over time for ants foraging on the toxic bait compared to ants foraging for a food resource in control colonies, using two-way repeated measures ANOVA with Greenhouse-Geisser correction (Mothapo & Wossler 2014). The Greenhouse-Geisser correction is used when the assumption of sphericity or equal variances are violated, and corrects by calculating new degrees of freedom and a new significance value (P) so that a valid F-ratio can be obtained.

Consumption and mortality. A paired samples *t*-test was used to compare consumption between a given bait type and corresponding food control, non-parametric Wilcoxon Signed-Ranks test was used for non-normal data. One way ANOVA, followed by Tukey HSD *post hoc* test, was used to compare the differences in consumption between the six toxic baits and the food resource (in control colonies). Cumulative worker mortality was calculated for the treatment groups (bait type) and the control colonies. Ant mortality per treatment and for the control colonies was assessed by first calculating the proportion of dead ants. The data were then arcsine transformed, compared using a non-parametric Kruskal-Wallis H test followed by pairwise comparisons using Mann-Whitney U tests (Pallant 2007). Statistical significance was adjusted following Bonferroni correction for multiple pairwise comparisons (P = 0.003). Queen mortality across the six treatments and control colonies was compared with a chi-square (χ^2) test for independence.

Survival time and bait toxicity. To determine the efficacy of baits in terms of the time taken for the colony to collapse, a survival analysis was conducted using a Kaplan-Meier test followed by a Cox-regression/proportional hazards modelling the survival time by the covariate bait type. The Kaplan-Meier test produces a survival table which indicates the time in days taken for 50 % of the workers to die. The Gehan-Wilcoxon test was used to compare the survival times between treatments and the control. The Cox's proportional hazards model assumes that the probability of mortality, or hazard ratio, varies over time for an ant but that the covariates influence the hazard/mortality risk by a proportion which does not change over time. The hazard ratio (exp β) indicates the toxicity of each bait type in comparison with the others. In this study the mortality of the control colonies was used as the base to which the efficacy of all baits in killing Argentine ants relative to natural mortality,

was compared in the regression model. The values of the hazard ratio >1 indicates risk of death higher than the reference group (control colonies), <1 is lower than the reference group and = 1 indicates no difference to the reference group. The Log Rank (Mantel-Cox), Breslow (generalised Wilcoxon) and Tarone-Ware tests were used to compare the equality of survival distributions between the baits. Analyses were conducted in STATISTICA 10.0 statistical software. Statistical significance was accepted at $P < 0.05$.

RESULTS

Recruitment intensity

There is a concomitant decrease in foraging as the insecticidal activity of the baits takes effect. In our study, ant foraging activity practically ceased after 6 h. Argentine ants were not recruited in larger numbers to the toxic bait than to the corresponding food control, except for bait C where the food was marginally more attractive than the bait (repeated measures ANOVA, $F = 4.22$,, d.f. = 1, 20, $P = 0.05$, Fig. 1c). Even though recruitment intensity to the toxic bait and corresponding food control were similar (Fig. 1), the recruitment effort decreased over time for the food control and the hydramethylnon baits A (repeated measures ANOVA, $F = 3.62$, d.f. = 2, 48, $P = 0.027$) and D (repeated measures ANOVA, $F = 3.94$, d.f. = 2, 41, $P = 0.026$), and similarly for imidacloprid bait F (repeated measures ANOVA, $F = 9.28$, d.f. = 1, 29, $P = 0.002$). Recruitment intensity, however, differed significantly over time between ants foraging on different toxic baits and those foraging on the food resource in control colonies (ANOVA, $F = 2.74$, d.f. = 6, 67, $P = 0.019$, Fig. 4a). *Post hoc* (Tukey HSD) pairwise comparison showed that only the recruitment intensity of workers to bait C and bait F was significantly different (Fig. 4a), with large numbers of workers recruited to bait F while very few workers foraged on bait C.

Consumption and mortality

Ants consumed similar amounts of hydramethylnon baits B, D and imidacloprid gel bait F compared to the corresponding food control in treatment colonies which supports the foraging effort data for these colonies (Figs 1, 2). Even though recruitment intensity to the food control and toxic bait were similar for hydramethylnon granular bait A and imidacloprid granular bait E, ants consumed significantly more amounts of the food provided than hydramethylnon granular A (paired *t*-test, $t = 2.65$, d.f.= 7, $P < 0.05$) and imidacloprid granular bait E (Wilcoxon, $Z = –2.52$, $n = 7$, $P < 0.05$, Fig. 2). More ants were recruited to the food control than pyriproxyfen granular bait C (Fig. 1) and the ants consumed more of the food supplied than the bait (Wilcoxon, $Z = –2.52$, $n = 7$, $P < 0.05$). Consumption rate of the various toxic baits and that of the food resource in the control colonies differed significantly (ANOVA, $F = 2.40$, d.f. = 6, 55, $P \leq 0.05$), with the consumption of bait C (0.03 ± 0.01 g) being significantly less than that of imidacloprid gel F (0.12 ± 0.03 g, Fig. 3, significant pairwise differences based on Tukey's HSD *post hoc* test).

