Research on Improving Containment of Argentine Ant Infestations

Richard Toft

Entecol Report: **ENT-014**

Prepared for: **Tasman District Council and Landcare Research NZ Ltd**
Envirolink Project TSDC 74

Date: **May 2011**

Entecol Ltd – PO Box 142 – Nelson – New Zealand 7040
Ph. +64 3 539 1474 – info@entecol.co.nz

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1. Background

Regional Councils have funded a series of field and laboratory trials on products and techniques to determine their potential for aiding the containment of Argentine ant populations. The analysis and reporting of results presented here has been funded by Envirolink Project TSDC 74 for Tasman District Council and completed by Entecol Ltd under contract to Landcare Research.

2. Introduction

Argentine ants (*Linepithema humile*) have been established in New Zealand since 1990 (Green, 1990), and are a major pest for householders and a recognised threat to biodiversity and horticulture (Harris, 2002; Vega & Rust, 2001; Ward, 2009). They have now spread into many towns and cities throughout the North Island and northern South Island, primarily through accidental human-mediated dispersal of propagules (Ward *et al.*, 2005).

Unlike many other ant species, Argentine ant queens do not disperse by flight, but rather by a process of "budding", in which a queen and workers split off from an existing nest to form a new colony close by. This limits the natural spread of Argentine ants to about 150m per year (Suarez *et al.* 2001). Effective and coordinated management to contain existing infestations could potentially restrict Argentine ant infestations to relatively small areas, and even lead to the possibility of localised eradication (Ward *et al.* 2010). However, improved tools and techniques are required before we can effectively manage and contain existing populations.

In New Zealand, Argentine ants are still primarily associated with urban and suburban areas, and these environments have their own challenges when it comes to managing ant populations. Concrete curbing, foot paths, and sealed surfaces provide ant highways, allowing them to move faster and over longer distances than if they had to move through grass or leaf-litter. The large expanses of concrete and paving also retain heat better, providing good over-wintering sites and protection from the elements. The alkaline nature of concrete also works against many insecticides that perform better in neutral or more acidic conditions. Another issue is that land tenure is often divided into small areas, especially in residential areas, leading to major difficulties in coordinating treatment activities. Containment operations in human environments will therefore require a suite of effective tools and techniques to deal with the range of situations encountered.

Wide area baiting techniques using Xstinguish Argentine ant bait is often successful at substantially reducing ant numbers (Ward *et al.* 2010), but remnant populations often remain and need "mopping up". In small-scale residential operations there may be a need to control reinvasion from adjacent infested properties. Xstinguish ant bait has a short field life and is not suitable to be left out as a control tool awaiting residual populations to bounce back or when long-term boundary protection is required. Advion® ant bait arenas provide long-lasting bait in a protective bait station that remains viable for long periods, and previous lab trials

have indicated it does have potential for killing of colonies (Toft & Rees, unpublished data). Field trials of the performance of Advion® arenas at controlling Argentine ants at low population levels were requested to determine whether they do provide an option for mop-up of residual populations and boundary protection.

Biff Ant, containing bifenthrin, is a new surface treatment product being developed by Key Industries that is specifically targeting the control of ants in urban situations. It is designed to have good performance on a wide range of surfaces, including concrete. In order to be effective for containment in urban areas it needs to remain active for as long as possible, be resistant to at least light-medium rainfall, and be non repellent to Argentine ants (otherwise they will avoid it rather than be killed and will look for alternative ways to cross into new areas). Regional Councils requested trials to determine the effectiveness of this new product as a potential means for containing populations and protecting boundaries from reinvasion.

Finally, potted plants have been identified as one of the main ways that Argentine ant propagules can be accidentally transported into new areas, and people living in infested areas need to be encouraged to take steps to ensure any plants being moved out of their properties are free of ant nests. Many residents would be keen to avoid using expensive and toxic chemical treatments and may be inclined to try other methods to treat their potted plants. The internet is a source of many home-remedies for treating ant nests, and two of the more promising remedies, lemon extract and peppermint tea, were selected for testing.

3. Objectives

- Assess the effectiveness of Advion® Ant Bait Arenas for treating residual populations of Argentine ants
- Assess Biff Ant surface treatment for repellency to ants and efficacy over time and following rainfall
- Trial two home remedies for removing Argentine ants from potted plants

4. Methods

4.1 Use of Advion Arenas for treating residual populations

We selected eight urban roadside sites with Argentine ants present. These sites were all narrow strips of either traffic islands or small ornamental garden areas on the street-frontage of industrial buildings. Most sites included varying amounts of landscaping bark on the ground surface. The area around the sites had all previously been subjected to some ant control measures applied by council contractors approximately one month before the trials began. The treatments applied previously were a range of surface spray treatments and are

likely to have resulted in a reduction in Argentine ant populations at all sites, but these treatments did not necessarily extend directly onto the gardened areas themselves.

