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Full title: Current insecticide treatments used in locust control have less short-term impact on Australian arid zone reptile communities than temporal variation

Running head: Locust control treatments and reptiles

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Abstract

Context: Despite the regular use of pesticides to control locusts, there is a general lack of information on the effects of locust control treatments on reptiles worldwide. Exposure to pesticides poses a significant potential hazard to reptiles, particularly small lizards, both from the direct effects of exposure, and indirectly due to their largely insectivorous diet and small home ranges.

Aims: Our study aimed to monitor the effects of two insecticides applied operationally for locust control in Australia.A phenyl pyrazole pesticide, fipronil, and a fungal biopesticide, *Metarhizium anisopliae* var. *acridium* (Green Guard®) were applied aerially in either a barrier or block treatment in the absence of high density locust populations, and effects on non-target Australian arid-zone reptiles were measured.

Methods: We monitored reptile abundance and community composition responses to treatment methods using a large field-based pitfall trapping experiment with replicated control and spraying treatments which approximated the scale of aerial-based locust control operations in Australia.

Key results: Neither reptile abundance nor community composition was significantly affected by locust control treatments. However, both abundance and community composition as detected by pitfall trapping changed over time, in both control and treatment plots, possibly due to a decrease in annual rainfall during the two years of the study.

Conclusions: The absence of any significant short-term pesticide treatment effects in our study suggests that the two locust control application methods studied present a relatively insignificant hazard to reptiles at our site, based on a single application. Similar to other areas of Australia, climate or climate driven vegetation change are likely to be stronger drivers of reptile abundance and community structure.

Implications: Monitoring over an area which approximates the scale of current locust control operations is an important step in understanding the possible effects of current pesticide exposure on reptile populations and will inform insecticide risk assessments in Australia. However, important information on the immediate response of individuals to insecticide application and any longer-term effects of exposure are still missing. The preliminary research reported in this paper should be complemented by future investigations on long-term and sublethal impacts of pesticide exposure on Australian native reptiles and the possible benefits provided to reptiles by the resource pulses represented in untreated high-density locust populations.

Summary

The effect of locust control on reptiles is unknown, despite high reptile species diversity in Australian arid ecosystems where locust control is commonly undertaken. Neither reptile abundance nor community composition changed after barrier application of fipronil (pesticide) or blanket application of *Metarhizium anisopliae* var. *acridium* (biopesticide), suggesting that these locust control methods pose a relatively insignificant hazard to reptile populations.

Introduction

Locust control operations worldwide expose extensive areas of arid land to pesticides (Peveling 2001). Despite the frequent use of pesticides to control locusts, there is a general lack of information on the effects of locust control on other components of arid ecosystems (Sanchez-Zapata *et al.* 2007). This lack of data hinders the ability of environmental managers and risk regulators to accurately assess the hazard presented by locust control and improve pesticide management practices. Risk assessment data to support pesticide registrations in Australia are based on laboratory acute toxicity studies involving a small number of non-endemic vertebrate species. These data do not necessarily define how native animals will respond to pesticide application in the field, and the tested animals do not often represent the native taxa likely to be exposed to the pesticides in arid regions (Köhler and Triebskorn 2013; Story and Cox 2001).

Both biological and chemical insecticides are aerially applied in Australia for locust control. Fipronil (5-amino-3-cyano-1-(2, 6-dichloro-4-trifluoromethylphenyl)-4-trifluoromethylsulfinylpyrazole) a phenyl-pyrazole compound, is a broad-spectrum, low-dose chemical insecticide that works via direct contact and, when ingested, stomach action. Although not as fast acting as some other insecticides currently used for locust control, it does work at very low doses and has a long residual activity with a half-life of 4-12 months in soil (Gunasekara *et al.* 2007). Fipronil is an extremely active molecule and is a potent disrupter of the insect central nervous system that works by interfering with the passage of chlorine ions through the chlorine channel regulated by c-aminobutyric acid (Story *et al.* 2005). The aerial application of fipronil for locust control in Australia utilizes an ultra-low volume (ULV) formulation as a barrier treatment whereby strips of pesticide (barriers) are laid down by spray aircraft at an angle of 90^0 to the prevailing wind direction, leaving untreated areas between each barrier. In this procedure it is assumed that locust bands within the unsprayed strips will move into a sprayed strip before the insecticide has lost potency, so the movement behaviour of the locusts reduces the need for full spray coverage. Typically, the Australian Plague Locust Commission (APLC) will only treat an area once during a locust control program, and sites did not require treatment in subsequent years (P Story, unpublished data). While the environmental effects of this application methodology are largely unstudied in Australia, alternative application techniques (full cover or “blanket” applications) using ULV fipronil formulations at higher doses in other countries have resulted in significant food chain perturbations. For example, the abundance of lizard species, *Chalarodon madagascariensis* and *Mabuya elegans* decreased significantly after the single application of fipronil (3.2 – 7.5 g active ingredient (a. i.) /ha) sprayed in continuous blocks in Madagascar, largely due to reductions in their arthropod prey (Peveling *et al.* 2003).