The average cumulative mortality over the 8-day experiment ($n = 8$ colonies/treatment) indicates that the hydramethylnon granular baits A, B and D, as well as the imidacloprid granular bait E were the most successful in controlling laboratory colonies of Argentine ants (Fig. 4b). There was a significant difference in the efficacy of the baits tested in killing Argentine ants (Kruskal-Wallis H, $\chi^2 = 38.20$, d.f. = 6, $P < 0.01$), with hydramethylnon granular baits A (93 %), B (100 %) and D (85 %) killing the highest percentage of the ants, followed closely by imidacloprid granular bait E (77 %) (Fig. 4c). Pyriproxyfen granular bait C and imidacloprid gel F were least successful, only killing 55 % and 47 % of the ants, respectively, while only 34 % of ant workers perished in the control colonies (Fig. 4b, c). Pairwise comparisons with Bonferroni correction ($P = 0.003$) showed that mortality differed significantly between the control and treatment colonies (Fig. 4c). Although workers suffered relatively high mortality rates, queens did not follow the same trend. There was no significant difference in queen mortality whether kept in treatment or control colonies (chi-square, $\chi^2 = 12.47$, d.f. = 12, $P > 0.5$) and many of the queens were still alive at the end of the experiment (day 8), particularly for pyriproxyfen granular bait C, imidacloprid gel bait F and the control colonies (Fig. 4c). Only 13 % of queens perished in the control colonies. Most queens on the bait treatments were killed between day 3 and day 4.

Survival time and toxicity

A Kaplan-Meier survival analysis, followed with Cox proportional hazards model was conducted

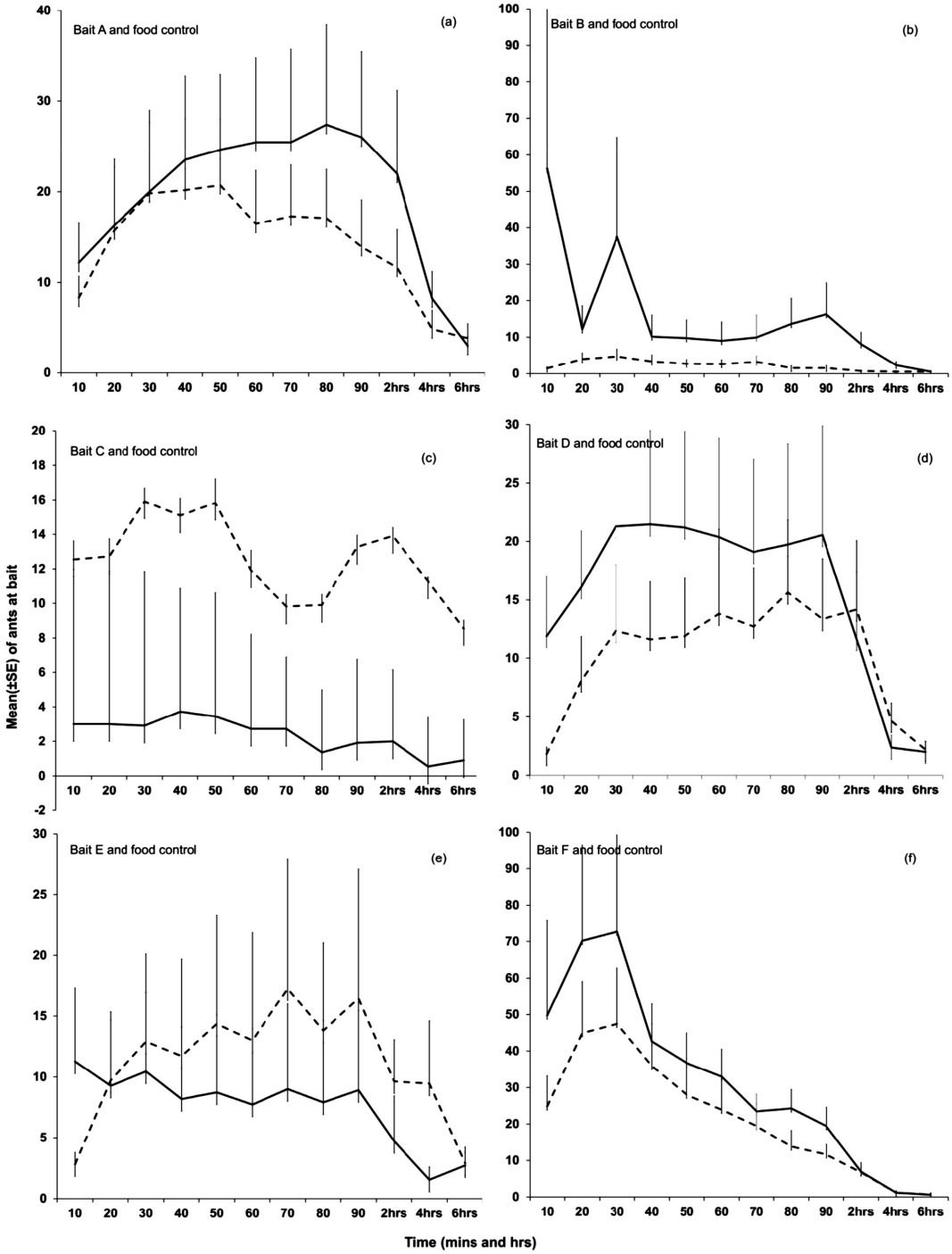

Fig. 1. Recruitment curves (means ± S.E.) showing Argentine ant foraging on toxic baits A–F (solid line) and their corresponding food controls (dashed line) over a six-hour time period. Foraging intensity was not significantly different between all toxic baits and corresponding food control, except for bait C (based on repeated measures ANOVA).