On 26 March 2010, the general abundance of Argentine ants at each site was assessed using 25 ml pottles baited with “Inform” ant monitoring bait. “Inform” is a non-toxic paste bait known to be highly attractive to Argentine ants. A 15 m long transect of pottles was placed through the centre of each site, with pottles 1 m apart (i.e. a total of 16 pottles per site). The baited pottles were left open and on their sides for 1 hour, and then their tops put on and ants counted back at the laboratory.

On 29 March, we placed eight Advion Arenas 2 m apart in the centre of four of the sites. The other four sites were used as the non-treated control sites.

On 12 April, two weeks after the Advion arenas were placed out, we repeated the baited pottle counts at all sites. We also did a rapid visual inspection of each site (including scratching away landscaping bark and lifting low vegetation) to determine the presence/absence of Argentine ants in case none were collected in the baited pottles. The visual search stopped as soon as Argentine ants were seen, but limited to 5 person-minutes maximum search time per site. A second visual inspection was undertaken at each site on 21 May, about 2 months after the Advion arena stations had been put out. The arenas were left on site for the whole period.

4.2 Trials of Biff Ant surface treatment

Repellency

A trial to assess the repellency of Biff Ant used 85 × 85 mm cement board tiles and was assessed 24 hours after application. A line of 24 stations was set up around a Nelson property infested with Argentine ants, with at least 5 m between each station. At each station, two treated tiles and one untreated tile was placed on the ground in a triangle pattern with approximately 10 cm between tiles. A blob of “Inform” ant monitoring bait was placed in the centre of each tile. Stations were re-visited after 15, 30 and 60 minutes and ant activity on the tiles assessed visually. It was not possible to do simple counts of ants on baits as was hoped because ants visiting the treated tiles were lethally affected by the toxin within minutes of exposure and therefore unable to recruit in larger numbers over time. Instead we were looking for evidence of ants turning away from treated tiles and therefore not visible on the tiles.

Effectiveness over time

Biff Ant (80 g/L bifenthrin) was mixed at 15 ml per litre of fresh water and sprayed on to 85 × 85 mm cement board tiles in an effort to replicate the highly alkaline conditions found on new concrete surfaces. A Chapin brand hand sprayer fitted with an adjustable cone nozzle was used and the flow rate of the sprayer was measured immediately prior to application to

ensure the coverage on the tiles equaled the recommended application rate of 250 ml per M^2 (equivalent to 0.3 g of bifenthrin per M^2). The tiles were sprayed at 1 pm on 14 February 2010. The weather was hot and sunny and the tiles appeared dry again within a few minutes.

An hour after treatment, tiles were arranged on large plastic baking trays for ease of handling and subjected to differing conditions. Group "A" tiles represented a worst case scenario and were left out in a completely exposed area and subjected to full sun and rain for the entire length of the experiment. Group "B" tiles were placed beneath clear plastic roofing on a north-facing deck where they were protected from the rain, but exposed to both filtered sunlight from directly overhead and unfiltered angled sunlight from the north (as might typically be found when treating surfaces around houses). Group "C" tiles were the control group and were not treated with Biff Ant, but put out in fully exposed conditions beside group A.

Tests on the relative effectiveness of the tiles at killing Argentine ants were conducted at 1, 2, 4, 8, 15, 22, 36, 57, and 85 days after spraying. On the morning of each test day, groups of ten worker ants were collected fresh from laboratory nests and placed into each of 30 small plastic vials (i.e. 300 ants total). Ten tiles of each treatment (A, B, and C) were brought into the laboratory and one group of ten ants deposited on to the surface of each tile for 5 – 10 seconds before replacing them back into their pottle.

The numbers of healthy, distressed, and dead ants in each pottle were recorded after 30 mins, 1, 2, 4, 7, 12, 24, 36, and 45 hrs, or stopped at any period before 45 hrs if all ants in treatments A and B were dead at that point. In the first test, it was found the mortality of the untreated control group was unexpectedly high, so in all subsequent tests a small square of filter paper soaked in a 30% sucrose solution was provided in each pottle to provide moisture and energy to the ants. This greatly reduced mortality in the control group. Occasionally an ant was accidently wounded or killed during handling and was left out of analyses, but in no case was the amount of ants in any one group less than 9.

Efficacy after rainfall

The cement board tiles used in the trials above were more absorbent than typical concrete or paved surfaces, so the effect of rainfall on the efficacy of Biff Ant was subsequently tested on 190 x 230 mm concrete pavers. Twenty-four pavers were treated with Biff Ant at 15 ml per litre of water. The pavers were left to dry in the sun for a day prior to testing the before-rain efficacy. Six pavers were not treated with Biff Ant and used as the experimental control group.

For efficacy tests, ten individual workers of Argentine ants were allowed to run across each paver for 5 – 10 seconds and then placed carefully in separate pottles and their health monitored for up to 48 hours (less if the ant died beforehand).