The native fungus, *Metarhizium anisopliae* var. *acridium* (Driver and Milner, isolate FI-985, marketed as Green Guard®), forms the basis of a biological insecticide used in aerial control of locust populations in Australia. *Metarhizium anisopliae* var. *acridium* (hereafter abbreviated to *Metarhizium*) is applied at a rate of 25g of spores suspended in a 500-ml mixture of mineral and corn oil per ha. Spores can either land on locusts directly during application or can be picked up on the cuticle as they move through vegetation (Scanlan *et al.* 2001). Live spores germinate when they contact orthopteran cuticle and then grow into the body. In the field, the host is usually killed within 1 to 2 weeks; although mortality can take 3 to 5 weeks when temperatures for fungal development are unfavorable (Story *et al.* 2005). While viable spores are not likely to survive on vegetation longer than 7 days, it is possible for *Metarhizium* spores to persist in soil for eight months in arid agricultural areas (Guerrero-Guerra *et al.* 2013). *Metarhizium* was selected as a biological insecticide in Australia by testing the virulence of Australian sourced spores of this subspecies towards orthopterans. Similar strains have been successfully used to control other arthropod pests, particularly various beetle larvae (Zimmermann 2007). Full cover blanket spraying of *Metarhizium* is standard practice in many countries, and some field evidence suggests that small block applications of *Metarhizium* has minimal effect on non-target arthropods and vertebrates compared to chemical pesticides (Arthurs *et al.* 2003; Zimmermann 2007). Although captive West African fringe-toed lizards (*Acanthodactylus dumerili*) were found to be sensitive to both fipronil and *Metarhizium* in captivity, mortality due to fipronil was much greater (Peveling and Demba 2003).

There is a particular dearth of information regarding the hazards that pesticides pose to reptiles globally, despite the likelihood that they have an impact (Hopkins 2000; Invin and Irwin 2006; Sparling *et al.* 2010). Research on the sublethal effects of fenitrothion on the Australian central bearded dragon (*Pogona vitticeps*) is the only recorded study of the direct response of an Australian reptile to pesticide exposure (Bain *et al.* 2004), and this study, and others on non-Australian reptiles are used to infer responses of multiple reptile species despite the high levels of diversity and endemism in this group within Australia (Story and Cox 2001). Pesticides pose a hazard to reptiles both directly and indirectly. Indirect impacts arise because many lizards have a largely insectivorous diet and small home ranges; factors which imply that reptiles are likely to ingest treated insects, and are less likely to be able to avoid treated areas than more mobile vertebrates. Despite this apparent hazard, field studies of reptile ecotoxicology are notoriously difficult and rarely attempted due to the low detectability and highly seasonal activity of many reptile species (Amaral *et al.* 2012b; Sánchez-Bayo 2011). Monitoring reptile responses to pesticide application on a large, field-relevant scale is also rarely reported, despite the large areas of arid lands subjected to locust control activities (Peveling 2001).

The Australian arid-zone has a variable climate and is prone to 'boom and bust' cycles of rainfall and nutrient cycling which influence the abundance and distribution of many arid zone species (Greenville *et al.* 2013; Nano and Pavey 2013). Arid-zone reptiles are well adapted to short-term reductions in prey availability resulting from climatic variation and they may be able to cope with equivalent reductions caused by pesticide applications. Long-term studies have shown that not all reptile species increase in abundance after rainfall, with factors such as temperature, vegetation cover, and intra- and interspecific reptile abundance better correlated with changes in population abundance (Pianka and Goodyear 2012; Read *et al.* 2012; Tinkle and Dunham 1986). Longer-lived reptiles can interrupt their yearly reproductive output to increase survival during drought or disturbance (Godfrey *et al.* 2013; James 1991), and they may be less affected by pulse disturbances compared to species that consistently breed each year. If pesticide application can be considered as yet another pulse disturbance, these arid zone species may be more likely to persist in a habitat periodically treated with pesticides. Nevertheless, some longer-lived species are more likely to be impacted by repeated pesticide applications that reduce reproduction in good years, and may rely on an occasional year of abundant resources to provide a pulse of recruitment to allow persistence in normally marginal habitat. If those abundant resources include increases in locust population densities, and if locust control measures deplete those resources, then reptile populations may be adversely affected despite their adaptations to persist through the drought years.

