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UV STABILITY OF SELECTED ANT BAITS USED IN ANT ERADICATION PROGRAMS IN AUSTRALIA

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Summary

The active ingredients in ant baits commonly used in eradication programs for invasive ant species are generally non-repellent, slow-acting, and relatively benign to other organisms and the environment because they degrade relatively quickly. However, few studies have ever assessed how quickly these active ingredients degrade when formulated into bait products. When various formulated baits containing the active ingredients pyriproxyfen, s-methoprene and hydramethylnon were exposed to different levels of UV irradiation, rates of degradation varied substantially. Over a period of 8.5 days, pyriproxyfen was relatively stable even under full UV exposure, whilst s-methoprene and more so hydramethylnon degraded rapidly under full and partial UV exposure. Under permanent cover with only side illumination, all three active ingredients were relatively stable.

Keywords: ant bait, UV stability, eradication, pyriproxyfen, s-methoprene, hydramethylnon

INTRODUCTION

The primary tool for eradication of invasive tramp ants is food-based insecticidal baits (Hoffmann et al. 2011, Lach et al. 2019, Webb 2013, Wylie et al. 2016). In Australia, the use of ant baits is a key tool in the current eradication programs for red imported fire ant *Solenopsis invicta* Buren, tropical fire ant *Solenopsis geminata* F., little fire ant or electric ant *Wasmannia auropunctata* (Roger), African bigheaded ant *Pheidole megacephala* (F.), yellow crazy ant *Anoplolepis gracilipes* (Smith) and various other species.

For baits to be effective the matrix needs to be attractive to ants but the active ingredients used also need to be non-repellent, slow acting and relatively specific to ants when presented in bait form (Hoffmann et al. 2010). The four key active ingredients used in eradication programs in Australia are the insect growth regulators pyriproxyfen and s-methoprene, the metabolic inhibitor hydramethylnon and the neurotoxin Fipronil. All are effective when applied in attractive matrices and at rates low enough to encourage bait retrieval and consumption. Both s-methoprene and hydramethylnon are relatively unstable, particularly under UV exposure (Chakraborty et al. 1993, Mallipudi et al. 1986, Quistad et al. 1975) with half-lives of a few hours whereas pyriproxyfen and fipronil are relatively more photostable (Sullivan and Goh 2008, Tingle et al. 2003) with half-lives of a week or more. Ant bait is very effective if harvested by ants quickly and consumed or cached in the nest, but if not, then the active ingredient degrades relatively quickly in the environment and this is considered a desirable feature (Drees et al. 2013, Etheridge and Phillips 1976, Spicer-Rice et al. 2012).

At least in theory, the encapsulation of active ingredient within the bait matrix should provide some protection of the active ingredient from degradation processes, particularly UV exposure, but the extent of this protection has not been validated. Chakraborty et al. (1993) reported an aqueous half-life for hydramethylnon of just 42 minutes under UV exposure and pH was found to have only a minimal effect on hydramethylnon. Vander Meer (1982) determined that Amdro™ Fire Ant Bait (hydramethylnon) degraded by ca. 40% within 12 hours (and 90% within 60 hours) under UV exposure which suggests that, relative to the physico-chemical result of Chakraborty et al. (1993) incorporation into a bait matrix may enhance resistance to photolysis. Nevertheless, such rapid degradation in the matrix is not conducive to a consistent successful eradication outcome unless bait is rapidly retrieved or other mitigating factors occur eg. (shading by vegetation, use of bait sheltered containers). Taniguchi et al. (2003) exposed plastic bait stations containing Amdro to UV light for up to 12 weeks and then tested the bait on small laboratory colonies of African bigheaded ant and achieved 100% mortality for all exposure periods indicating that the bait remained active whilst protected by the plastic bait station. To my knowledge there is no similar validation data for the other commonly used active ingredients.

The purpose of this study was to evaluate the degradation of pyriproxyfen, s-methoprene and hydramethylnon, when formulated as ant bait, under various UV exposure scenarios. Freshly formulated bait was exposed to full sun, half-shade and full shade for 8.5 days under typical weather conditions in Sydney during the peak summer period (late January). While fipronil is a common active ingredient used in baits for invasive species eradication, a suitable

fipronil-based corn-based bait was not available at the time of this study.

MATERIALS AND METHODS

Bait Products

Samples of recently manufactured baits commonly used in programs for control or eradication of invasive ants in Australia were sourced. The four baits were Distance®, Engage®, Campaign® and Synergy Pro® manufactured by Sumitomo Chemical

Australia. Details of the four baits are shown in Table 1. Distance and Engage are used extensively in the eradication program for red imported fire ant in Brisbane administered by Biosecurity Queensland; Campaign is used in the eradication program for little fire ant (electric ant) in Cairns (also administered by Biosecurity Queensland) and Synergy Pro is used in the various control programs for yellow crazy ant in various locations in Queensland and New South Wales.

Table 1: Ant bait products used in the trial.

Trade Name	Active Ingredient	Manufacture Date and Batch Number	Assayed content
Distance Ant Bait ¹	5g/kg pyriproxyfen	9 August 2017 (D/103230)	5.39g/kg
Engage Ant Bait ²	5g/kg s-methoprene	7 September 2017 (E/Round1/1)	6.06g/kg
Campaign Ant Bait ²	7.3g/kg Hydramethylnon	16 October 2017 (C+/103250)	7.3g/kg
Synergy Pro Ant Bait ¹	2.5g/kg pyriproxyfen, 3.65g/kg Hydramethylnon	1 November 2017 (S/103272)	2.67g/kg, 3.28g/kg

¹. Registered products under APVMA legislation ². Approved by the APVMA under special permits for use against invasive ant species.