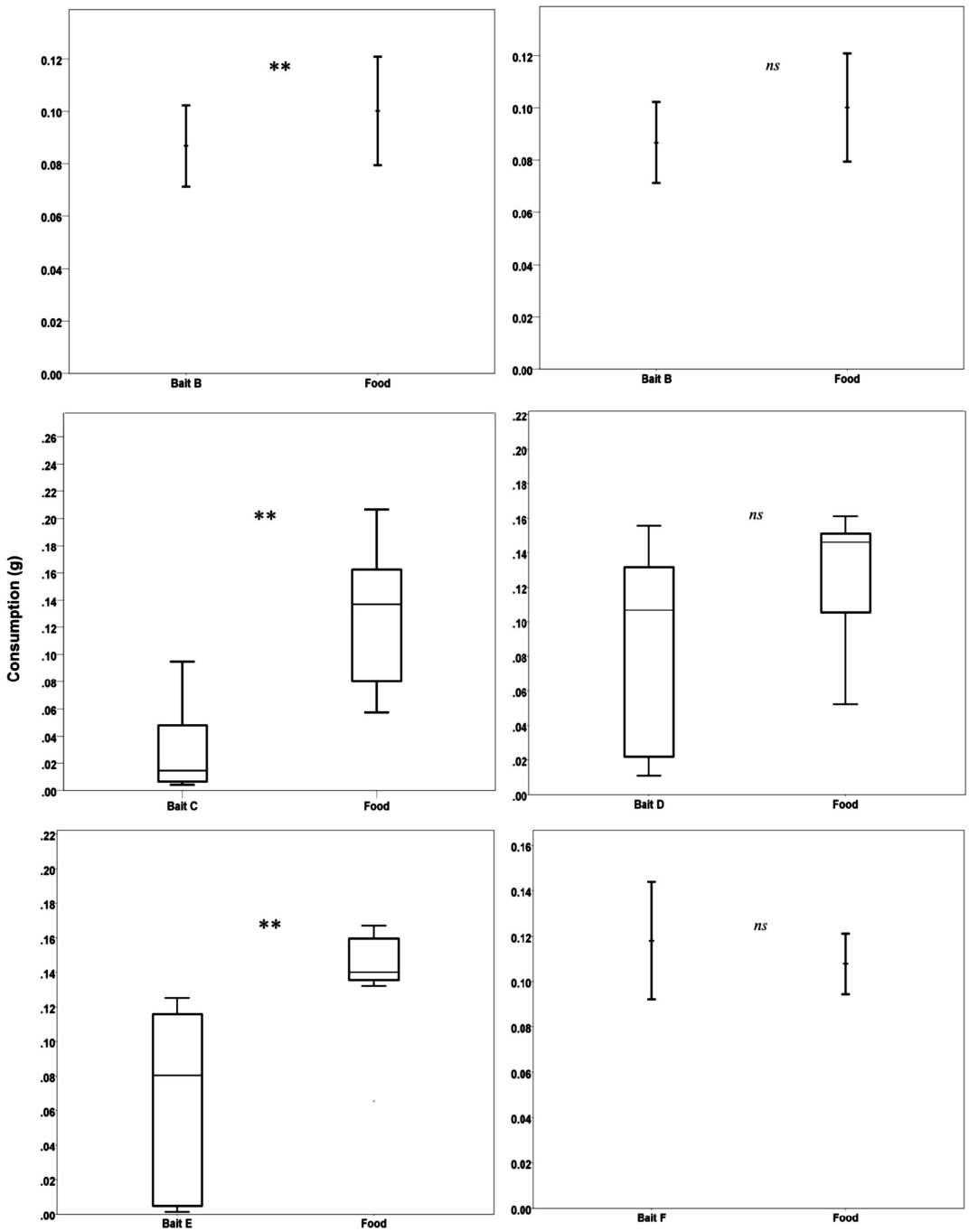

Fig. 2. Amount (g) of bait and food consumed across all trials. Graphs **a**, **b**, and **f** show error bars with mean ± S.E (**= $P \leq 0.01$ and *ns* based on paired *t*-test), while graphs **c**, **d**, and **e** show median ± min/max (** = $P \leq 0.01$ and *ns* based on Wilcoxon (*H*) signed-ranks test).

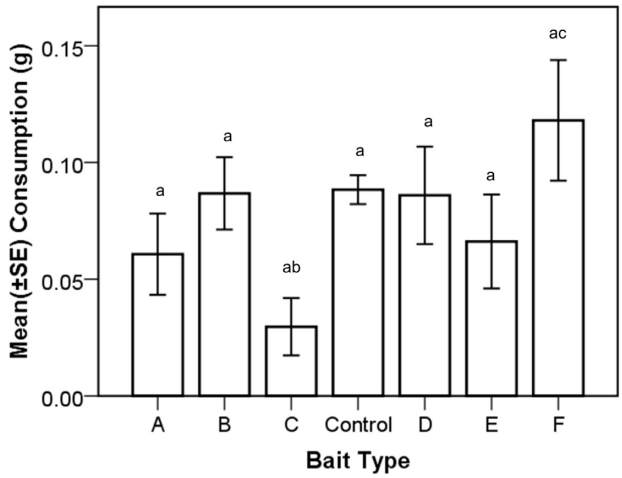

Fig. 3. Mean consumption (± S.E.) of the six toxic baits and food resource (control) across all trials. Argentine ants consumed less of bait C compared to F (Tukey's HSD *post hoc* test).