The following day, the treated pavers were divided into 4 groups of six and subjected to different levels of simulated rainfall: nil, 2 mm, 4 mm, and 8 mm. This was considered

typical of light – moderate rainfall where it would be hoped a treatment would retain some efficacy, rather than very heavy rain where it is unreasonable to expect a treatment to survive. Rain was simulated using a garden hose with a spray nozzle set to a fine drop size and sprayed out from a balcony at a height about 5 m above the pavers. The amount of simulated rain that fell on the tiles was measured at the tiles themselves using an electronic rain gauge that relayed information to a control unit placed on the balcony and allowing the rain to be turned off at the appropriate time. The weather was threatening to put additional rain on the pavers, so they were allowed to dry in a carport for 24 hours before repeating the efficacy tests.

After discussion with Key Industries, an additional test was undertaken a couple of weeks later when the pavers previously given the highest rainfall (8 mm) were exposed to the sun for a day along with the nil rainfall pavers and then had the efficacy test repeated to determine if further exposure to UV increased insecticidal activity.

4.3 Home remedies for removing ants from potted plants

Thirty-three ornamental tussock and flax plants in PB5 planter bags were obtained from a nursery. All the plants were well established in their planter bags to simulate a typical situation with potted plants. All the plants were placed in a residential garden with a heavy infestation of Argentine ants in October 2010 and left for 3 months to ensure ants were well acclimatised to them. To promote the plants as a place for ants to inhabit we also placed a pottle of sugar solution with wicking tape on the top of each plant for the ants to feed from. The plants were watered occasionally when it became very dry.

In late January the plants were assigned into one of three treatment groups, with 11 plants in each treatment. The treatments were (A) peppermint tea solution, (B) lemon extract solution, and (C) pure water. Treatments A and B followed typical remedies for ant removal that are reported on a range of internet websites. Treatment C represented the experimental control group to ensure it wasn't just the water component of treatments that drove ants out. There was at least 2 m between plants of differing treatments to ensure it was unlikely that any effect from one treatment would be impacting on plants of a differing treatment.

The recipes used were as follows:

Peppermint tea solution: Woolworths brand "pure peppermint herbal infusion" teabags were used at a rate of one tea bag per 250 ml of water. The tea bags were placed in a jug and near boiling water added. They were left to stew for 15 minutes to ensure a strong solution, and then allowed to cool. One drop of Sunlight dishwashing liquid was added to 3 L of cold peppermint tea to aid dispersion through the potting mix when applied.

Lemon extract solution: The juice and blended rind of one lemon was added to each litre of water. The lemon rind was first grated into a bowl and then the juice added. A kitchen stick blender was juiced to finely blend the juice and rind. Hot water was then added to the bowl and allowed to stew for 15 minutes and then the mix was poured through a sieve to remove

any solid material. One drop of Sunlight dishwashing liquid was added to the 3 L of lemon extract solution.

On 27 January 2010, the plants were each treated with 250 ml of their assigned solution. The treatments were poured on slowly to avoid run-off and ensure maximum uptake of solution into the potting mix. Any immediate reaction of ants were recorded. After 24 hours, each plant had the planter bag sliced off and the contents of the soil and root ball examined for evidence of ants, reproductive castes, and immatures.

5. Results

5.1 Use of Advion Arenas for treating residual populations

The average counts at the four sites treated with Advion® ant bait arenas had significantly fewer ants both before and after treatment, but the decline in average ant catch two weeks after treatment was about the same for both the treated and untreated sites (Fig. 1). There was substantial variation between sites within treatments in terms of change over the two weeks, and no significant difference in comparing changes between treated and untreated sites. There was a 97% reduction in ant counts at one of the treated sites after 2 weeks, but another treated site had a 3-fold increase in ant abundance, and one of the non-treated sites experienced a 75% reduction.

Using simple presence/absence data for ants in each pottle provided the proportion of pottles containing Argentine ants along the 15 m transect and reduced variance, but there was still no difference between treated and non-treated sites when comparing the change in proportion of pottles with ants after two weeks. In fact. the mean percentage change of both treated and untreated sites was exactly the same, (-15.6 percent).

Two weeks after Advion arenas were put out, the visual inspection very quickly revealed the presence of Argentine ants at all sites (usually within 30 seconds of starting to look). Bait remained present in all of the arenas, although some showed signs of feeding by slugs or small snails. We saw no ants feeding at the stations, although it is possible some had been fed on by ants during the 2 weeks.

The arenas were left out and the sites revisited after 2 months. There was still no difficulty in detecting the presence of Argentine ants at any of the sites with less than a minute of searching. About half of the arenas were missing (possibly removed by dogs or rodents) and 2 had apparently been run-over by a vehicle. Of those that remained, one appeared to still have a full loading of bait, while others showed variable signs of being fed on (mostly consistent with slug feeding) and several appeared empty.