Location and Trial Design

The trial was conducted at Cronulla (NSW, Australia) on a residential property. The site was orientated in an east-west direction, high on a ridge which received direct solar exposure during most of the day (from 7am to 7pm). Five gram samples of each bait were placed into 90mm diameter plastic petri dishes and sealed around the rim using white electrical tape. Petri dishes were placed horizontally into large translucent plastic trays with similar translucent lids. One tray each was exposed to three levels of UV exposure (full sun, covered with shade cloth and fully covered under a hard structure (Figure 1) from 6am on 24 January 2018. Single petri dishes were extracted at each time point from the 3 trays and placed in the freezer for subsequent analysis. Hence there is only a single replicate of each exposure/time combination. The cost of analysis prohibited a more extensive replicated study. During the analysis, certain exposure time combinations were selected for replicate analysis to ensure method repeatability.

All three tubs were fitted with DS1921G i-Button probes (Thermodata Pty Ltd, Brisbane, Australia) under the lid which were capable of recording both temperature (-40 to 85°C) and humidity (0-100%). Probes were active for the entire period of the study.

Shade cloth was Coolaroo® brand Extreme Exterior Fabric manufactured by Gale Pacific Ltd. The shade cloth was advertised as blocking 50-60% of ultraviolet light.

Weather Conditions

During the period of the trial just 0.6mm of rain fell and generally conditions were warm and sunny (Table 2). On 31 January and 1 February maximum daily temperature declined from ca. 30°C to ca. 22 °C which corresponded with the small amount of rain on 31 January. On each day during the study (at 5 times from 7am till 7pm), UV intensity was measured using a Digitech QM1587 light meter. UV readings were consistent with typical summer conditions (Table 2) with mid-day readings up to ca. 100,000 LUX. Readings were taken from beneath the translucent lid of the tubs and in the case of the tub kept beneath shade cloth, beneath both the tub lid and shade cloth.

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Figure 1: Exposure scenario for ant bait a. Sheltered tub shown without lid b. Sheltered tub with lid in place c. Full sun and shaded tubs with lids and shade cloth in place. NB petri dishes are labelled out to 14 days of exposure but these were adjusted during to the study for a maximum exposure of 8.5 days.

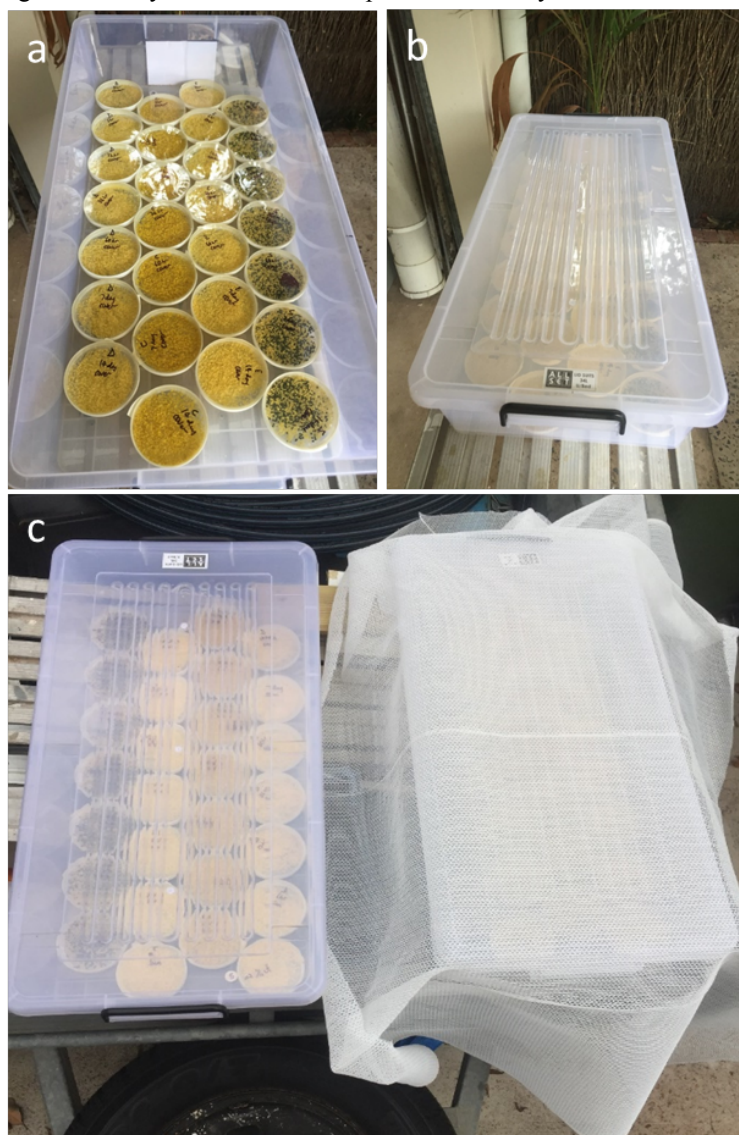


Table 2: Weather conditions during the trial (Sydney Airport weather station) for the period 24 January 2018 to 1 February 2018.