to determine the cumulative survival and toxicity of each bait treatment and the control (Table 2). Our results show that there was a significant difference in the survival time of the colonies on each of the treatments compared to the control (Gehan Wilcoxon test = 109.47, d.f. = 6, $P < 0.01$) with ants exposed to bait B having the shortest survival and those exposed to bait F having the longest survival of all treatments (Table 2). Ant worker survival was longest in the control colonies, as expected. However, all baits can be considered effective in terms of their delayed toxicity based on the survival times (Table 2). The control was used as the base to which mortality risk of all the baits was compared. Based on the mortality risk (exp β) of each bait relative to the control, hydramethylnon granular bait B is the most lethal bait followed by hydramethylnon granular bait D > hydramethylnon granular bait A > pyriproxyfen granular bait C > imidacloprid granular bait E and imidacloprid gel bait F (Table 2). Even though imidacloprid gel bait F was attractive to ants (Fig. 1f) and readily consumed (Fig. 2f), it was not effective due to the long survival time and the low mortality rate.

DISCUSSION

We found a considerable difference in the efficacy of the six baits in controlling laboratory colonies of Argentine ants. Argentine ant workers foraged readily on all baits, except they recruited more to the food resource than to pyriproxyfen granular bait C, and consequently consumed significantly less of this toxic bait, suggesting that this active ingredient and/or formulation in this study was not highly attractive or palatable to the Argentine ants (Nelson & Daane 2007; Nyamukondiwa & Addison 2011). Moreover, ant foragers recruited a similar number of nest mates to a food resource in control colonies as they did to toxic baits in treatment colonies, in most instances consuming similar amounts of both, indicating that the baits assessed were attractive and palatable to the Argentine ants tested. Recruitment intensity, however, was not always consistent with consumption or mortality rates. Recruitment intensity, measured as actual ant presence on the bait, is a good indicator of bait preference (Nyamukondiwa & Addison 2011). However, it cannot exclusively be used as a measure of bait performance. Rather, the composite results of consumption and mortality rate must be evaluated concomitantly as evident from this study.

Toxic bait is considered to be effective if it is highly attractive and palatable to the ants, non-repellent, slow-acting and can eradicate the colony within 2–4 days after treatment (Nelson & Daane 2007; Nyamukondiwa & Addison 2011). All the baits containing hydramethylnon as the active ingredient, in granular formulation (baits A, B and D), were fast acting and most effective in killing between 85 % and 100 % of all the worker ants respectively within 4 days. Under field conditions, hydramethylnon is considered a slow-acting toxicant killing <15 % of Argentine ant populations

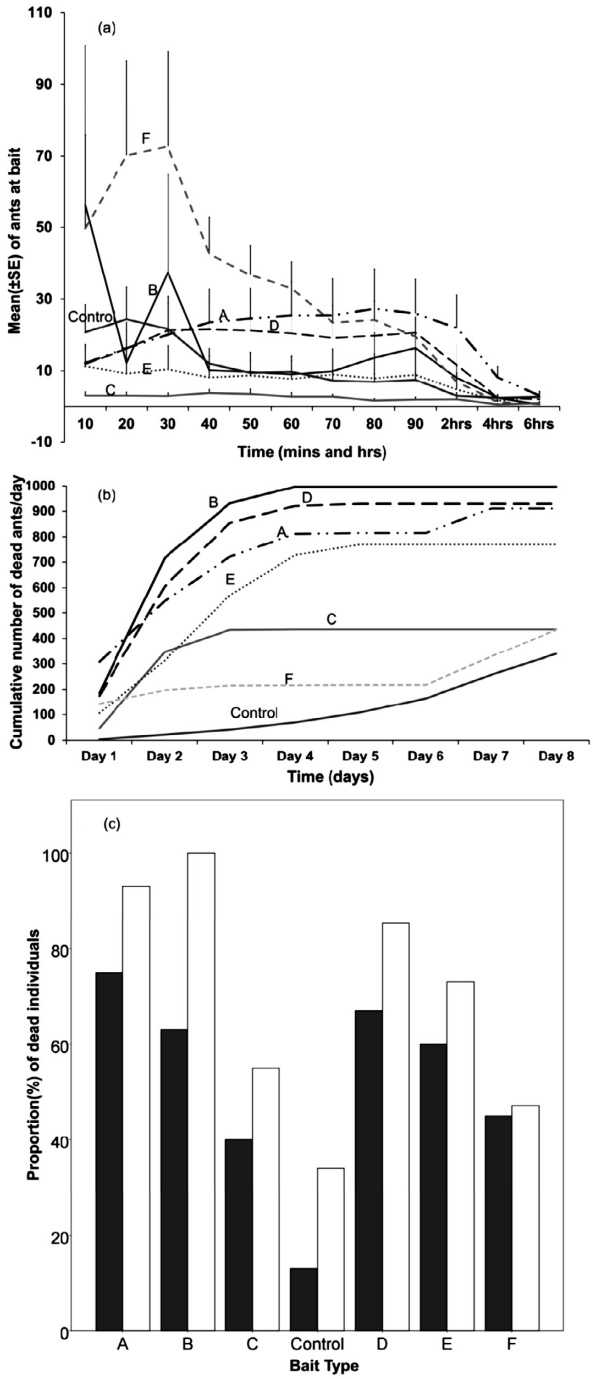

Fig. 4. **a**, Mean (± S.E.) recruitment intensity of Argentine ants to toxic bait treatments and to the food resource in control colonies (compared using repeated measures ANOVA, only the recruitment intensity of workers to bait C and bait F differed, Tukey's HSD *post hoc* test); **b**, average cumulative mortality of Argentine ants on each bait A–F and in control colonies over 8 days ($n = 8$); **c**, Mortality rates for Argentine ant workers (white bars) and queens (grey bars) between the six baits and for control colonies across all trials (Kruskal-Wallis H test based on proportions of dead individual workers). None of the baits had a significant effect on queen mortality (χ^2 test of independence).