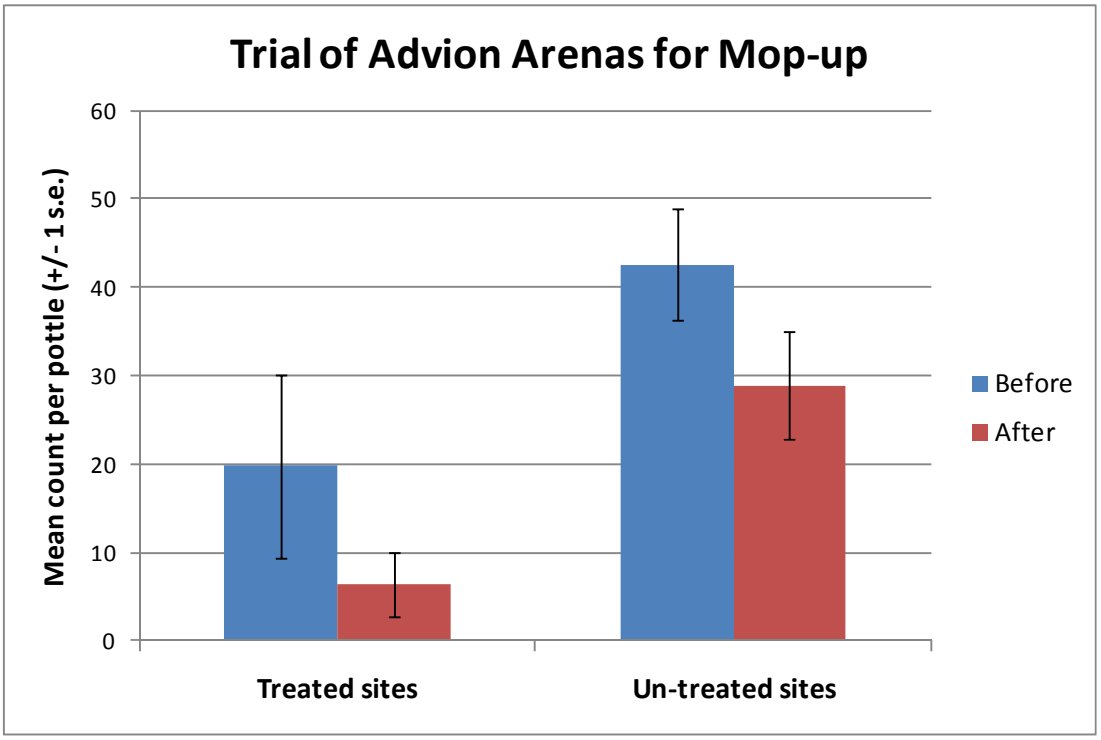

Figure 1: Comparison of average ant counts in baited pottles at four sites treated with Advion® ant bait arenas and four untreated sites, both before treatment and 2 weeks after treatment.

5.2 Trials of Biff Ant surface treatment

Repellency

One day after application there was no evidence that tiles treated with Biff Ant were repellent to Argentine ants. At sites where ants were already actively foraging over the ground surface, ants were observed to run on to A, B and C tiles before any bait was placed on the tiles. Once bait was placed on the tiles, ants quickly moved on to the tiles to investigate, regardless of treatment (Fig 2).

Within 30 minutes, it was clear that there were greater numbers of ants feeding at baits on the untreated control tiles than either the A or B treatments, but this would be expected because of the toxicity of the treatment after 1 day. Laboratory observations showed that the tiles had a dramatic physical effect on ants within 15 minutes of contact, so they would have been unable to return to the nest and establish recruitment trails to those tiles. However, new ants were observed continuing to move onto treated tiles to investigate the bait an hour after they were put out.

Figure 2: Fifteen minutes after placement, Argentine ants investigate bait on a cement board tile treated with Biff Ant the previous day. Although these ants would soon die, it was clear the treated surface was not repellent.

Effectiveness over time

The Biff Ant treatment on both A & B tiles was remarkably effective in the first two days after the trial, with 70 - 90% mortality after just 30 mins, and close to 100% mortality within 2 hrs on both days (Figs. 3a&b). On the night before the 4th day test there was 1.3 mm of rain recorded in an electronic rain gauge near the tiles. Both the "A" treatment and control tiles were still wet when the lab trial began. The effectiveness of the wet "A" tiles was much reduced with over half the ants still alive after 36 hours. By comparison, 90% of the ants exposed to the dry "B" tiles were dead within an hour, and all were dead within 12 hours (Fig. 3c).

By the eighth day, the unsheltered tiles (A) had been exposed to a total of 3.8 mm of rain, but there were two sunny days before the trial day and all tiles were completely dry for the test. The mortality rate of ants exposed to the A tiles was very low and no different to the untreated control tiles. The B tiles remained highly lethal to ants, although the speed of action was slightly reduced with 90% mortality being reached after 12 hours, and total mortality within 24 hours (Fig 3d).

