Optimizing Pitfall Sampling for the Detection of Argentine Ants, *Linepithema humile* (Hymenoptera: Formicidae)

by

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ABSTRACT

Effective sampling devices and optimized sampling strategies play an important role in the detection of invasive species and monitoring outcomes of pest management. Many ant species, such as the globally invasive Argentine ant, *Linepithma humile* (Mayr), are transported by human trade activity and establish in new regions. Because of their small size and their cryptic nature, they often go unnoticed upon arrival and survivors are hard to detect when controlled. This study compared the effectiveness of different pitfall trap designs (use of fish oil, Teflon coating on the trap) and trapping durations for the detection of Argentine ants in two urban reserves in Auckland, New Zealand. Detectability differences between pitfall traps and monitoring baits were also evaluated. The probability of detecting the presence of Argentine ants increased sixteenfold with the addition of fish oil. There was no significant change in detection if Teflon was used. The probability of detecting Argentine ants also increased with increasing duration of pitfall trapping. Pitfall trapping, particularly over 4-weeks duration, was consistently better at detecting the presence of Argentine ants than baiting. Optimizing sampling devices can play an important role in the detection of invasive species.

Keywords: Argentine ants, detection, monitoring, pitfall traps, surveillance

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INTRODUCTION

The detection of low numbers of individuals is a common problem in many biological areas, but one that can be extremely difficult and costly to answer (Venette *et al.* 2002). The problem of detection is a fundamental issue for pest management, as failure to detect (and thus control) a pest may have considerable flow-on effects in terms of pest density, spread, and long-term persistence. Control programs frequently have the tools to kill pests but are unable to detect survivors, or even determine whether survivors exist. As a consequence, a large proportion of control program budgets can be spent attempting to kill the last 1-10% of the population (Myers *et al.* 1998). Methods to increase the probability of detecting pest populations can therefore be extremely valuable in terms of utilising resources.

Pest ant species cause significant economic and agricultural impacts, affect human health and disrupt natural ecosystems (Williams 1994; Holway *et al.* 2002). Attempts have been made to eradicate invasive ants, although the inability to detect survivors remains a serious issue for the success of these attempts. The most successful attempt to date has been the eradication of little fire ant (*Wasmannia auropunctata*) from 3 ha of Santa Fe Island in the Galapagos, after decades of intensive work (Abedrabbo 1994). The success of a recent attempt to eradicate little fire ant (*Wasmannia auropunctata*) from 21 ha of Marchena Island in the Galapagos Archipelago awaits confirmation from additional monitoring, due to uncertainty of whether any small ant populations remain (Causton *et al.* 2005). Early indications suggest *Pheidole megacephala* (10 ha) and *Solenopsis geminata* (3 ha) have been eradicated from Kakadu, Australia (Hoffmann & O'Connor 2004). However, confirmation of eradication necessitates at least 2 years of post-treatment assessment, due to the inability to detect survivors without extremely intensive and costly monitoring (Hoffmann & O'Connor 2004).

The Argentine ant, *Linepithema humile* (Mayr), is one of the world's worst invasive ant species (Holway *et al.* 2002). It now occurs on all continents except Antarctica (Suarez *et al.* 2001), and has negative impacts in many natural ecosystems by out-competing native ant species (Bond & Slingsby 1984; de Kock; 1989, Christian 2001; Sanders *et al.* 2003). In 1990, the first Argentine ant population was found in New Zealand (Green 1990) and it has quickly spread. The first eradication attempt in New Zealand was in 2001 from a 9 ha infestation on Tiritiri Matangi Island, a conservation sanctuary (Harris *et al.* 2002). Although ant numbers were reduced to <1% of pre-treatment levels, eradication has still not been confirmed, primarily because of the uncertainty in detecting survivors.

Argentine ants are increasingly found in many other urban centres around New Zealand, spread primarily by human-mediated dispersal (Ward *et al.* 2005). This has prompted a number of regional-based surveillance initiatives to detect new populations of Argentine ants. Not surprisingly, the number of attempts to control and eradicate Argentine ants has also increased. To date, there has been no comparison of the effectiveness of devices for detecting Argentine ants, nor any attempt to determine what the optimal use of the devices might be. The aim of this study was to compare the effectiveness of different pitfall trap designs in the detection of Argentine ants. We also compared pitfall traps deployed for differing durations and used monitoring baits to compare detectability between pitfall traps and baits.