Table 2. Kaplan-Meier survival analysis and Cox-regression results showing the mortality risk (expβ) of each of the toxic baits relative to the control.

	Survival time (days)	B	S.E.	Wald	d.f.	Sig.	Exp(β)	95.0 % CI for exp(β)	
Constant				83.37	6	0.00			
A	2.77 ± 0.287	0.81	0.22	3.47	1	0.04	2.24	0.98	3.31
B	1.82 ± 0.18	1.39	0.23	22.63	1	0.00	3.99	0.89	4.98
C	2.27 ± 0.19	0.75	0.23	10.52	1	0.00	2.11	1.35	3.32
D	2.84 ± 0.19	1.08	0.27	16.52	1	0.00	2.93	1.75	4.92
E	3.00 ± 0.20	0.38	0.20	3.46	1	0.06	1.46	0.98	2.17
F	3.20 ± 0.25	0.28	0.203	1.946	1	0.163	1.33	0.159	0.394
Control	6.57 ± 0.22								

within 24 h and over 90 % or complete eradication within 18 days after application allowing sufficient time for the toxicant to be distributed within the colony (Stringer *et al.* 1964; Klotz *et al.* 2004; Nondillo *et al.* 2014). The efficacy of imidacloprid in eradicating *L. humile* is ambiguous, with this study exhibiting greater effectiveness when in a granular formulation (77 % mortality compared to 47 % in gel form) while Blight *et al.* (2011) found that imidacloprid in gel formulation was more effective. Yet Nondillo *et al.* (2014) found that imidacloprid in gel formulation was not at all effective in eradicating workers of *Linepithema micans*, a sister species of Argentine ant, in greenhouse experiments. It was not surprising that the pyriproxyfen granular bait C was not that effective since it targets larval growth and consequently high and rapid adult mortality is not expected (Vail & Williams 1995; Vail *et al.* 1996; Oi *et al.* 2000; Invest & Lucas 2008). Pyriproxyfen was initially targeted for fire ants, *Solenopsis invicta* (Rust *et al.* 2003) and our results suggest that this toxicant was not particularly attractive to Argentine ants since they did not recruit efficiently to it, nor was it effective in killing Argentine ant workers and queens. In addition, the overly high concentration of the toxicant may have repelled the workers and/or the formulation used was not attractive to the ants (Nelson & Daane 2007; Nondillo *et al.* 2014). Further testing should be done using other bait formulations such as liquid or gel or possibly the re-assessment of the concentrations of the toxicant and the duration of the experiments extended.

The concentration of the toxicant in the bait is important because high concentrations of the toxicant may deter foraging by killing ants too quickly, while those of low concentrations may not kill the ants even though ants may be attracted to the bait (Silverman & Roulston 2001; Nyamukondiwa 2008). In contrast to Klotz & Reid (1993), imidacloprid appears to be concentration dependent in this study, and particularly in its efficacy in killing Argentine ants. Imidacloprid granular bait E was more effective than imidacloprid gel bait F in killing ants, with the granular formulation having higher toxicant concentration (Table 1). In another study, imidacloprid gel bait, at a concentration similar to that of the granular bait in this study, applied *in situ* on nests of Argentine ant was highly effective (Blight *et al.* 2011). Consequently, a higher concentration of imidacloprid in the gel formulation may have increased its efficacy on the Argentine ants used in this study since trophallaxis between workers would result in dilution effects and conceivably affect the efficacy of imidacloprid in gel formulation. Declines in effectiveness with horizontal transfer for given toxicants are, however, not always predictable, with efficacy shown to be unchanged for certain toxicants with trophallactic interactions (Klotz & Reid 1993). However, based on the cumulative mortality curves of the imidacloprid gel bait, it is possible that there is a delayed action through the accumulation of imidacloprid in colonies of Argentine ants in this study. Perhaps if the experiments were run over a longer period, the cumulative mortality for imidacloprid gel bait F would match those of the other baits tested, since this mortality curve is the only one that has not reached an asymptote even after the 8-day period. In solid formulation, the efficacy of imidacloprid may have been increased due to the direct contact of the ants with the bait and directly with contaminated workers (Soproeno & Rust 2004), through allogrooming (Klotz *et al.* 2000b). Solid baits are thought to be more effective mainly due to the handling time required

(Soproeno & Rust 2004; Cooper *et al.* 2008). Grain size is thus important in bait uptake and should be small enough for ants to easily collect and take back into the nest to maximise efficacy in the field (Krushelnycky & Reimer 1998a, b; Silverman & Roulston 2003). The effectiveness of hydramethylnon bait B may well be attributed to particle size (Table 1).