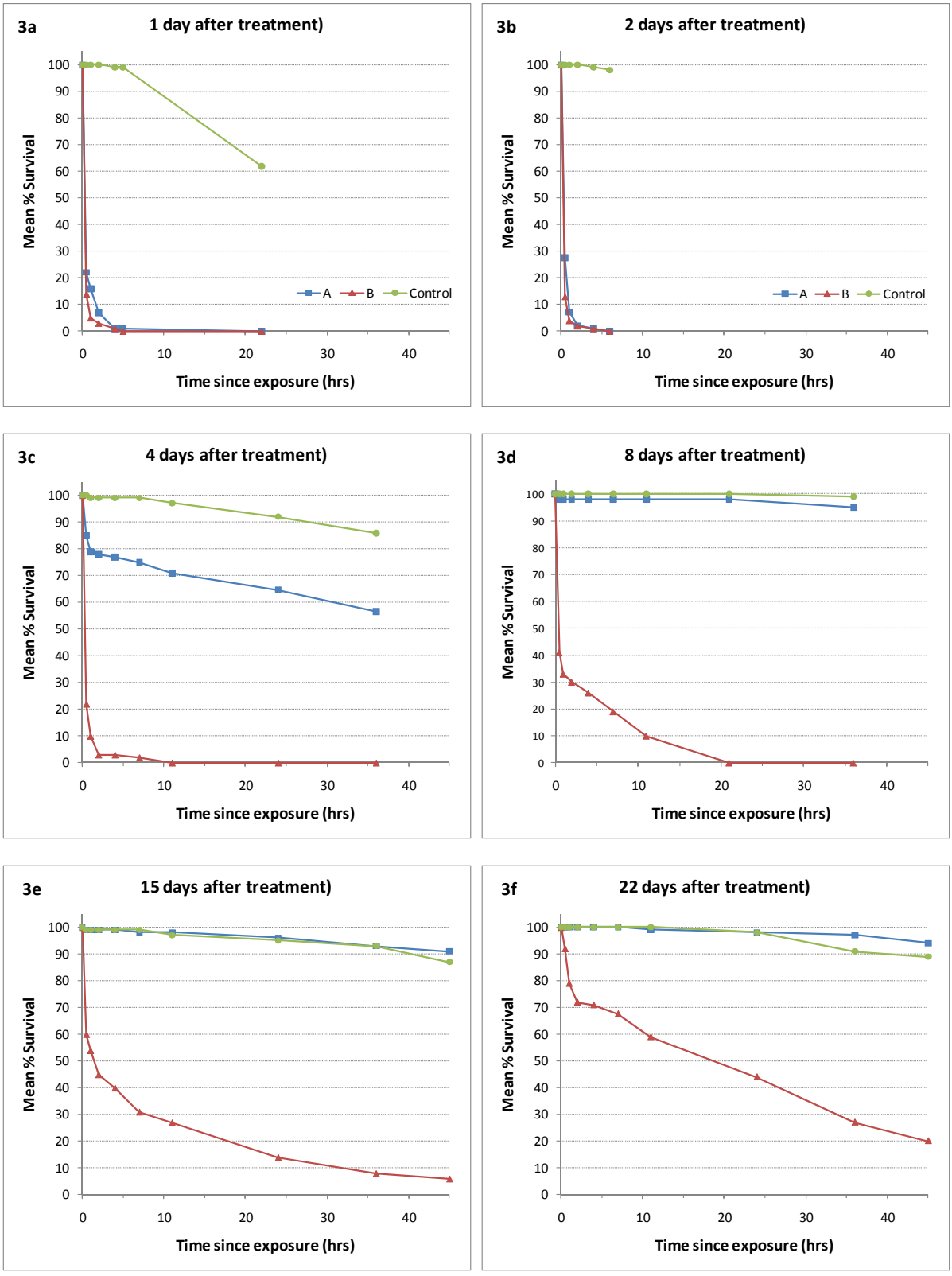

Figure 3: Mean percentage of ants surviving after exposure to Biff Ant-treated tiles exposed to all weather (Treatment A), Biff Ant treated tiles sheltered from rain (Treatment B), and untreated tiles (Control).