METHODS

Sites

Trials were conducted in two urban reserves in Auckland, New Zealand, where Argentine ants were known to be present. Waikowhai reserve, (Waikowhai Rd, Waikowhai, Auckland; 36°55.981S, 174°44.167E) is a one of the largest remnants of native forest remaining in Auckland city (ca. 12ha), and has several recently replanted areas of native coastal forest. Rotary reserve (Lake Rd, Northcote, Auckland; 36°48.32S, 174°44.68E) is a small reserve consisting of open grass areas surrounded by replanted native vegetation. At both reserves, the vegetation consisted primarily of trees and shrubs, such as mahoe (*Melicytus ramiflorus*), kawakawa (*Macropiper excelsum*), karaka (*Corynocarpus laevigatus),* cabbage tree (*Cordyline australis*), and several *Coprosma* species, used as pioneer species in restoration plantings. The trials were conducted in winter, when Argentine ant populations are expected to contract (Gordon *et al.* 2001). This was to approximate low-density Argentine ant populations, similar to what would be expected from a newly established population.

Trial 1: Optimizing pitfall trap design

Trial 1 was designed to determine whether adding fish oil to pitfall traps or coating the top 2 cm of the pitfall trap in Teflon enhanced the probability of detecting ants. The fish oil was expected to act as an attractant for Argentine ants (Stanley 2005), and the Teflon was expected to increase the probability that once an individual ant fell into a pitfall trap it would be unable to get out.

Four treatments were used: 1) TF = pitfall traps with fish oil attractant and with Teflon coating; 2) NTF = pitfall traps without Teflon coating, but with the fish oil attractant; 3) TNF = pitfall traps with Teflon coating, but without the fish oil coating; and 4) NTNF = pitfall traps with neither the Teflon coating nor the fish oil attractant.

Trials were carried out at two plots (at least 50 m apart) in each of the two reserves, giving four sites. At each site, five replicates of each treatment (TF, NTF, TNF, NTNF) were randomly allocated to a 5 x 4 pitfall trap grid, with traps spaced 5 m apart. In total there were 20 pitfall traps at each site (80 traps in total) and a total of 20 replicate traps for each treatment across all sites. Pitfall traps were deployed for 2 weeks (13–27 May 2004).

Pitfall traps were 25 ml plastic vials, 55 m deep, with a 25 mm diameter opening. The traps were dug into the ground using a soil corer, and 15 ml of preservative (50% monoethylene glycol : 50% water) was added to each vial. Tin lids (20 cm diameter) were used as covers to minimize trap flooding and disturbance by birds. Lids were secured to the ground with wire pegs with a 2 cm gap between the litter and the lid.

For the Teflon treatments, the top 2 cm of the vials were coated by dipping the open end of the vial into liquid Teflon® (Siltech Industries Ltd, Auckland, New Zealand). Vials were left to dry for 12 hours. For the fish oil attractant treatment, three drops of tuna oil (Kilwell Ltd, Rotorua, New Zealand) were added to the preservative once the pitfall trap was dug into the soil.

At the end of the 2 week period all traps were collected, and a vial with 5 g of non-toxic monitoring bait (Xstinguish™; Bait Technology Ltd, Auckland, New Zealand) was placed on the surface of the ground where each of the pitfall traps had been in the grid (i.e. 20 baits at each site). The baited vials were collected after 3 hours and taken back to the lab. This has been a standardized technique for sampling Argentine ants in New Zealand. All ants collected in pitfall traps and in bait vials were identified to species.

Trial 2: Optimizing duration of pitfall trap deployment

Trial 2 was designed to determine whether the duration of sampling using pitfall traps influenced the probability of detecting Argentine ants. There were three treatments in this trial: durations of 1 week, 2 weeks or 4 weeks. The pitfall traps used in this trial are described above, except no Teflon or fish oil were used.

Trials were carried out at Waikowhai and Rotary reserves. At Waikowhai reserve, 24 replicate pitfall trap stations were established in two plots (each with 12 stations); and at Rotary reserve, 26 replicate pitfall stations were established in three plots (plot 1 = 15 stations , plot 2 = 6 stations, plot 3 = 5 stations), giving a total of 50 replicate pitfall stations. A pitfall trap station consisted of three pitfalls spaced equidistantly 1 m apart. Each treatment (1 week, 2 weeks, 4 weeks) was represented at each station (allocated randomly) by a pitfall trap. Pitfall trap stations were 5 m apart.

All pitfall traps were deployed on 31 May 2004. At the end of each week, all 1-week traps were collected and replaced with fresh 1-week traps. After 2 weeks, all 2-week traps were collected and replaced with 2-week traps. At the end of the trial (4 weeks, 28 June 2004), all 1-week, 2-week and 4-week traps were collected. Thus over the trial, each of the three treatments had the same trap effort; 200 trap weeks (4 sets of 50 traps at 1-week duration; 2 sets of 50 traps at 2-weeks duration; 1 set of 50 traps at 4-weeks duration)

At the end of the trial, after all the pitfall traps had been collected a vial with 5 g of non-toxic monitoring bait (Xstinguish™) was placed on the ground where each replicate bait station had been (n = 50). The baited vials were collected after 3 hours and taken back to the lab. All ants in collected in pitfall traps and in bait vials were identified to species.