Our study monitored the short-term effects of the two locust control treatmentss used in Australia on non-target Australian arid-zone reptiles. Because the aim of the research was to determine the relative impacts of pesticide applications on non-target species, spray was applied when locusts were sparse. Both control agents are normally applied aerially, fipronil as a barrier application and *Metarhizium* as a full cover blanket spray. We predicted that the impact would be greater and the reptile community would be slower to recover when fipronil was used compared to an unsprayed control and *Metarhizium* treatments. Because fipronil takes longer to degrade than *Metarhizium*, recolonization of reptiles from adjacent areas may also be delayed. The speed with which the ecosystem recovers from either treatment is likely to inform strategies for locust control.

Core to our approach was a large field-based experiment with replicated control and sprayed treatments located in arid grasslands in western NSW, Australia. The nine replicate 70 ha sites approximate the scale of aerial locust control operations in Australia. While laboratory and field tests often suggest that pesticides impact individuals, the relative impact of field pesticide applications on populations and ecological communities are difficult to predict using only toxicology data, making the analysis of risks to populations problematic (Story *et al.* 2005; Weir *et al.* 2010). The use of a manipulative experiment at realistic, field-relevant scales should lead to more informed decisions on locust control both in Australia and elsewhere.

Methods

Study Site

Research was conducted at Fowlers Gap Arid Zone Research Station, near Broken Hill, NSW Australia (31.087034, 141.792201). Although there were no locust outbreaks at the time of the study, this site is within the geographical region of western New South Wales, where destructive locust outbreaks periodically occur. The property has not been previously treated with pesticide for locust control and is a working sheep station also managed for biodiversity conservation. It has cool winters and hot summers (average maximum temperature for Jan: 36°C) with rainfall totals of 526.2mm in 2011, 321 mm in 2012, 97.8 mm in 2013 and 194.4 mm in 2014 (Australian Bureau of Meterology). The research station contains a mixture of arid woodlands and grasslands, but all sites in the current study were located in arid grassland habitat, with no trees and a ground layer dominated by perennial grasses and low shrubs. Dominant genera of grasses included *Astrebla*, *Dichanthium, Panicum* and *Eragrostis*. The shrub layer was dominated by Chenopodiaceae species.

Study Design and Setup

We used a BACI (before, after, control, impact) experimental design to test the effects of pesticide treatments on native reptiles (Green 1979). We used nine sites, each approximately 1 km in diameter and spaced at least 2 km apart. Three sites were randomly allocated to each of three treatments; control, fipronil treatment and *Metarhizium* treatment (Fig 1). We monitored sites during summer months before treatment in December 2012 and early

February 2013, applied the pesticide spray in late February 2013, and then monitored sites after treatment in March 2013, December 2013 and February 2014. Each site contained six monitoring arrays with five arrays placed in a circular pattern around a central array. Placement was determined by random number generation determining an angle within each of five sections of a circle and between 200-500 m from the central array. All arrays were at least 200 m apart. Each array contained five 15 cm diameter pitfall traps. Pitfall traps were 50 cm deep with a mesh base and were each supplied with a piece of non-absorbent cotton to protect animals from heat, cold and drowning. Pitfall traps within arrays were arranged in a cross formation, with one pitfall placed in the centre, and the other four pitfalls placed 10 m north, south, east and west of the centre pitfall. The traps were connected by 30 cm tall black plastic drift fences, which extended 2 m past each outer pitfall trap. The 30 pitfall traps in each of the nine sites were monitored each morning for 5 days during each of the five monitoring sessions (total 2700 trap days before spraying; 4050 trap days after spraying). Fences were removed and pitfall traps were covered with a plastic lid between trapping sessions. Traps were also closed if high rainfall was predicted, and then reopened so that all traps were open for a total of five days during each trapping session. All captured reptiles were identified to species, individually marked with non-toxic paint pens (to avoid counting recaptures within a trapping session), and released close to the point of capture. We found that paint marks lasted up to 3 months (based on two recaptures), but it is likely that there were undetected recaptures between trapping sessions. Most small reptile species captured have a life span of two to seven years, and high site fidelity has been recorded for several of the skink species in this study (James 1991; Read 1999; Read *et al.* 2012).

Figure 1 should be positioned here

We used the number of reptiles captured in the pitfall traps as an index of abundance. We recognise that lower capture numbers may simply reflect a reduction in activity under altered climatic conditions, but our major hypothesis was that there would be relatively fewer captures in sprayed than unsprayed sites that were surveyed at the same time and under similar climatic conditions.