	January									February
	24	25	26	27	28	29	30	31	1	
Min. Temp ^(°C)	21.0	22.4	23.4	23.6	23.8	22.8	21.6	17.9	16.9	
Max. Temp ^(°C)	31.3	28.9	28.7	30.4	29.2	30.8	32	21.8	22.1	
Rainfall (mm)	0	0	0	0	0	0	0	0.6	0	
RH (%) (9am)	78	90	90	88	89	76	59	79	66	
RH (%) (3pm)	58	80	83	79	77	52	48	60	64	
Sunrise (AEST)	0609	0610	0611	0612	0613	0614	0615	0616	0617	
Sunset (AEST)	2004	2003	2003	2002	2002	2001	2000	2000	1959	
Daylength (hrs)	13.55	13.53	13.51	13.50	13.48	13.47	13.45	13.43	13.42	

Chemical Analysis

Analysis of S-methoprene in Engage Ant Bait (nominal 5 g/Kg) - A 0.5 gram sub-sample of the original 5 gram sample was taken. S-methoprene was extracted in 50 mL 80:20 acetonitrile: Milli Q aided by ultrasonification for 30 minutes. The liquid extracts were filtered through 0.45 μ nylon filters and 10 μ l was injected onto a Waters Symmetry Shield C18 column (100 x 4.6 mm, 3.5 μ m) using a Waters 2695 Alliance HPLC system. Detection was by Diode-array (W996) at 265 nm. S-methoprene was eluted at 5.8 minutes using a mixture of Acetonitrile and MilliQ water (70: 30) at a flow rate of 1.8 mL/min.

Analysis of Pyriproxyfen in Distance Ant bait (nominal 5 g/Kg) - A 1 gram subsample of the original 5 gram samples was taken. pyriproxyfen was extracted with 40 mL of acetonitrile aided by ultrasonification for 30 minutes. The extract was transferred to a 50 mL volumetric flask and the remaining bait was re-extracted with 5 mL of acetonitrile and 10 minutes ultrasonification. The two extracts were combined and adjusted to a final volume of 50 mL with MilliQ water. The combined extract was then filtered through 0.45 μ nylon filter and 10 μ l was injected onto a Waters CSH C18 column (50 x 3 mm, 2.5 μ m) using a Waters HPLC system (W600E quaternary pump, W717 autosampler, W996 Diode-array). pyriproxyfen was eluted at 2.0 minutes using a mixture of acetonitrile, water and 2% triethylamine (aq) (65:15:20) at a flow rate of 0.8 mL/min, and was detected at 270 nm.

Analysis of Hydramethylnon in Campaign Ant Bait (nominal 7.3 g/Kg) - A 1 gram subsample of the original 5 gram samples was taken. Hydramethylnon was extracted with 35 mL of acetonitrile aided by mechanical shaking for 60 minutes and then ultrasonification for 30 minutes at 50^oC. The extract was transferred to a 50 mL volumetric flask and the remaining bait was re-extracted with 10 ml of acetonitrile and 15 minutes ultrasonification at 50^oC. The two extracts were combined and adjusted to a final volume of 50 mL at 20^oC with MilliQ Water. The combined extract was then filtered through 0.45 μ nylon filter and 10 μ l was injected onto a Waters CSH C18 column (50 x 3 mm, 2.5 μ m) using a Waters HPLC system (W600E quaternary pump, W717 autosampler, W996 Diode-array). Hydramethylnon was eluted at 2.6 minutes using a mixture of acetonitrile, water and 2% triethylamine (aq) (70:10:20) at a flow rate of 0.8 mL/min, and was detected at 270 nm.

Analysis of Pyriproxyfen and Hydramethylnon in Ant Bait (nominal 2.5 and 3.65 g/Kg respectively) - 1

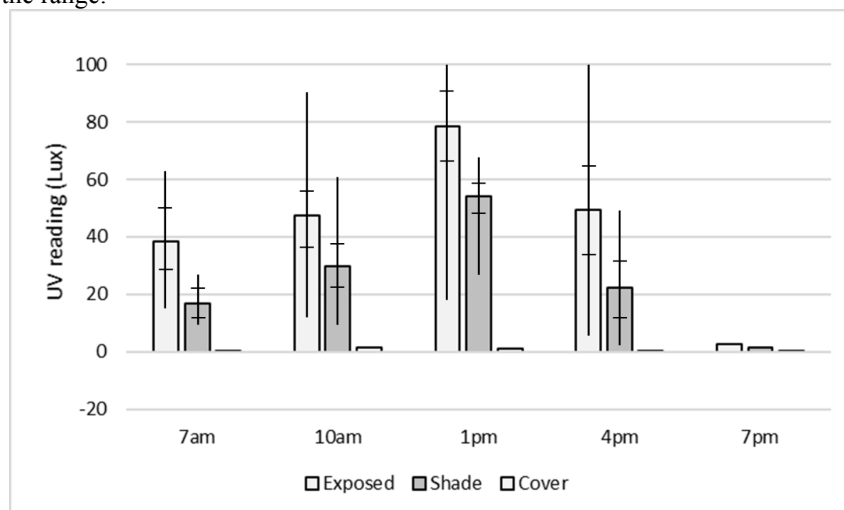
gram portions of Ant bait granules were extracted with 35 mL of acetonitrile aided by mechanical shaking for 60 minutes and then ultrasonification for 30 minutes at 50^oC. The extract was transferred to a 50 mL volumetric flask and the remaining bait was re-extracted with 10 mL of acetonitrile and 15 minutes ultrasonification at 50^oC. The two extracts were combined and adjusted to a final volume of 50 mL at 20^oC with MilliQ Water. The combined extract was then filtered through 0.45 μ nylon filter and 10 μ l was injected onto a Waters CSH C18 column (50 x 3mm, 2.5 μ m) using a Waters HPLC system (W600E quaternary pump, W717 autosampler, W996 Diode-array). pyriproxyfen and hydramethylnon were eluted at 1.7 and 2.6 minutes respectively using a mixture of acetonitrile, water and 2% triethylamine (aq) (70:10:20) at a flow rate of 0.8 mL/min, and were detected at 270 nm.