Linepithema humile prefers foraging on gel formulations (Silverman & Roulston 2001; Nondillo *et al.* 2014), which was apparent from the high consumption of bait F, the only gel formulation in this study. However, in this study Argentine ants were equally attracted to dry bait as to a corresponding food resource, except for pyriproxyfen dry bait to which they had low recruitment. In previous studies, the liquid bait formulations containing hydramethylnon were extremely fast acting (Klotz & Reid 1993; Hooper-Bui & Rust 2000), killing the ants too quickly and failing to eliminate queens even at low concentrations (Knight & Rust 1991; Klotz & Reid 1993; Krushelnycky & Reimer 1998a; Hooper-Bui & Rust 2000; Silverman & Roulston 2001). Moreover, queens may be sensitive to hydramethylnon, particularly in liquid sucrose form and consequently limit feeding directly on liquid baits (Klotz *et al.* 2000a). However, queens have been shown to consume the toxicant when in low concentrations as fed by workers *via* trophallaxis. This is possibly due to the dilution of the toxicant through horizontal food transfer, even eliminating queens at sufficiently low concentrations (Klotz & Reid 1993; Hooper-Bui & Rust 2001). We found no significant differences in queen mortality on any of the toxicants tested, including hydramethylnon. In ant workers, hydramethylnon acts as a feeding stimulant and increases feeding rates (Hooper-Bui & Rust 2001). A gel formulation has been shown to slow down toxicant ingestion in comparison to a liquid formulation, thus stimulating multiple feedings which then slows down uptake (Silverman & Roulston 2001). In consequence, the multiple feeding by workers would ensure that the queen is fed (Klotz & Reid 1993; Hooper-Bui & Rust 2001). In contrast, Klotz & Reid (1993) showed that hydramethylnon was rendered non-lethal by trophallaxis, having no effect on both secondary donors and recipient workers. However, we found that hydramethylnon baits were most effective in killing Argentine ant workers in comparison to both imidacloprid and pyriproxyfen baits.

This study showed that the most promising toxicants for the eradication of Argentine ants in South Africa are hydramethylnon in granular formulation and potentially imidacloprid in granular formulation. However, it is important to conduct these baiting experiments in the field before conclusive recommendations on bait efficacy can be made. The bait formulations with hydramethylnon in our study were all in solid formulation and would most likely be effective in the field environment as well since it was the most attractive and palatable to the ants. Furthermore, imidacloprid was more effective in solid formulation, however, concentrations of this toxicant differed between the gel and granular baits therefore we cannot make appropriate conclusions on the efficacy of this toxicant between the two matrices. We suggest further testing be conducted with similar concentrations of imidacloprid in both gel and granular matrices. Although liquid formulation is supposedly preferred by Argentine ants (Silverman & Roulston 2001; Nyamukondiwa & Addison 2011), granular formulations were more effective in this study and therefore further testing using gel/liquid matrices is needed (Haack & Vinson 1990; Nondillo *et al.* 2014). Additionally, combinations of toxicants such as pyriproxyfen and hydramethylnon need to be assessed both in the laboratory and field (Webb 2011), in order to maximise efficacy in eradicating the entire colony from brood to queens.

ACKNOWLEDGEMENTS

We thank Bayer Africa and DST/NRF Centre of Excellence for Invasion Biology for funding (K. Cloete and N.P. Mothapo); M. Watkins-Baker, K. Cloete, K. Whitehead, N. Gartrell and D. Phair for help with ant collection, sorting and running experiments. Thanks to O. Wittridge for collection permit at Helderberg Nature Reserve.

REFERENCES

ADDISON, P. & SAMWAYS, M.J. 2000. A survey of ants (Hymenoptera: Formicidae) that forage in vineyards in the Western Cape Province, South Africa. *African Entomology* **8**: 251–260.

BLIGHT, O., ORGEAS, J., RENUCCI, M. & PROVOST, E. 2011. Imidacloprid gel bait effective in Argentine ant control at nest scale. *Sociobiology* **58**: 23-30.

BOND, W. & SLINGSBY, P. 1984. Collapse of an ant-plant

mutualism: the Argentine ant (*Iridomyrmex humilis*) and myrmecochorous Proteaceae. *Ecology* **65**: 1031–1037.

BRIGHTWELL, R.J., BAMBARA, S.B. & SILVERMAN, J. 2010. Combined effect of hemipteran control and liquid bait on Argentine ant populations. *Journal of Economic Entomology* **103**: 1790–1796.

BUCZKOWSKI, G. & BENNETT, G.W. 2008. Detrimental effects of highly efficient interference competition: invasive Argentine ants outcompete native ants at toxic baits. *Environmental Entomology* **37**: 741–747.

CHOE, D.H. & RUST, M.K. 2008. Horizontal transfer of insecticides in laboratory colonies of the Argentine ant (Hymenoptera: Formicidae). *Journal of Economic Entomology* **101**: 1397–1405.

COOPER, M.L., DAANE, K.M., NELSON, E.H., VARELA, L.G., BATTANY, M.C., TSUTSUI, N.D. & RUST, M.K. 2008. Liquid baits control Argentine ants sustainably in coastal vineyards. *California Agriculture* **62**: 177–183.

DAANE, K.M., COOPER, M.L. & SIME, K.R. 2008. Testing baits to control Argentine ants (Hymenoptera: Formicidae) in vineyards. *Journal of Economic Entomology* **101**: 699–709.

DAANE, K.M., SIME, K.R., HOGG, B.N., BIANCHI, M.I. & COOPER, M.L. 2006. Effects of liquid insecticide baits on Argentine ants in California's coastal vineyards. *Crop Protection* **25**: 592–603.