Statistical Analysis

All statistical analysis was completed in R 2.4.1 (R Development Core Team 2006). For Trial 1, a binary logistic model was fitted to the presence/absence of Argentine ants in the 80 pitfall traps as a response to the four treatments (TF, NTF, TNF, NTNF). Six traps went missing during the trial and are excluded from analysis. A 2x2 chi-square test was used to compare the association between the presence/absence of Argentine ants in pitfall traps and baits.

For Trial 2, the probability of detection over different time periods was compared with the generalized linear mixed effect model function. The response was binary (detected or not). Sites and time period were included as fixed effects, and pitfall traps at a station are included as a random effect. No ants were found in one plot at Waikowhai, which has therefore been omitted from all the analyses in Trial 2. The probability of detection at baits versus pitfall traps was compared with a generalized linear mixed effect model.

For Trials 1 and 2 a binomial test was also used to compare "success" (presence of Argentine ants) between pitfall traps and baits. The comparisons involving baits and pitfall traps were carried out in the final week of the trials, as this was when baits were placed out.

RESULTS

Trial 1. Optimizing pitfall trap design

Adding fish oil (TF and NTF combined) increased sixteenfold the odds of detecting the presence of Argentine ants (Table 1). There was no significant increase in detection for the combined Teflon treatments (TF and TNF) or for other differences between the four individual treatments (TF, NTF, TNF, NTNF).

Argentine ants were detected at 38 pitfall traps (from a total of 74, 6 went missing during the trial), and at 31 baits. Pitfalls detected Argentine ants 14

Table 1. Binary logistic model of the probability of detection of Argentine ants in response to the pitfall trap design treatments.

Treatment	Log (odds) (P value)	Odds	Probability of detection
Teflon-Fish oil (TF)	1.01	2.75	0.73
No Teflon-Fish oil (NTF)	1.80	6.05	0.86
Teflon-No Fish oil (TNF)	-1.52	0.22	0.18
No Teflon-No Fish oil (NTNF)	-1.2	0.30	0.23
Average SEM	0.77		
Fish oil effect	2.78 (0.00)	16.19	
Teflon effect	-0.54 (0.47)	0.58	
Interaction	0.28 (0.74)	1.33	
Effect SE	-0.68	0.51	

times when baits did not, and baits detected Argentine ants seven times when pitfall traps did not. This was not significantly different (Binomial test from a 50:50 distribution, $p = 0.19$).

Combining the four sites, there was a significant association between the zero counts (absence) from pitfall traps and baits (Chi-square = 12.8, $p < 0.001$). That is, zero pitfall counts happened more frequently with zero baits (and vice versa). However, this association was variable between the four sites, with no common pattern. At one site there was a very significant association, at two sites no significant association, and one site captured very few Argentine ants.

Trial 2: Optimizing duration of pitfall trap deployment

The probability of detecting Argentine ants increased with increasing duration of pitfall trapping, and was highest for pitfalls left for a 4-week duration (Table 2). Comparing the differences with their standard errors (SE Difference) shows a real trend consistent with the log odds of capture increasing in proportion to the duration of trapping (Table 2).

After the end of the 4-week sampling period, all the duration treatments had the same trap effort (200 trap weeks). Therefore, the three duration treatments should have the same number of traps with Argentine ants (after 4 weeks). This was the case, with no statistical difference between the number of traps with Argentine ants between the three duration treatments (Chi-square = 0.400, d.f. = 2, $p = 0.819$). Thus, the difference in the probability of capture between different duration treatments was not caused by a bias towards certain traps at a station.

Table 2. The probability of detecting Argentine ants in pitfall traps for different durations. P-values are based on the z ratio of difference to standard error.

Treatment	Log(odds) (P value)	Odds	Probability of detection
One week	-0.073	0.930	0.48
Two weeks	0.488	1.629	0.62
Four weeks	1.883	6.573	0.87
SE Difference (1 – 2 weeks)	0.342 (0.10)		
SE Difference (1 – 4 weeks)	0.557 (0.00)		
SE Difference (2 – 4 weeks)	0.588 (0.02)		

All pitfall trap duration treatments had a higher probability of detecting Argentine ants than baits (Table 3). The trend of increasing detection probability with pitfall trap duration (Table 2) is shown again, and the detection probability for baits is always lower than for pitfall traps. However, the SE Differences shows that detection probabilities at baits could equal the 1-week pitfall trap value (p = 0.25), and there is only weak evidence (p = 0.09) that it is less than for 2-week pitfalls (Table 3).