RESULTS

On most days during the study period there was either full or partial direct solar exposure, with minimal cloud cover, with the exception of the final two days where some rainfall occurred and general conditions were cool and overcast (Table 2). The mean maximum daily UV reading was 79 LUX (at 1pm) but ranged up to 100 LUX for both the 1pm and 4pm readings) (Figure 2). The claimed reduction of 50-60% UV light by the shade cloth was generally validated by the light meter readings (Figure 2) at most times with the exception of 1pm where mean reduction was only ca. 30%.

Under full and partial exposure to sun, temperatures peaked at ca. 50-55^oC within the tubs but in the covered position the temperature maximum more closely approximated ambient temperature (ca. 30^oC) (Figure 3). Although humidity was recorded during the study it is unclear how important this might have been for active ingredient stability. Humidity fluctuated dramatically during the day within the trays either fully or partially exposed to sunlight with peaks during the night and early morning of ca. 90 % declining to 20-30 % in the late afternoon. In the covered tray the fluctuation was generally confined to the range of 60-90 % with the peak also occurring during the night and early morning. It is also unclear how humidity recorded in the trays directly relates to humidity in the petri dishes given that they were sealed with electrical tape.

Figure 2: Mean LUX readings across the day, during the trial period. Due to scale, no error or range bars are included for the covered tub nor for any 7pm reading. The inner cross bars represent the SEM and the full length of the bar represents the range.



Bait samples exposed to full sun and partial shade faded in colour intensity over the course of the study, at least for the corn component in each bait (Figure 4). The decay curves for the three active ingredients varied. For sheltered samples, pyriproxyfen and s-methoprene remained at or near starting concentration for the full period of the study (Figs. 5,6). In contrast, hydramethylnon declined slowly over this period – 38 % decline for Campaign® and 17 % decline for Synergy Pro® (Fig.7). Under UV exposure, pyriproxyfen was the most stable maintaining close to actual starting concentrations. For Distance Ant Bait (nominally 5 g/kg), concentration started declining around 2.5 days for both exposed and semi-exposed samples, but still stayed within 80 % of starting concentration through to the end of the trial (8.5 days) (Figure 5). For the pyriproxyfen component of Synergy Pro® (nominally 2.5 g/kg), the actual recorded values all remained in excess of 2.5 g/kg at all times in all exposure scenarios (Figure 5). This was largely due to the fact that the original batch analysis was higher than the nominal concentration at 2.67 g/kg. S-methoprene in Engage® (nominally 5

g/kg) maintained above nominal concentration for 2.5 days but then declined rapidly to around 20 % in full sun and 60 % under partial shade (Figure 6). Note that the starting level was higher than nominal concentration at 6.0 g/kg. Under UV exposure, hydramethylnon was the most photo-unstable molecule. For Campaign® (nominally 7.3 g/kg), hydramethylnon concentration declined rapidly from 6 hour onwards in full and partial sun to almost zero at the completion of the study (Figure 7). A similar result was evident for the hydramethylnon component of Synergy Pro® (nominally 3.65 g/kg). All data points were single analyses with the exception of repeat analyses for select exposure/time combinations to ensure method repeatability. These were consistently within the range of 3-4% of average (Table 3). Ideally, repetition of trays would have been preferable but the expense of chemical analysis prevented such repetition. However, chemical analysis did confirm that initial active ingredient levels in bait samples were in line with batch analyses and nominal concentrations.

Table 3: Variability in repeat analyses of selected samples.

Product	Exposure type	Sample time	Mean analysis value (g/kg)	Range and sample size (N)
Distance®	Shadecloth	1 hour	4.90	4.72-5.04 (N=5)
Engage®	Full sun	1.5 days	5.55	5.40-5.65 (N=5)
Campaign®	Full cover	1 hour	6.50	6.30-6.77 (N=5)
Synergy Pro® - pyriproxyfen	Shadecloth	1 hour	2.71	2.67-2.74 (N=2)
Synergy Pro® - Hydramethylnon	Shadecloth	1 hour	3.32	3.30-3.34 (N=2)
Synergy Pro® - pyriproxyfen	Full cover	6 hour	2.76	2.73-2.78 (N=2)
Synergy Pro® - Hydramethylnon	Full cover	6 hour	3.32	3.30-3.34 (N=2)

Figure 3. Temperature (a) and humidity (b) profiles in the tubs over the study period as recorded by Thermodata DS1921G i-Button probes.