DAVIDSON, D.W. 1997. The role of resource imbalances in the evolutionary ecology of tropical arboreal ants. *Biological Journal of the Linnaean Society* **61**: 153–181.

EDWARDS, J.P. & BAKER, L.F. 1981. Distribution and importance of the Pharaoh's ant *Monomorium pharaonis* (L.) in National Health Service Hospitals in England. *Journal of Hospital Infection* **2**: 249–254.

FORSCHLER, B.T. & EVANS, G.M. 1994. Argentine ant (Hymenoptera: Formicidae) foraging activity response to selected containerized baits. *Journal of Entomological Science* **29**: 209–214.

HAACK, K.D. & VINSON, S.B. 1990. Foraging of pharaoh ants *Monomorium pharaonis* (L.) in the laboratory. In: Van der Meer, R.K., Jaffe, K. & Cedeno, A. (Eds) *Applied Myrmecology: a World Perspective*. 452–460. Westview, Oxford, U.K.

HOOPER-BUI, L.M. & RUST, M.K. 2000. Oral toxicity of abamectin, boric acid, fipronil, and hydramethylnon to laboratory colonies of Argentine ants (Hymenoptera: Formicidae). *Journal of Economic Entomology* **93**: 858–864.

HOOPER-BUI, L.M. & RUST, M.K. 2001. An oral bioassay for the toxicity of hydramethylnon to individual workers and queens of Argentine ants, *Linepithema humile*. *Pest Management Science* **57**: 1011–1016.

HU, X.P.D., SONG, P.D. & SCHERER, C.W. 2005. Transfer of indoxacarb among workers of *Coptotermes formosanus* (Isoptera: Rhinotermitidae): effects of dose, donor: recipient ration and post-exposure time. *Pest Management Science* 61: 1209–1214.

INVEST, J.F. & LUCAS, J.R. 2008. Pyriproxyfen as a mosquito larvacide. Proceedings of the Sixth International Conference on Urban Pests. 239–245. Robinson, W.H. & Bajomi. D. (Eds). OOK Press kft, H200-Veszprém papai út 37/9, Hungary.

KING, J.R. & TSCHINKEL, W.R. 2008. Experimental evidence that human impacts drive fire ant invasions and ecological change. *Proceedings of the National Academy of Sciences of the United States of America* **105**: 20339–20343.

KLOTZ, J.H. & SHOREY, H. 2000. Low-toxic control of Argentine ants using pheromone enhanced liquid baits. Report, California Department of Consumer Affairs, CDCA84SA802007.

KLOTZ, J.H., RUST, M.K., GREENBERG, L., FIELD, H.C. & KUPFER, K. 2007. An evaluation of several urban pest management strategies to control Argentine ants (Hymenoptera: Formicidae). *Sociobiology* **50**: 1–8.

KLOTZ, J.H., RUST, M.K. & PHILLIPS, P. 2004. Liquid bait delivery systems for controlling Argentine ants in citrus groves (Hymenoptera: Formicidae). *Sociobiology* **38**: 695–708.

KLOTZ, J.H., GREENBERG, L., AMRHEIN, C. & RUST, M.K. 2000a. Toxicity and repellency of borate-sucrose water baits to Argentine ants (Hymenoptera: Formicidae). *Journal of Economic Entomology* **93**: 1256–1258.

KLOTZ, J.H., GREENBERG, L. & VENN. G. 2000b. Evaluation of two hydramethylnon granular baits for control of Argentine ants (Hymenoptera: Formicidae). *Sociobiology* **36**: 201–207.

KLOTZ, J.H., GREENBERG, L. & VENN, G. 1998. Liquid boric acid bait for the control of the Argentine ant (Hymenoptera: Formicidae). *Journal of Economic Entomology* **91**: 910–914.

KLOTZ, J.H. & REID, B.L. 1993. Nocturnal orientation in the black carpenter ant *Camponotus pennsylvanicus* (DeGeer) (Hymenoptera: Formicidae). *Insectes Sociaux* **40**: 95–106.

KNIGHT, R.L. & RUST, M.K. 1991. Efficacy of formulated baits for control of Argentine ant (Hymenoptera: Formicidae). *Journal of Economic Entomology* **84**: 510–514.

KRUSHELNYCKY, P.D. & REIMER, N.J. 1998a. Efficacy of Maxforce bait for control of the Argentine ant (Hymenoptera: Formicidae) in Haleakala National Park, Maui, Hawaii. *Environmental Entomology* **27**: 1473–1481.

KRUSHELNYCKY, P.D. & REIMER, N.J. 1998b. Bait preference by the Argentine ant (Hymenoptera: Formicidae) in Haleakala National Park, Hawaii. *Environmental Entomology* **27**: 1482-1487.

NELSON, E.K. & DAANE, K.M. 2007. Improving liquid bait programs for Argentine ant control: bait station density. *Environmental Entomology* **36**: 1475–1484.

MATHIESON, M., TOFT, R. & LESTER, P.J. 2012. Influence of toxic bait type and starvation on worker and queen mortality in laboratory colonies of Argentine ant (Hymenoptera: Formicidae). *Journal of Economic Entomology* **105**(4): 1139–1144.

MOTHAPO, N.P. & WOSSLER, T.C. 2014. Resource competition assays between the African big-headed ant, *Pheidole megacephala* and the invasive Argentine ant, *Linepthima humile*: mechanisms of inter-specific displacement. *Ecological Entomology* **39**: 501–510.