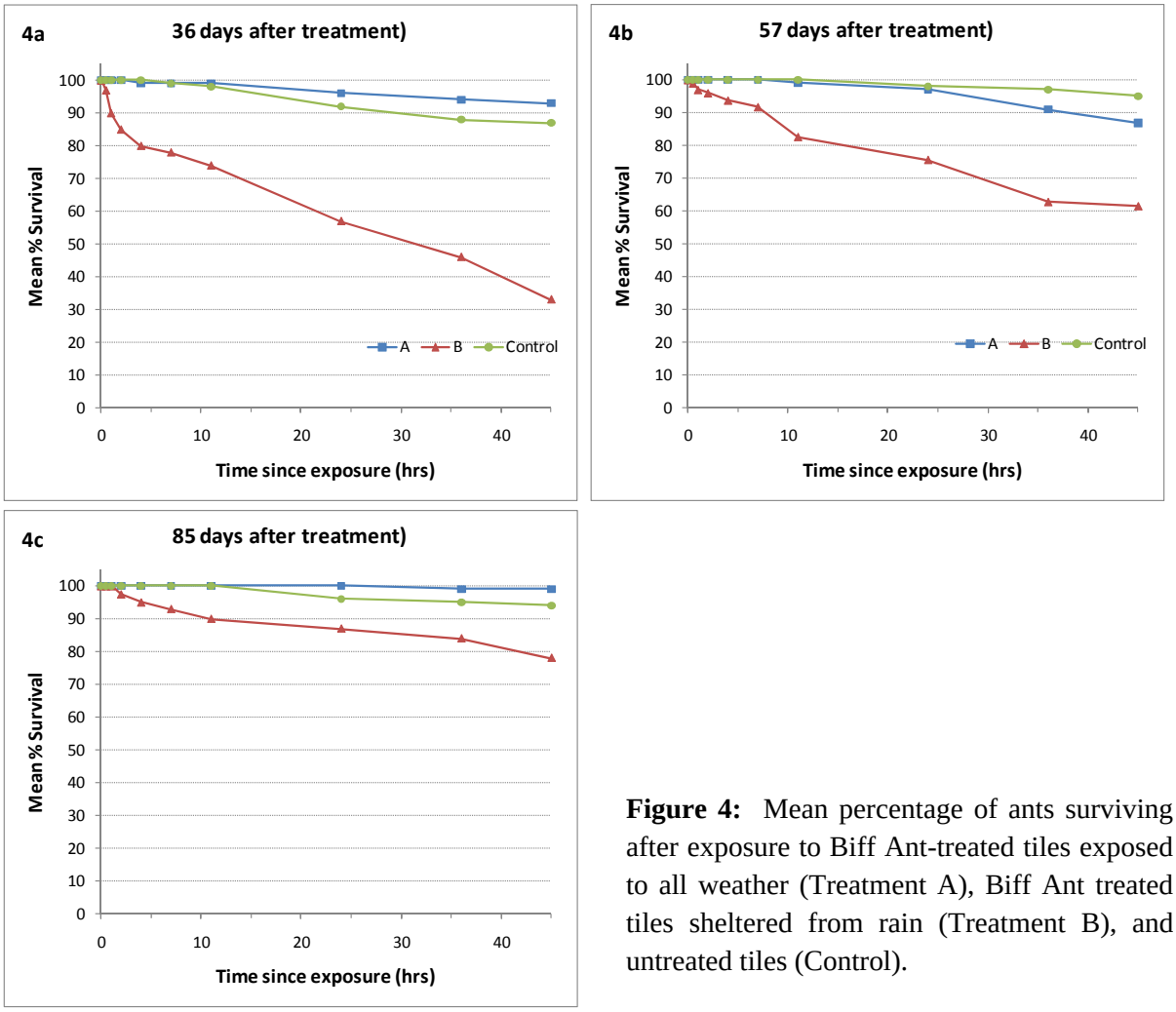

Figure 4: Mean percentage of ants surviving after exposure to Biff Ant-treated tiles exposed to all weather (Treatment A), Biff Ant treated tiles sheltered from rain (Treatment B), and untreated tiles (Control).

For the remainder of the trial, the A tiles had no greater mortality than the untreated control tiles (Figs. 3e-f, 4a-c). There was a gradual reduction in the effectiveness of the sheltered (B) tiles, so that by 36 days after spraying it took more than 24 hours to obtain a 50% mortality rate (Fig. 4a). After 57 days, more than 60% of ants still survived two days after being exposed to the B tiles (Fig 4b). Three months (87 days) after the tiles were sprayed, there was still a measurable effect on ants exposed to B tiles, but almost 80% survived 2 days after exposure (Fig. 4c).

Efficacy after rainfall

Before the simulated rain treatment, all the concrete pavers that were treated with Biff Ant were near 100% lethal to ants within 4 hours. Two days later, and after simulated rainfall treatments, the rate of mortality on all treated pavers was slower, even for the pavers that were not exposed to simulated rain. After 48 hours all the ants exposed to pavers without rain were dead, but the mortality in the other pavers were clearly differentiated, with pavers having the most rain having the lowest death rate other than those that were not treated at all (see Fig. 5a). The pavers exposed to 8 mm of rain had less than 50% mortality after 48 hours.

It was subsequently decided to see if more exposure to UV would release a new layer of insecticide and improve performance of the pavers exposed to 8 mm of rain (the pavers had been kept dry in a carport). The rate of mortality of ants from pavers with no rain had dropped off further in the two weeks that had elapsed since the previous trial, as would be expected with time, but still reached 93% after 48 hours. However, the eventual mortality of ants from pavers that had previously been exposed 8 mm rain was increased after the additional exposure to UV, and reached 65% after 48 hours (Fig. 5b). This indicates that there is indeed a level of renewed insecticidal activity with further exposure to UV after rainfall.