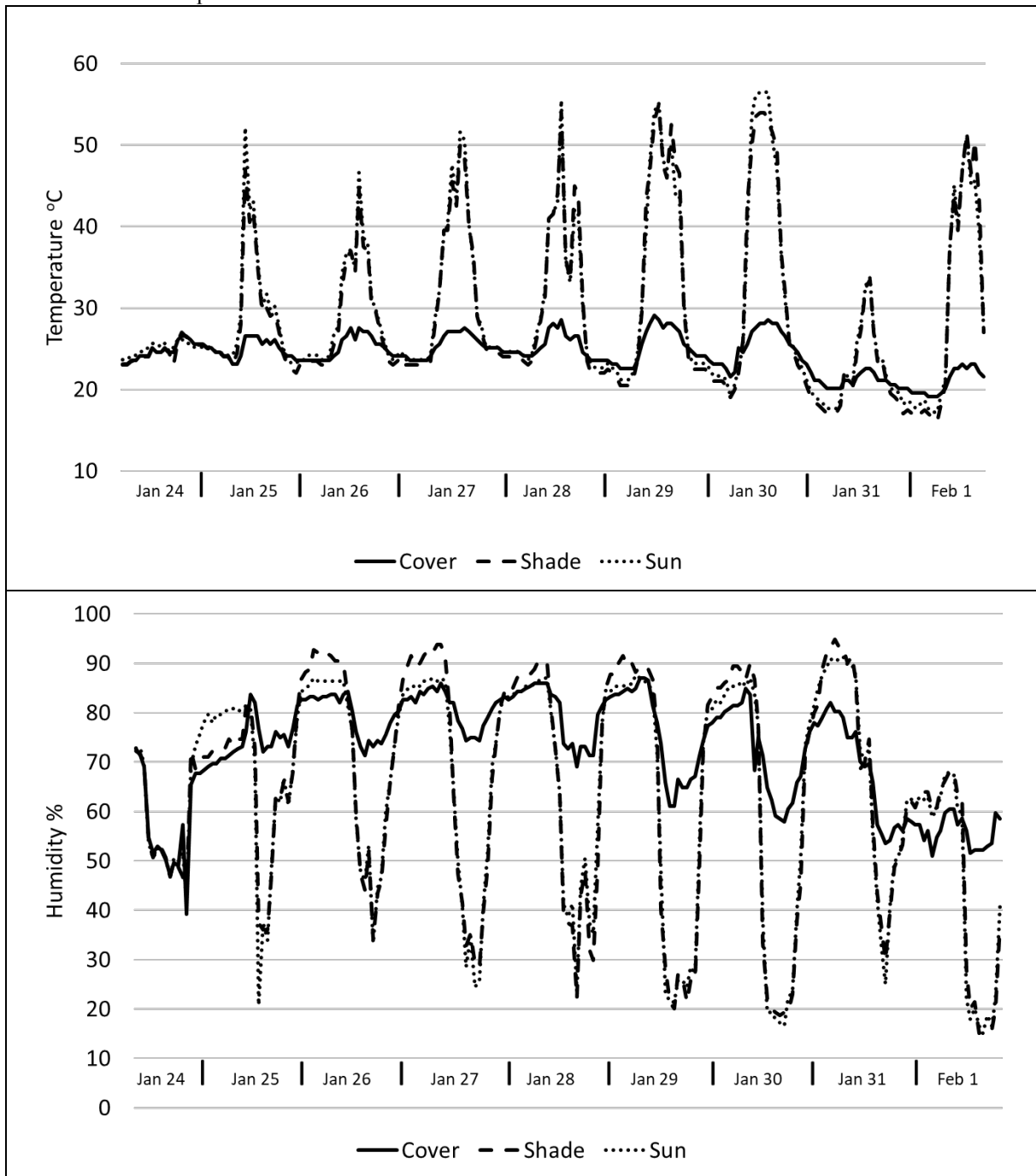


Figure 4: Colour change in bait following exposure to sunlight. A. Campaign full cover vs full sun at 8.5 days. B. Engage full cover vs full sun at 8.5 days. C. Distance full cover vs full sun at 8.5 days. D. Synergy Pro full cover vs full sun at 8.5 days.