NONDILLO, A., CHAVES, C.C., FIALHO, F.B., BUENO, O.C. & BOTTON, M. 2014. Evaluation of insecticides for the control of *Linepithema micans. Journal of Economic Entomology* **107**(1): 215–222.

NYAMUKONDIWA, C. & ADDISON, P. 2011. Preference

of foraging ants (Hymenoptera: Formicidae) for bait toxicants in South Africa vineyards. *Crop Protection* **30**: 1034–1038.

NYAMUKONDIWA, C. & ADDISON, A. 2014. Food preference and foraging activity of ants: recommendations for field applications of low-toxicity baits. *Journal of Insect Science* **14**: 48.

NYAMUKONDIWA, C. 2008. Assessment of toxic baits for the control of ants (Hymenoptera: Formicidae) in South African vineyards. M.Sc. thesis, Stellenbosch University, Stellenbosch, South Africa.

OI, D.H., VAIL, K.M. & WILLIAMS, D.F. 2000. Bait distribution among multiple colonies of Pharaoh ants (Hymenoptera: Formicidae). *Journal of Economic Entomology* **93**: 1247–1255.

PALLANT, J. 2007. *SPSS Survival Manual*. Two Penn Plaza, New York, U.S.A.

RUST, M.K., REIERSON, D.A. & KLOTZ, J.H. 2004. Delayed toxicity as a critical factor in the efficacy of aqueous baits for controlling Argentine ants (Hymenoptera: Formicidae). *Journal of Economic Entomology* **97**: 1017–1024.

RUST, M.K., REIERSON, D.A. & KLOTZ, J.H. 2003. Pest management of Argentine ants (Hymenoptera: Formicidae). *Journal of Entomological Science* **38**: 159–169.

RUST, M.K., REIERSON, D.A., PAINE, E. & BLOOM, L.J. 2000. Seasonal activity and bait preferences of the Argentine ant (Hymenoptera: Formicidae). *Journal of Agricultural and Urban Entomology* **17**: 201–2127.

RUST, M.K., HAAGSMA, K. & REIERSON, D.A. 1996. Barrier sprays to control Argentine ants (Hymenoptera: Formicidae). *Journal of Economic Entomology* **89**: 134–137.

SHEETS, J.J., KARR, L.L. & DRIPPS, J.E. 2000. Kinetics of uptake, clearance, transfer, and metabolism of hexaflumuron by eastern subterranean termites (Isoptera: Rhinotermidae). *Journal of Economic Entomology* **93**(3): 871–877.

SILVERMAN, J. & BRIGHTWELL, R.J. 2008. The Argentine ant: challenges in managing an invasive unicolonial pest. *Annual Review of Entomology* **53**: 231–252.

SILVERMAN, J. & ROULSTON, T.H. 2003. Retrieval of granular bait by the Argentine ant (Hymenoptera: Formicidae): effect of clumped versus scattered dispersion patterns. *Journal of Economic Entomology* **96**: 871–874.

SILVERMAN, J. & ROULSTON, T.H. 2001. Acceptance and intake of gel and liquid sucrose compositions by the Argentine ant (Hymenoptera: Formicidae). *Journal of Economic Entomology* **94**: 511–515.

SOEPRONO, A.M. & RUST, M.K. 2004. Effect of horizontal transfer of barrier insecticides to control Argentine ants (Hymenoptera: Formicidae). *Journal of Economic Entomology* **97**: 1675–1681.

STEVENS, M.M., JAMES, D.G. & SCHILLER, J. 2002. Attractiveness of bait matrices and formulation/toxicant combinations to the citrus pests *Iridomyrmex purpureus* (F. Smith) and *Iridomyrmex rufoniger* (Hymenoptera: Formicidae). *Journal of Applied Entomology* **126**: 490–496.

STRINGER, C.E., LOFGREN, C.S. & BARTLETT, F.J. 1964. Imported fire ant bait studies: evaluation of toxicants. *Journal of Economic Entomology* **57**: 941–945.

SUAREZ, A.V., HOLWAY, D.A. & CASE, T.J. 2001. Patterns of spread in biological invasions dominated by long distance jump dispersal: insights from Argentine ants. *Proceedings of the National Academy of Sciences of the United States of America* **98**: 1095–1100.

VAIL, K.M. & WILLIAMS, D.F. 1995. Pharaoh ant (Hymenoptera: Formicidae) colony development after consumption of pyriproxyfen baits. *Journal of Economic Entomology* **89**: 1501–1507.

VAIL, K.M., WILLIAMS, D.F. & OI, D.H. 1996. Perimeter treatments with two bait formulations of pyriproxyfen for control of Pharaoh ants (Hymenoptera: Formicidae). *Journal of Economic Entomology* **89**: 1501–1507.

VAN WILGENBURG, E., TORRES, C.W. & TSUTSUI, N.D. 2010. The global expansion of a single ant supercolony. *Evolutionary Applications* **3**: 136–143.

VOGEL, V., PEDERSEN, J.S., GIRAUD, T., KRIEGER, M.J.B. & KELLER, L. 2010. The worldwide expansion of the Argentine ant. *Diversity and Distributions* **16**: 170–180.

WEBB, G. 2011. Evaluation of an ant bait against *Linepithema humile* in Australia. In: Robinson, W.H. & de Carvalho Campos, A.E. (Eds) *Proceedings of the 7th International Conference on Urban Pests*. 77–84. Instituto Biológico, São Paulo, SP, Brazil.

Accepted 28 January 2016