Binomial tests for the presence of Argentine ants between pitfall traps and baits also showed a similar trend (Table 4). Argentine ants were present in pitfall traps more often than on baits for each duration treatment, but this was only statistically significant for the 4-week duration.

DISCUSSION

Fish oil was a powerful attractant for Argentine ants. The probability of detecting the presence of Argentine ants in pitfalls traps was significantly increased with the addition of fish oil by a ratio of 16. There was no signifi-

Table 3. The probability of detecting Argentine ants in pitfall traps and on baits for different durations. Pitfalls were collected in the final week of the trial. Baits were placed out at each pitfall station and left for 3 hours. P-values are based on the z ratio of difference to standard error.

Treatment	Log (odds) (P value)	Odds	Probability of detection
One week	-1.363	0.256	0.20
Two week	-1.056	0.348	0.26
Four week	0.705	2.024	0.67
Bait	-1.967	0.140	0.12
SE Difference (Bait – 1-week)	0.501 (0.25)		
SE Difference (Bait – 2-week)	0.531 (0.09)		
SE Difference (Bait – 4-week)	0.658 (0.00)		

Table 4. Binomial test of the presence of Argentine ants in either pitfall traps or baits for different durations. Data from trial 2 (from 4 sites and 50 pitfalls), from pitfall traps which had been out 1, 2 and 4 weeks (collected in the final week of the trial). Baits were placed out at each pitfall station and left for 3 hours.

Occurrence of Argentine ants	Duration		
	One week	Two weeks	Four weeks
Present on baits but absent in pitfall traps	4	3	2
Present in pitfall traps but absent on baits	8	9	18
P-value	0.39	0.15	0.00

cant increase in detection if Teflon was used. The probability of detecting Argentine ants also increased with increasing duration of pitfall trapping. Pitfall traps left for longer durations would be expected to increasingly accumulate the presence of Argentine ants, particularly if there was short-term variation in the abundance or activity of Argentine ants. If the abundance of Argentine ants changed often (e.g., different foraging trails, weather), then traps with shorter durations would be less likely to detect their presence in periods when their abundance was very low. Having a longer duration of trapping would increase their capture, because a longer duration covers periods where abundance/activity are both high and low. Gordon *et al.* (2001) has previously shown that the abundance Argentine ants varies on a weekly basis and is strongly related to weather conditions.

Pitfall trapping was consistently better at detecting the presence of Argentine ants than baiting. The strongest difference in detection probability was between 4-week pitfall traps and baits. We acknowledge that the comparison of baits to pitfalls was limited to only one bait type and of short duration (although this has been a standardised baiting approach throughout New Zealand). The main purpose of the paper was to examine pitfalls traps, and further research on optimizing surveillance strategies with a wider range of sampling methods is needed.

However, no sampling method is optimal in all situations. Although pitfall traps were better than baits at detecting Argentine ants, traps can only be used where they can be dug into the ground. Thus, they are less useful in many urban areas, or for surveillance around sea/airports that have hardened surfaces (e.g. concrete). Pitfalls are also more time-consuming to use because they have to be dug-in and pulled out of the ground and require more time to sort their contents, especially if the traps become full of debris. Baits overcome these but they can be easily tampered with by birds, dogs and people in urban environments. Bait attractiveness and palatability are also important issues for invasive ants (Stanley 2005). Thus, a tailored response is needed for the surveillance and detection of Argentine ants, and this depends largely on the environment.

The importance of detection

Invasive species are a global problem, affecting productive agroforestry sectors, human health and natural ecosystems (Williams 1994; Holway *et al.* 2002). Optimizing devices and sampling strategies plays an important role in two key areas of invasive species management.

First, they are essential in determining whether local control or eradication programs have been successful. For example, the first eradication attempt of Argentine ants in New Zealand was initiated in 2001 from a 9.3 ha infestation on Tiritiri Matangi Island, a conservation sanctuary (Harris *et al.* 2002). Although Argentine ant numbers were reduced to <1% of pre-treatment levels, the use of pitfall traps would have also been extremely useful in helping detect very small surviving colonies; which to date has prevented eradication being confirmed.

Second, optimal sampling plays an important role in surveillance programs designed to detect species in their initial stages of establishment/colonisation. Such programs are commonly employed at points of entry along borders and transport hubs (shipping ports, airports, mail centres) where goods arrive, given that invasive ants are well known for their ability to be dispersed by human trade and transportation (McGlynn 1999; Suarez *et al.* 2005; Ward *et al.* 2006). If invasive species are detected early, there is a greater chance of them being successfully contained and/or eradicated (Simberloff 2003).

For pest managers to have confidence in their ability to detect low density Argentine ant populations, it is critical they know which device(s) will optimize detectability. Currently pitfall trapping is not part of routine surveillance for Argentine ants in New Zealand, but would be a valuable addition.

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