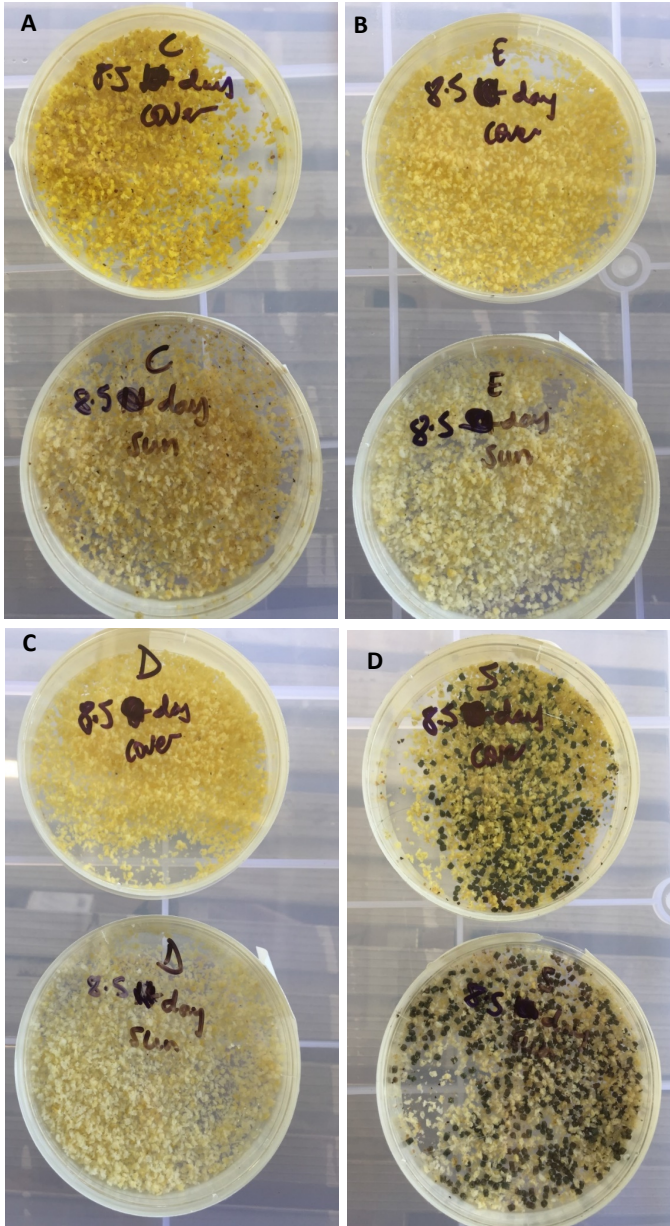


Figure 5. Degradation of active ingredient over time - pyriproxyfen. A. Distance