Application of Treatments

To reflect the normal pattern of locust control, we used a single pesticide application. The experimental spraying was conducted at a time when there was no locust threat, and when no other spraying was conducted in the region. However, our late summer treatments coincided with when spraying would occur historically (when locust population increases requiring treatment in the region are often found). Chemical pesticide (fipronil) treatments were applied cross-wind from a Piper Brave (PA36) fixed-wing aircraft equipped with two Micronair AU5000 rotary atomizers (Micron Sprayers). The spray plane was equipped with a Satloc differential global positioning system (Hemisphere GPS) for spray guidance using a constant flow rate. Spray application and meteorological data for each day of treatment are given in Table 1. Within each treated site, three arrays were directly sprayed and three were not. Oil sensitive cards confirmed that only targeted arrays were sprayed

Fipronil (Adonis 3UL formulated at 3 g a. i. /L) was applied using barrier treatments, which involved the spray plane applying a swath of pesticide (one swath per array) allowing the cross-wind to drift pesticide across each array corresponding to a dose per unit area of 0.25 - 1.25 g a. i. /ha). Green Guard ULV (*Metarhizium* conidia suspended in corn oil) was applied as a blanket treatment using cross-wind spraying with slightly overlapping tracks resulting in a continuous area or 'block' of treatment over half of each site, including three arrays. Several grasshoppers showing pink coloration indicative of *Metarhizium* infection were found near the sites during the week after spray, confirming that viable conidia were used in our application of this biological insecticide.

Table 1 should be positioned here

Statistical analysis

The effect of treatment (control, fipronil or *Metarhizium*) and trapping session (December 2012, February 2013, March 2013, December 2013 and February 2014) on mean reptile abundance per site was analysed using repeated measures MANOVA (JMP Pro 11.0.0, SAS Institute Inc. 2013). Analyses that only included data from December and February samples, before and after spraying, produced identical trends and are not presented here. We also separately analysed the effect on reptile abundance of fipronil (comparing the sprayed and unsprayed arrays within the three sprayed sites) and trapping session using repeated measures MANOVA (JMP Pro 11.0.0, SAS Institute Inc. 2013). We used a similar analysis for *Metarhizium.* Where the data were spherical we used the exact multivariate F values. When the condition of sphericity was not met, Wilks' Lambda calculation was used to determine approximate F and P values for within subject effects. We used Tukey – Kramer HSD post hoc analysis of reptile abundance to explore the direction of significant effects. We used retrospective power analysis based on our study design and the standard deviation from our reptile abundance data to estimate the effect size of our sampling procedure (JMP Pro 11.0.0, SAS Institute Inc. 2013).

The effect of treatment and trapping session on untransformed reptile community composition within sites was analysed using PerMANOVA (PRIMER 6.1.11 & PERMANOVA+ 1.0.1, PRIMER-E Ltd, 2008). We used Dec 2012, Feb 2013 data with equivalent sampling periods for before spraying treatment and Dec 2013 and Feb 2014 for after spraying samples. Then we used the similarity percentages module (SIMPER) in PRIMER to identify species that accounted for dissimilarities between these two time periods, and visualised the data using a nonmetric MDS. The effect of spray within treatments (sprayed and unsprayed arrays within fipronil or *Metarhizium* sites) and trapping session on untransformed reptile community composition data was analysed separately for fipronil and *Metarhizium* using PerMANOVA (PRIMER 6.1.11 & PERMANOVA+ 1.0.1, PRIMER-E Ltd, 2008).

Results

We captured 289 individual reptiles from 22 species during 6750 pitfall trap-days. Recaptures within survey periods were not included in this study. Five species were only detected with single captures (see online appendix).

Reptile abundance did not differ among treatments, but abundance changed among trapping sessions (Table 2). Mean numbers of reptiles captured declined over time, showing a significantly lower abundance or activity of reptiles in the second year of the study (Fig 2). Within treatment sites, there was no significant change among sessions, and sprayed and unsprayed arrays had similar reptile abundance, though differences among arrays were nearly significant for *Metarhizium* sites (Table 3, Fig 3). Based on retrospective power analysis, our design had an effect size of 0.57 among mean reptile abundance at different treatment sites (n = 9, alpha = 0.05, SD = 4.74).

PerMANOVA showed a significant difference in detected community composition among treatments; however the differences were consistent between pre and post-spray trapping sessions, suggesting that there was no treatment effect (Table 4). Rather this analysis implies that the detected reptile communities differed among the sites selected for each treatment before the spraying began, and that they retained those differences despite different spray treatments. Pairwise tests showed that although *Metarhizium* and control sites were similar, fipronil sites were consistently significantly different from other sites before and after treatment (Table 5, Fig 3). Further analysis using SIMPER of before and after spray captures showed that the detected abundance of 7 of the 11 most commonly trapped reptile species declined over time (*Delma tincta* disappeared from the trap captures at a control site), *Diplodactylus tessellatus* abundancedid not change, and 3 species increased (Table 6). Analysis using SIMPER also suggested these changes in abundance accounted for 90% of the dissimilarities between community composition in samples before and after spraying (Table 6). Sprayed and unsprayed arrays had different detected reptile community composition within both of the sprayed treatments before and after treatments; however, there were significant changes among trapping sessions for *Metarhizium*, but not fipronil sites (Table 7). Once again there were no significant treatment x time interactions to indicate a specific effect of either type of spraying, and the significant treatment effects represent the heterogeneity of the detected reptile community even among different arrays within sites.

