# Research on Biff Ant for the Control of Argentine Ant Infestations

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# 1. Background

Regional Councils 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 from these trials was funded by Envirolink Project TSDC 74 for Tasman District Council and completed by Entecol Ltd under contract to Landcare Research. The information provided here is a direct extract of the components of the Envirolink project that dealt specifically with Biff Ant, and is provided to Key Industries for their interest.

# 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.

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.

## 3. Objective

• Assess Biff Ant surface treatment for repellency to ants and efficacy over time and following rainfall

# 4. Methods

## 4.1 Repellency

A trial to assess the repellency of Biff Ant used  $85 \times 85$  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.

#### 4.2 Effectiveness over time

Biff Ant (80 g/L bifenthrin) was mixed at 15 ml per litre of fresh water and sprayed on to 85  $\times$  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<sup>2</sup> (equivalent to 0.3 g of bifenthrin per M<sup>2</sup>). 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.

## 4.3 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.

# 5. Results

## 5.1 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.

#### 5.2 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).

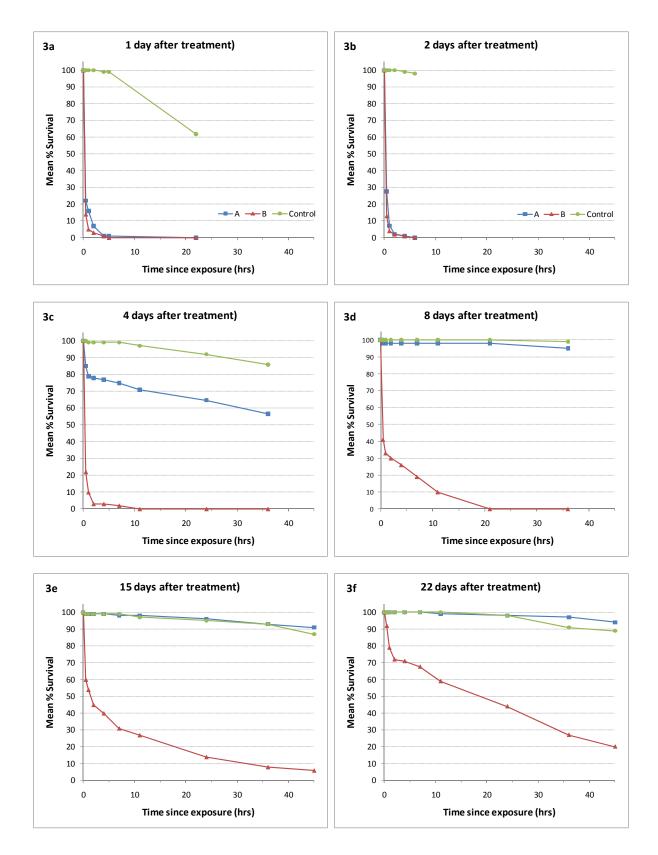
By the eighth day, the unsheltered tiles (A) had been exposed to a total of 3.8 mm of rain, but there were two sunny days before the trial day and all tiles were completely dry for the test. The mortality rate of ants exposed to the A tiles was very low and no different to the untreated control tiles. 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).

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).

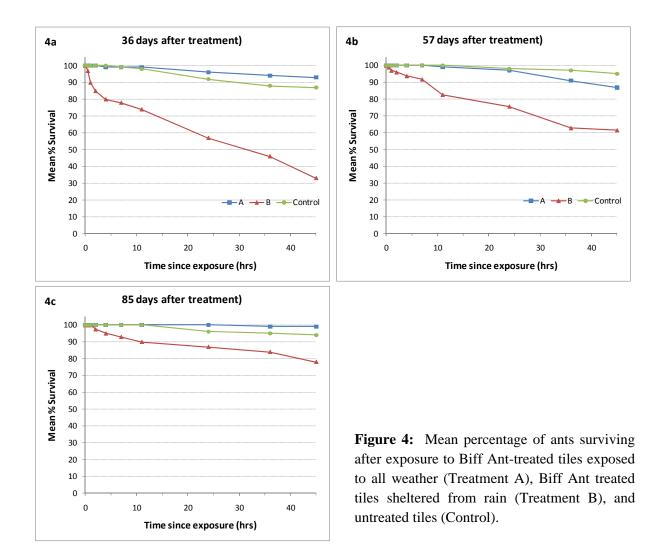
#### 5.3 Efficacy after rainfall

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 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).



#### 6. Discussion

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.

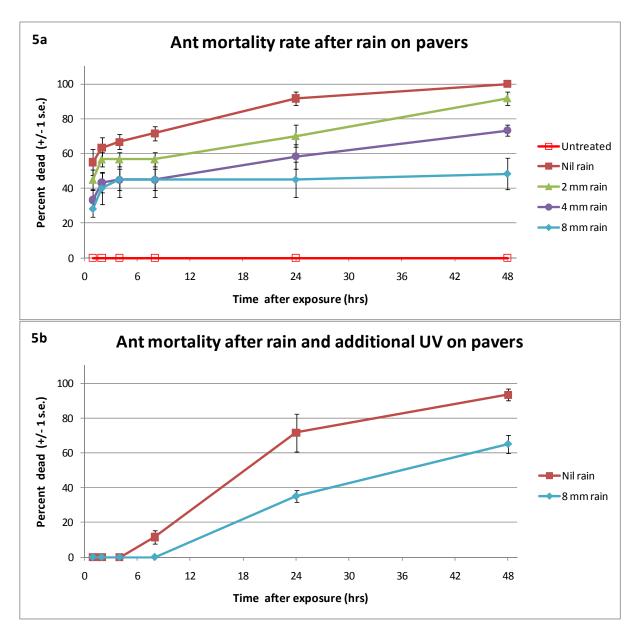


Figure 5: (a) Ant mortality after exposure to concrete pavers treated with Biff Ant and varying quantities of simulated rainfall, and (b) after 1 day of additional exposure to sunlight for the nil and 8mm rain pavers.

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 rainfastness, 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.

# 7. Conclusion

• 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.

## 8. Acknowledgements

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