B. Synergy Pro

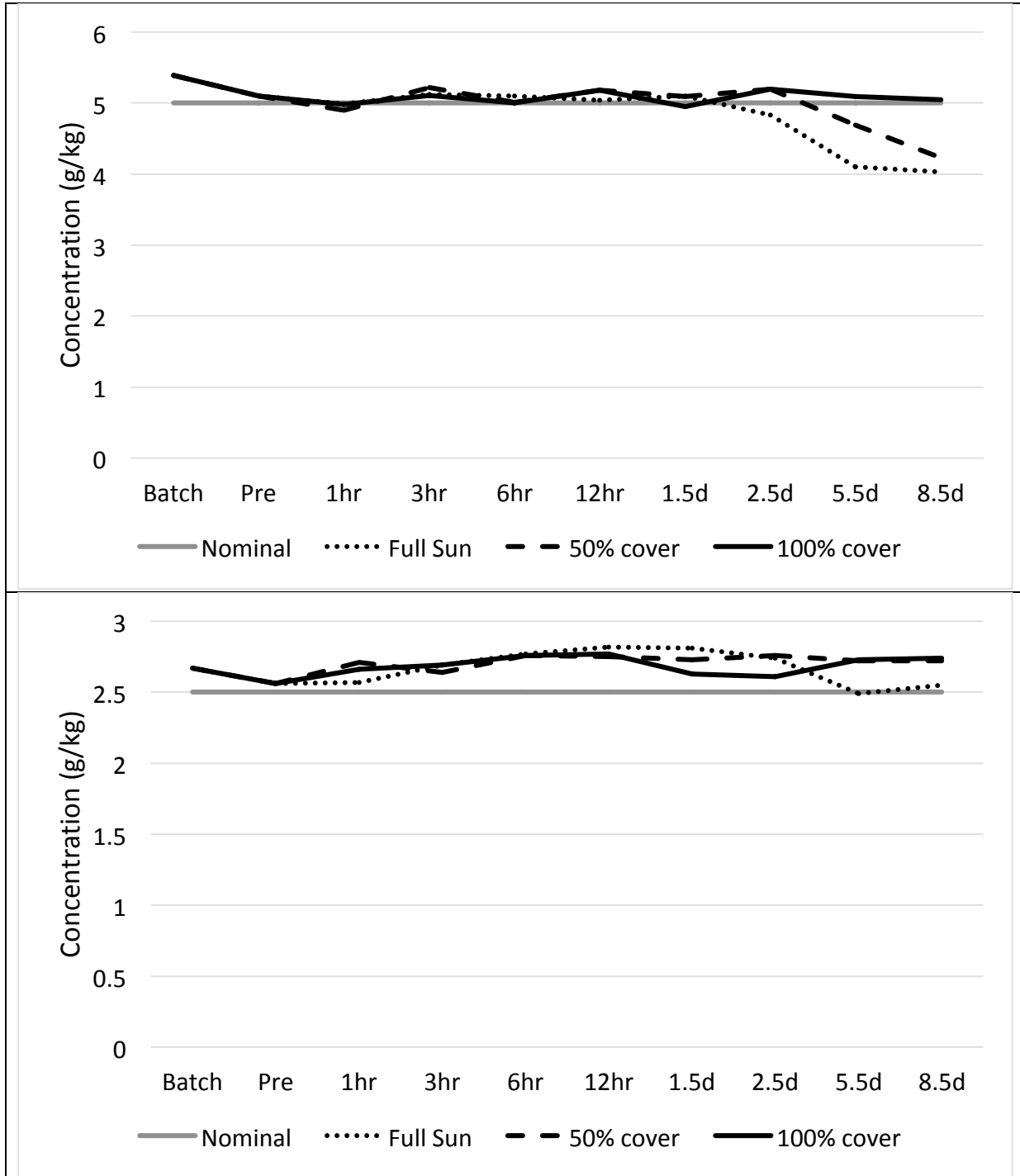
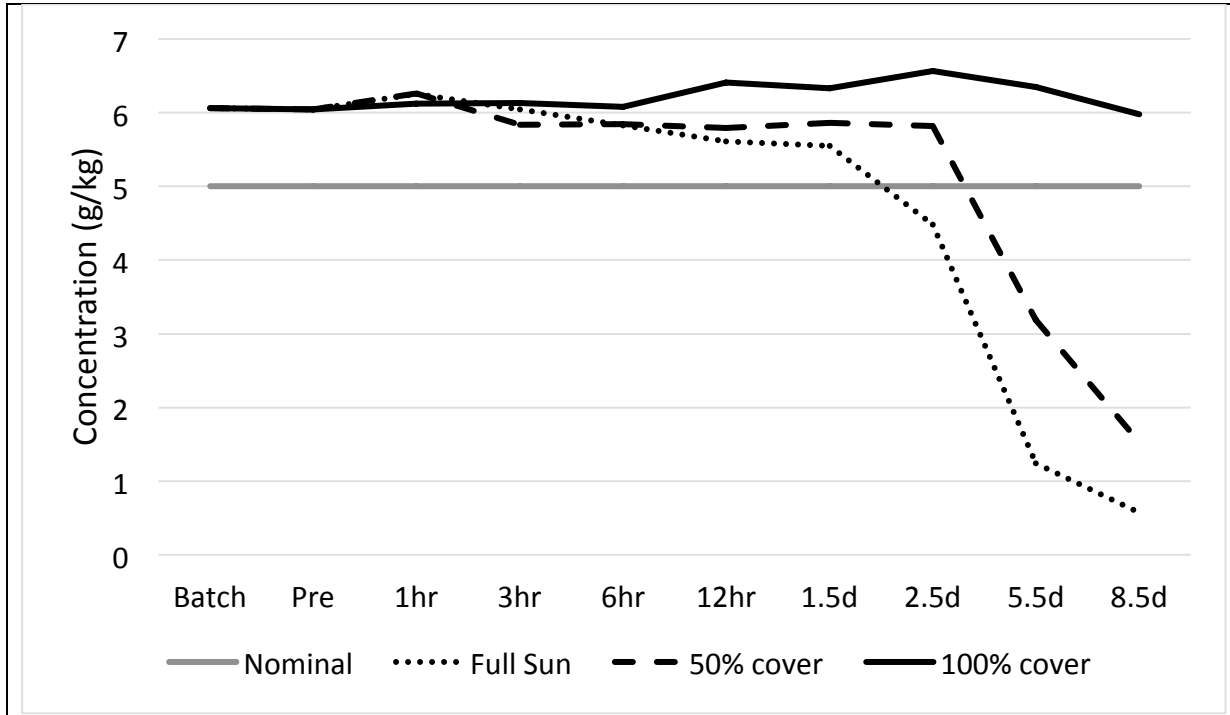