Tables 2 through 7 and Figures 2 through 4 should be positioned here.

Discussion

Our results showed no detectable effects of locust control spray applications on native Australian reptiles at our site at the time of our surveys. We found neither a reduction in reptile abundance nor a change in reptile community composition within sites after pesticide treatment. The treatments used appeared not to affect the reptile populations in the treated areas in the short-term. Our results contrast with previous studies showing reductions in the abundance of two common lizards in Madagascar (Peveling *et al.* 2003). One possible explanation is that the maximum dose applied in our experiment was 1.25 g a. i. /ha, while the Madagascar study used a 560% higher maximum application rate of 7 g a. i. /ha. This comparison supports the hypothesis that a single application of fipronil using the APLC's current spray protocols and dosages, while being effective in the control of locusts, will not have any measureable short-term effects on lizard communities. Similarly *Metarhizium* has been shown to affect reptiles under laboratory conditions, but only when they were forced to consume high doses not likely to be experienced by reptiles in the field (Austwick and Keymer 1981; Peveling and Demba 2003). Even if sub-lethal effects were experienced by exposed reptiles at our sites, it is possible that they would recover quickly after the single application of pesticide or biopesticide agent. Our monitoring was timed to investigate the possible short to medium-term effects of each of the two insecticide application methods over two years, and commenced 3-10 days after insecticide spray, because not all sites could be open at one time. Therefore this sampling may have missed instantaneous effects of treatments on reptile populations. Research has shown that the recovery of individuals after a single high dose application of an acutely toxic organophosphate or organochloride pesticide can occur within days or weeks, but prolonged pesticide exposure can cause long-term population depressions (Amaral *et al.* 2012a; Guillette Jr and Edwards 2008). It is possible that sublethal effects from exposure to less toxic low dose fipronil and *Metarhizium* experienced by reptiles at our sites would not be recorded by our monitoring. Our study area had not been previously treated with pesticides, and our results represent the possible effect of reptile exposure to the normal single application of pesticide used in locust control. Arid Australian locust control operations do not consist of repeated treatments at sites over time (P Story, unpublished data). Repeated exposure represents a very different scenario, and is likely in intensively managed agro-ecosystems where repeated pesticide applications are necessary for control of crop pests.

If there was a short-term treatment effect, it may be un-measurable relative to the strong site and year effects that we observed. The abundance and community structure of reptiles differed among trapping sessions. Reptile abundance, or at least the number of reptiles captured in pitfall traps during a survey period, declined soon after the first session of monitoring and the species composition of communities changed over time in both control and treated sites. Changes in reptile communities, as detected by trapping, may have been caused by the dramatic drop in rainfall that occurred over the course of our study. Annual rainfall shifted from an above average 300-500 mm per year in 2010-2012 to a below average 97.8 mm in 2013, bringing on drought conditions at our study sites (BOM 2014). Low rainfall conditions cause vegetation to dry out and arthropod prey numbers and activity to decrease (Bell 1985). This possible reduction in cover and prey may have caused either low survival or low activity levels in reptiles (or both) at our site. There was temporary relief from drought in early 2013, when 25 mm of rainfall occurred four days after our spray treatments on 28 February – 1 March 2013. The rain may have boosted arthropod prey numbers diminishing the possible effects of the spray on reptiles and their prey. In that sense, our single experimental trial may not represent the responses that would be expected if there had been different climatic conditions. However, locust spraying in the area represented by our study site historically occurs in late summer and even though there was no locust outbreak during our experiment, spray was applied in conditions that realistically replicated the time of year, and climatic conditions, when locusts could be controlled (Hunter *et al.* 2001).

Relative to other studies which have documented effects of environmental disturbances on reptile populations and communities, our trapping effort was adequate to detect small changes that may have resulted from the spray treatments. We conducted surveys using 18 sampling arrays per treatment with spacing of 200 m or more between arrays, within three sites that were up to 3 km apart, per treatment. Our high trapping effort and the spacing of our sites ensured that we should have detected any response to treatments. Other reptile studies using nine or fewer replicate sampling arrays per treatment spaced as little as 60 m apart have reported changes both in reptile communities and in abundance of individual species in response to disturbances (Jellinek *et al.* 2004; Letnic *et al.* 2004; Peveling *et al.* 2003; Pianka and Goodyear 2012; Read 2002; Read and Cunningham 2010). This suggests that an increase in our trapping effort would not have increased the probability of detecting a response.