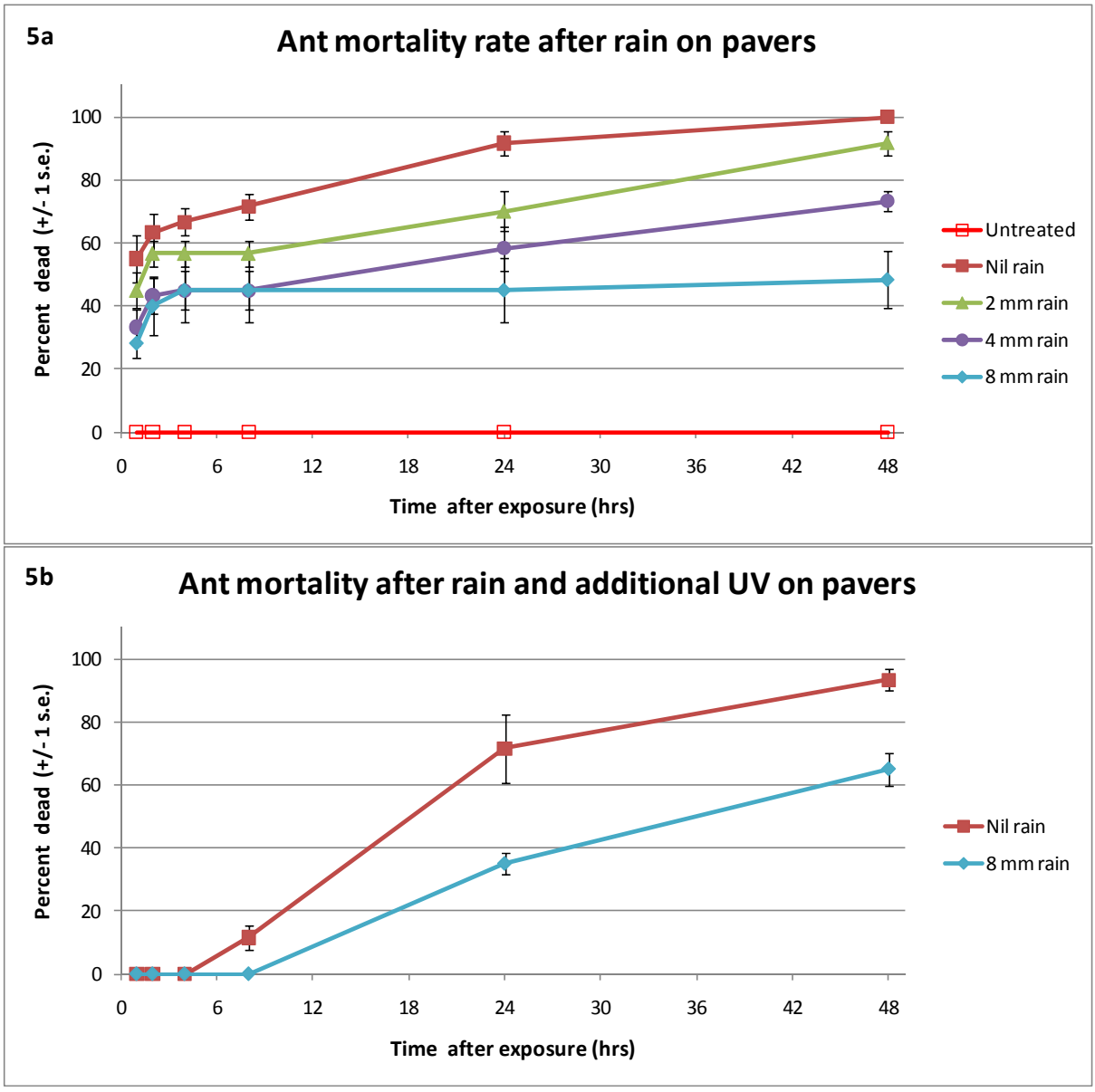

Figure 5: (**a**) Ant mortality after exposure to concrete pavers treated with Biff Ant and varying quantities of simulated rainfall, and (**b**) after 1 day of additional exposure to sunlight for the nil and 8mm rain pavers.

5.3 Home remedies for removing ants from potted plants

Upon application of the lemon extract solution, at least a few ants were observed to move out from either the top or bottom of all the potted plants treated, with a large eruption of ants recorded from one of those plants. For both the pure water and peppermint tea treatments, about half the plants had at least a few ants move out of them when the treatment was applied, with one of the water-treated plants having a large eruption.

After 24 hours, when planter bags were removed and contents examined, there were at least a few worker ants in all but 2 plants, one of which was a peppermint tea treatment, and the other a lemon extract treatment. Two plants were seen to have large numbers of worker ants, and these were the same plants that had an eruption of ants when treated, one a lemon extract and the other a water treatment. The former of these also had a male ant present. Only one plant had larvae present, and this was another of the lemon extract plants (not the one with the large numbers of workers).