Figure 6. Degradation of active ingredient over time – s-methoprene.



DISCUSSION

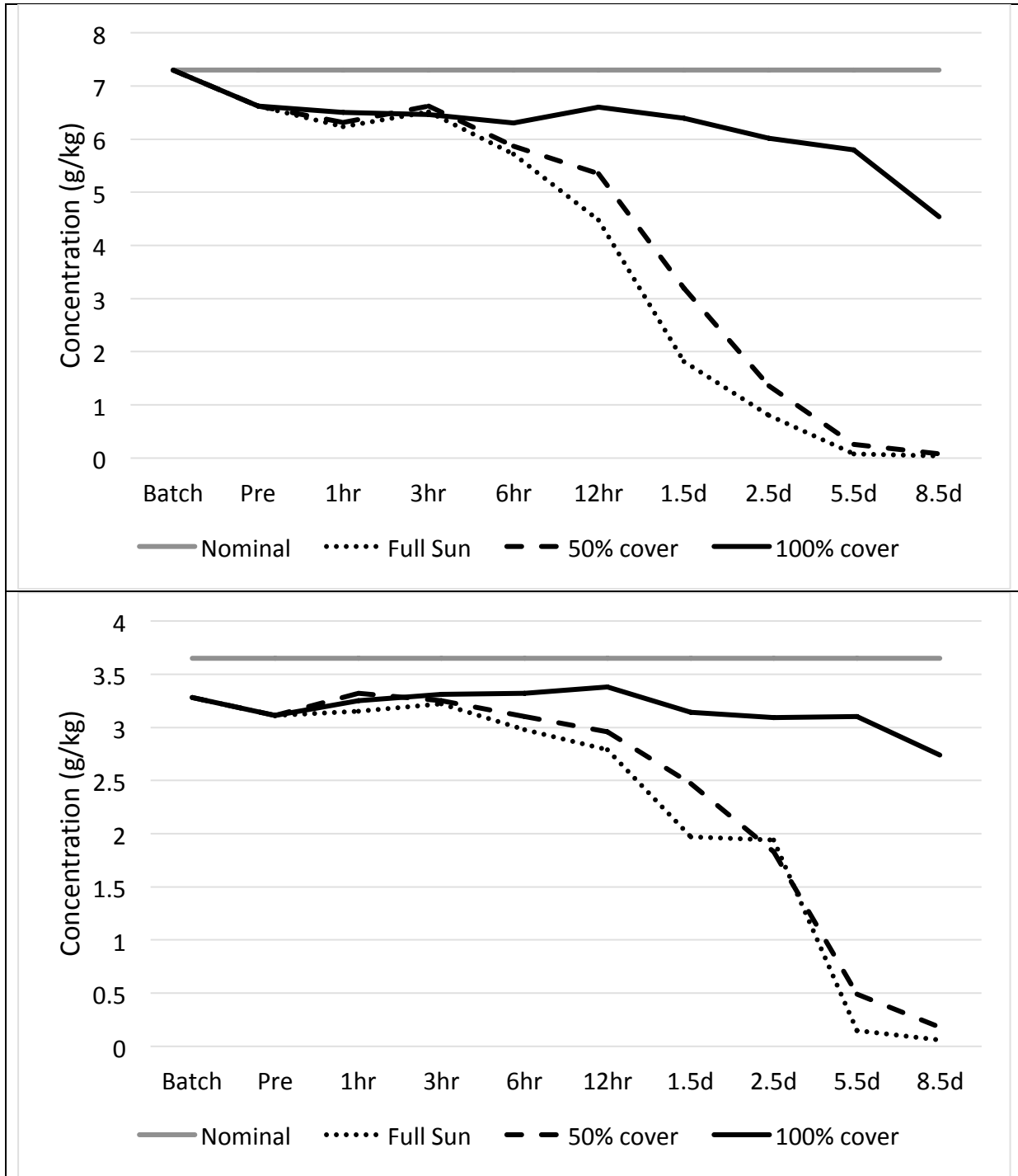
Bait products are a key part of the eradication process for many invasive tramp ant species (Hoffmann et al. 2016, Wylie et al. 2016). There are a limited range of insecticidal active ingredients used in bait products because they must have certain properties that make them amenable to success viz. they must be slow acting allowing safe bait retrieval (without mortality or morbidity), the bait must be accepted into the nest as viable food and processed quickly. Further, the bait matrix in which the active ingredient is incorporated needs to be highly attractive to ants to ensure rapid retrieval by foraging ants. This is particularly important because two of the key active ingredients used worldwide - hydramethylnon and methoprene (including its S isomer) are relatively unstable under UV exposure (Chakraborty et al. 1993, Mallipudi et al. 1986, Quistad et al. 1975). Vander Meer (1982) demonstrated that the hydramethylnon concentration in field exposed Amdro rapidly declined over 60 hours under UV exposure. In this study, the decline of hydramethylnon under full UV exposure was almost identical to that reported by Vander Meer (1982) ie. ca. 40% decline over 12 hours and 90% decline over 60 hours.

Of the three compounds tested pyriproxyfen was the most stable with relatively little decline in active ingredient content after 8.5 days - 10-20% from exposed and semi-exposed samples of Distance® and just 7% for exposed samples of Synergy Pro®. This is consistent with the known physico-chemical properties of pyriproxyfen (Sullivan and Goh 2008). S-methoprene has the same mode of action as pyriproxyfen, but is more UV susceptible (Quistad et al. 1975). However, under fully shaded conditions, the concentration of s-methoprene in Engage did not decline at all. Under exposed and semi-exposed conditions significant decline occurred from 2.5 days onwards with declines of 91% and 75% respectively after 8.5 days.

Taniguchi et al. (2003) demonstrated that Amdro kept in bait containers under UV exposure for 14 days did not decline in efficacy against bigheaded ant. However, Vander Meer (1982) tested Amdro, exposed directly to UV light over a period of 144 hours, against red imported fire ant and found a marked decline in efficacy from 100% within 3 days for fresh bait to less than 50% within 14 days following 12 hour UV exposure or longer. To our

knowledge there are no similar data for bait products containing pyriproxyfen or s-methoprene.

Figure 7. Degradation of active ingredient over time – Hydramethylnon. A. Campaign B. Synergy Pro.



The significance of the marked colour change for the corn component of each of the baits is unclear. While blank bait or pure corn granules were not included in the study to evaluate colour change, blank corn is known to fade from pale yellow to whitish yellow under prolonged UV light (Webb pers. obs.). Aside from the colour change, the bait samples from later exposure times remained similar in consistency to earlier samples and for that matter to bait maintained under full cover. There was no apparent increase in moisture despite the fluctuating humidity levels in the tubs and the bait did not aggregate and flowed freely in the hand. It is therefore likely that UV exposure was the sole reason for the colour change.

For all three molecules tested here, the decline in active ingredient content was minimal to zero under full shade. This suggests that application of bait to locations under cover of vegetation or application at times when UV exposure is minimised – early morning or late afternoon, or under heavy cloud cover – may aid in ensuring longevity of bait in the field. However, this is likely to be impractical in large scale eradication programs utilising helicopters to spread bait over wide areas and where warm sunny conditions are required to ensure maximum foraging of ants. Similarly, the use of bait stations to protect the bait is unlikely to be feasible in all but small-scale eradication or control programs. Aside from regulatory constraints on use, the choice of bait would therefore be dependent on factors such as population density and intensity of foraging activity, daytime temperature, likelihood of rainfall, and the program management intent eg. the desire to “infect” the population with insect growth regulators rather than more rapid nest kill using metabolic or neurotoxic compounds.

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