Of the seven species of reptile that declined in capture rates over time in our study, several similar species have been shown to decline in response to drought in other areas of Australia, notably the annual breeding gecko *Rhynchoedura ornata* (Read 1999; Read *et al.* 2012; Schlesinger *et al.* 2011). However, in another study *R. ornata* persisted and increased in abundance in heavily burnt habitats while other lizards declined (Pianka and Goodyear 2012). If *R. ornata* populations respond more dramatically to a decrease in rainfall than they do to vegetation change in other parts of Australia, we suggest that drought was the most likely cause of its decline in our study. We detected a decline in numbers of *Ctenotus leonhardii* over our study, although one long-term study showed this long-lived skink increased in abundance during lower rainfall years, possibly due to opportunistic breeding (Read *et al.* 2012). In other shorter studies, *C. leonhardii* and similar large *Ctenotus* species have declined in abundance or reproductive activity during periods of low rainfall, and have shown reduced abundance after disturbance from grazing and fire (Frank *et al.* 2013; Kutt and Woinarski 2007; Pianka and Goodyear 2012; Read 1998; Read and Cunningham 2010; Schlesinger *et al.* 2011). A common pygopod species, *Delma tincta*, was only detected at our control sites in the first year of this study. A similar species, *Delma impar*, is now endangered due to the destruction of grass cover habitat in agricultural areas (Dorrough and Ash 1999). We speculate that *D. tincta* may have been less active or abundant at our control sites in the second year due to the reduction of grass and litter cover at most sites (K Maute, personal obs.), which was possibly caused by both grazing and drought. This suggests a complex response of reptile species to climate and habitat change, and that drought may have differential effects on populations in different locations and circumstances.

While the pattern of decline seen in most species supports the hypothesis that decreased rainfall leads to reduced population density, several species did not decline. The capture levels of *Diplodactylus tessellatus* remained stable, and *Menetia greyii*, *Ctenotus schomburgkii* and *Heteronotia binoei* increased over time. All four species are common and have a wide distribution, and three have been shown to be little affected by climate or habitat disturbances such as grazing than rarer species (Read 1998; Read 2002; Read and Cunningham 2010). However, the increase in *Menetia greyii* captures is inconsistent with past literature, which showed declines in this species in response to reduced vegetation and litter cover (Read 2002; Valentine *et al.* 2012). The reason for this discrepancy is unknown, and highlights the possibility that temporal changes in other unmeasured factors, such as activity levels and catchability, microsite characteristics, interspecific competition, predation pressure and prey availability may also influence apparent reptile abundance and activity at traps. Recent research has found that arid zone reptile abundance can change dramatically, with unpredictable positive responses in some cases to apparently adverse climate, fire, grazing and feral predation (Pastro *et al.* 2013; Read and Cunningham 2010; Read *et al.* 2012). Because of the likely complexity of responses of each reptile species to this multitude of factors, it is unlikely that climate alone explains variation in reptile communities.

Reptile communities not only changed over time, but also differed in composition among our sites, and among the sampling arrays within our sites both before and after spray treatments. It is probable that this has resulted from small scale heterogeneities in soil structure, vegetation or other aspects of microhabitat, microclimate or predator and prey abundance. All sites were located in arid grassland dominated by *Astrebla* and Chenopodiaceae spp. However, unrecorded observations suggested slight differences in vegetation, soil and arthropod abundance among sites. Other studies of interactions between Australian reptiles and their habitat and prey suggest that these factors could influence the distribution of reptiles at our sites (Craig *et al.* 2006; Frank *et al.* 2013; Jellinek *et al.* 2004). Although this was not a central question of our research, further investigation of diets and habitat requirements of individual reptile species as well as measurements of site characteristics would be necessary to resolve this issue and better inform pesticide risk assessments in Australia.

Conclusions

Further research into the long-term, sublethal and landscape scale effects of fipronil and *Metarhizium* applications on native reptiles will better inform managers about the hazards that locust control methods pose to arid zone fauna. However, the lack of clear treatment effects in our study suggests that current locust control treatments for these two control agents are a relatively insignificant hazard to native reptiles at our site. As in other areas globally, and particularly in arid regions, climate and vegetation change are likely to be the major drivers of reptile abundance and community structure (Jellinek *et al.* 2004; Pianka and Goodyear 2012; Read and Cunningham 2010). Similar to resident and migratory bird populations which benefit from feeding on abundant locusts in the African Sahel, reptiles may also rely on an occasional year of abundant prey to provide a pulse of recruitment or increase the success of individual dispersal attempts (Sanchez-Zapata *et al.* 2007). By following the response of reptile populations to high locust abundance in treated and untreated areas, important insight into the possible costs of removing this resource pulse could be gained. Only then can the full impacts of locust control operations on reptile populations be quantified.