6. Discussion

6.1 Use of Advion Arenas for treating residual populations

There was no evidence in these experiments that the use of Advion® ant bait arenas provided an effective way to mop-up residual populations of Argentine ants. Earlier trials of the Advion® arena bait demonstrated that it was lethal to Argentine ant colonies in the laboratory (Toft & Rees, unpublished data), and there is good anecdotal evidence of arena stations being cleaned out when placed in high-density Argentine ant populations in the field. However, when field populations of ants are low, and there is less pressure on other food resources, it would seem the Advion® arena bait is not attractive enough to Argentine ants to achieve effective control. We observed no ants feeding at the arena stations, even though we could get good numbers of ants attracted to the “Inform” ant monitoring bait used in the baited pottles at the same time. This relatively low level of attractiveness has also been confirmed in recent trials to compare the relative attractiveness of various baits (Melissa Mathieson, MSc thesis, in preparation).

6.2 Trials of Biff Ant surface treatment

There was no evidence that surfaces treated with Biff Ant became repellent to ants. This is crucial for the success of a surface treatment as the target is to provide control by killing ants moving across a sprayed zone rather than just redirecting them away from the sprayed area and potentially finding a bridge across it. Argentine ants living in human environments are able to use roadside curbing and other concrete surfaces as highways, allowing them to move rapidly to new foraging areas. An effective, non-repellent treatment that can be applied to concrete has the potential to provide a greater impact on ant populations in urbanised areas than a treatment applied to gardens and lawns, and can also be used as a potential barrier to

movement as infested neighbourhoods may be entirely surrounded by concrete curbs and paths.

The Biff Ant treatment was highly effective at killing Argentine ants when applied to an alkaline surface and provided a good level of control for a month after application on cement board tiles that were not exposed to the rain. Residual toxic activity was still apparent 3 months after treatment on the sheltered tiles. When the tiles got wet, however, the effectiveness deteriorated rapidly, to the point where they were providing little control after 1.3 mm of rain, and nil control after 3.8 mm of rainfall. However, the cement board tiles were much more water absorbent than would be the case with typical concrete surfaces, so we conducted separate rain-fastness trials using concrete pavers.

When applied to concrete pavers, Biff Ant proved to have significantly improved rain-fastness, with treated pavers exposed to 8 mm rainfall still achieving near 50% mortality after 48 hours. Key Industries indicated that insecticidal performance of pavers exposed to rain may increase again following additional exposure to UV because additional insecticide would be released from polymer components in Biff Ant. This proved to be correct, with the 48-hr mortality of ants exposed to the 8 mm rainfall pavers increasing to 65% following an additional day in the sun.

Our trials exposed the ants to the surface of the treated tiles for 5 - 10 seconds, and thus represented the type of exposure an ant might face in walking quickly across a sprayed band of treatment on a pathway, rather than the prolonged exposure they might be exposed to in walking along a sprayed curb. The rate and speed of mortality is likely to have been higher if the ants spent more time on the sprayed surface.

In New Zealand, and many other parts of the world, Argentine ants are still primarily a pest of urban environments, which is a function of their human-mediated dispersal. The effectiveness of Biff Ant on cement surfaces indicates it is potentially a very useful tool in the management of Argentine ants in human environments.

6.3 Methods for removal of ants from potted plants

Neither of the home remedies trialed appeared to provide effective removal of Argentine ants from potted plants, and would not provide any level of assurance in preventing ant propagules being transported to new locations. Although there is good anecdotal evidence that Argentine ants find citrus oils in citrus-based cleaners repellent, it seems this cannot simply be replicated with a home blend of lemon extracts, despite reports on the internet of people killing red imported fire ant nests with such simple solutions.

A number of commercial insecticide products are available that are designed for controlling insect infestations in soil, and these can be applied to the top of potted plants that are potentially infested with ants. These include products such as Ant Stop G, Brigade, and No Insects Lawngard Prills. If there are only a few plants to be treated, another approach is sustained flooding to literally drown the ants out. In this case the potted plants should be

placed in a large sink or bucket and covered with water above the level of the soil and left for at least 2 hours. The most certain approach is to remove the plant from the pot and wash off all the soil from the root ball, and then re-pot the plant in new potting mix.

7. Conclusions

- Advion Ant Bait Arenas will not provide control of residual Argentine ant populations, probably because the bait is not sufficiently attractive when ant populations are at low levels.
- Biff Ant surface treatment shows considerable promise as a control tool in urban and sub-urban areas where there are extensive concrete surfaces. It is not repellent to Argentine ants (even when recently applied), remains active for a considerable time, and has resistance to light-medium rainfall.
- Treating potted plants with either lemon extract solution or peppermint tea will not provide confidence that the plants are free of potential Argentine ant propagules, and it is recommended that potted plants being moved from infested areas are treated with an appropriate insecticide or the ants removed by drowning in water for an extended period or removing all the soil from the root ball and replacing.

8. Acknowledgements

Thanks are due to Melissa Mathieson, Helen Barwick, and Joanna Rees for their assistance with field trials. Peter Visser, from Key Industries, provided materials and useful discussion for the testing of Biff Ant. The spraying of tiles and pavers were conducted by Stephen Fryer, from Pest Management Training and Services.

Thanks also to the land owners who allowed access to their properties, and especially to the Martin's who put up with our scruffy potted plant collection in their garden for several months.

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