Our monitoring at a scale which represents real locust control operations is important in understanding the possible effects of these spraying procedures on native Australian reptiles. However, important information on the immediate and long-term response of individuals to insecticide applications is missing. Future work should focus on understanding the effects of locust control pesticides in free living and captive populations and relating this information back to the pesticide risk assessment framework. We suggest following the activity and survival of individuals directly before and after single exposure to pesticides concomitantly with comprehensive pesticide residue analysis. This will provide insight into small pulse or sublethal effects on behaviour and reproduction which could impact populations in the longer term. Many native Australian reptiles are already kept in captivity and tracked in the wild, and would provide ideal test subjects for ecotoxicology studies in field, laboratory or mesocosm experiments.

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References

Amaral, M. J., Bicho, R. C., Carretero, M. A., Sanchez-Hernandez, J. C., Faustino, A. M., Soares, A. M., and Mann, R. M. (2012a). The usefulness of mesocosms for ecotoxicity testing with lacertid lizards. *Acta Herpetologica* **7**, 263-280.

Amaral, M. J., Carretero, M. A., Bicho, R. C., Soares, A. M., and Mann, R. M. (2012b). The use of a lacertid lizard as a model for reptile ecotoxicology studies-Part 1 Field demographics and morphology. *Chemosphere* **87**, 757-764.

Arthurs, S., Thomas, M. B., and Langewald, J. (2003). Field observations of the effects of fenitrothion and *Metarhizium anisopliae* var. *acridum* on non-target ground dwelling arthropods in the Sahel. *Biological Control* **26**, 333-340.

Austwick, P. K. C. and Keymer, I. F. (1981). Diseases of the Reptilia. In 'Fungi and Actinomycetes'. (Eds J. E. Cooper and O. F. Jackson) pp. 193-231. (Academic: London, UK.)

Bain, D., Buttemer, W. A., Astheimer, L., Fildes, K., and Hooper, M. J. (2004). Effects of sublethal fenitrothion ingestion on cholinesterase inhibition, standard metabolism, thermal preference, and prey-capture ability in the Australian central bearded dragon (*Pogona vitticeps*, agamidae). *Environmental Toxicology and Chemistry* **23**, 109-116. doi: 10.1897/02-555.

Bell, H. (1985). Seasonal variation and the effects of drought on the abundance of arthropods in savanna woodland on the Northern Tablelands of New South Wales. *Australian journal of ecology* **10**, 207-221.

BOM, A. B. o. M. (2014). Weather Station Directory. pp. http://www.bom.gov.au/climate/data/stations/. (Commonwealth of Australia.)

Craig, M., Withers, P., and Bradshaw, S. (2006). Patterns of diet and microhabitat use by four species of sympatric Ctenotus lizards: Do they reveal foraging specialisation? *Journal of the Royal Society of Western Australia* **89**, 1-5.

Dorrough, J. and Ash, J. E. (1999). Using past and present habitat to predict the current distribution and abundance of a rare cryptic lizard, Delma impar (Pygopodidae). *Australian journal of ecology* **24**, 614-624.

Frank, A. S., Dickman, C. R., Wardle, G. M., and Greenville, A. C. (2013). Interactions of grazing history, cattle removal and time since rain drive divergent short-term responses by desert biota. *PloS one* **8**, e68466.

Godfrey, S. S., Sih, A., and Bull, C. M. (2013). The response of a sleepy lizard social network to altered ecological conditions. *Animal Behaviour* **86**, 763-772. doi: http://dx.doi.org/10.1016/j.anbehav.2013.07.016.

Green, R. H. (1979) 'Sampling design and statistical methods for environmental biologists.' (John Wiley and Sons: New York.)

Greenville, A. C., Wardle, G. M., and Dickman, C. R. (2013). Extreme rainfall events predict irruptions of rat plagues in central Australia. *Austral ecology* **38**, 754-764.

Guerrero-Guerra, C., del Roció Reyes-Montes, M., Toriello, C., Hernández-Velázquez, V., Santiago-López, I., Mora-Palomino, L., Calderón-Segura, M. E., Fernández, S. D., and Calderón-Ezquerro, C. (2013). Study of the persistence and viability of Metarhizium acridum in Mexico's agricultural area. *Aerobiologia* **29**, 249-261.

Guillette Jr, L. and Edwards, T. (2008). Environmental influences on fertility: can we learn lessons from studies of wildlife? *Fertility and sterility* **89**, e21-24.

Gunasekara, A. S., Truong, T., Goh, K. S., Spurlock, F., and Tjeerdema, R. S. (2007). Environmental fate and toxicology of fipronil. *Journal of Pesticide Science* **32**, 189-199.

Hopkins, W. A. (2000). Reptile toxicology: Challenges and opportunities on the last frontier in vertebrate ecotoxicology. *Environmental Toxicology and Chemistry* **19**, 2391-2393. doi: 10.1002/etc.5620191001.

Hunter, D. M., Walker, P. W., and Elder, R. J. (2001). Adaptations of locust and grasshoppers to the low and variable rainfall of Australia. *Jouranl of Orthopteran research* **10**, 347-351.

Invin, L. and Irwin, K. (2006). Global threats affecting the status of reptile populations. In 'Toxicology of reptiles'. (Eds S. C. Garder and E. Oberdorster) pp. 9-34. (CRC Press: Boca Raton, FL.)

James, C. D. (1991). Population dynamics, demography, and life history of sympatric scincid lizards (Ctenotus) in central Australia. *Herpetologica*, 194-210.

Jellinek, S., Driscoll, D. A., and Kirkpatrick, J. B. (2004). Environmental and vegetation variables have a greater influence than habitat fragmentation in structuring lizard communities in remnant urban bushland. *Austral ecology* **29**, 294-304. doi: 10.1111/j.1442-9993.2004.01366.x.

Köhler, H.-R. and Triebskorn, R. (2013). Wildlife ecotoxicology of pesticides: can we track effects to the population level and beyond? *science* **341**, 759-765.

Kutt, A. S. and Woinarski, J. C. (2007). The effects of grazing and fire on vegetation and the vertebrate assemblage in a tropical savanna woodland in north-eastern Australia. *Journal of Tropical Ecology* **23**, 95-106.

Letnic, M., Dickman, C., Tischler, M., Tamayo, B., and Beh, C.-L. (2004). The responses of small mammals and lizards to post-fire succession and rainfall in arid Australia. *Journal of arid environments* **59**, 85-114.

Nano, C. E. and Pavey, C. R. (2013). Refining the 'pulse - reserve'model for arid central Australia: Seasonal rainfall, soil moisture and plant productivity in sand ridge and stony plain habitats of the Simpson Desert. *Austral ecology* **38**, 741-753.

Pastro, L. A., Dickman, C. R., and Letnic, M. (2013). Effects of wildfire, rainfall and region on desert lizard assemblages: the importance of multi-scale processes. *Oecologia* **173**, 603-614.

Peveling, R. (2001). Environmental conservation and locust control-possible conflicts and solutions. *Journal of Orthoptera Research* **10**, 171-187.

Peveling, R. and Demba, S. A. (2003). Toxicity and pathogenicity of Metarhizium anisopliae var. acridum (Deuteromycotina, Hyphomycetes) and fipronil to the fringe-toed lizard Acanthodactylus dumerili (Squamata: Lacertidae). *Environmental Toxicology and Chemistry* **22**, 1437-1447.

Peveling, R., McWilliam, A., Nagel, P., Rasolomanana, H., Rakotomianina, L., Ravoninjatovo, A., Dewhurst, C., Gibson, G., Rafanomezana, S., and Tingle, C. (2003). Impact of locust control on harvester termites and endemic vertebrate predators in Madagascar. *Journal of applied ecology* **40**, 729-741.

Pianka, E. R. and Goodyear, S. E. (2012). Lizard responses to wildfire in arid interior Australia: Long-term experimental data and commonalities with other studies. *Austral ecology* **37**, 1-11. doi: 10.1111/j.1442-9993.2010.02234.x.

Read, J. (1998). The ecology of sympatric scincid lizards (Ctenotus) in arid South Australia. *Australian Journal of Zoology* **46**, 617-629.

Read, J. L. (1999). Longevity, reproductive effort and movements of three sympatric Australian arid-zone geckos. *Australian Journal of Zoology* **47**, 307-316.

Read, J. L. (2002). Experimental trial of Australian arid zone reptiles as early warning indicators of overgrazing by cattle. *Austral ecology* **27**, 55-66.

Read, J. L. and Cunningham, R. (2010). Relative impacts of cattle grazing and feral animals on an Australian arid zone reptile and small mammal assemblage. *Austral ecology* **35**, 314-324.

Read, J. L., Kovac, K.-J., Brook, B. W., and Fordham, D. A. (2012). Booming during a bust: Asynchronous population responses of arid zone lizards to climatic variables. *Acta Oecologica* **40**, 51-61. doi: http://dx.doi.org/10.1016/j.actao.2011.09.006.

Sánchez-Bayo, F. (2011). Impacts of agricultural pesticides on terrestrial ecosystems. In 'Ecological Impacts of Toxic Chemicals.'. (Eds F. Sánchez-Bayo, P. J. van den Brink, and R. M. Mann) pp. 63-87. (Bentham Science Publishers, Online.)

Sanchez-Zapata, J. A., Donázar, J. A., Delgado, A., Forero, M. G., Ceballos, O., and Hiraldo, F. (2007). Desert locust outbreaks in the Sahel: resource competition, predation and ecological effects of pest control. *Journal of applied ecology* **44**, 323-329.

Scanlan, J., Grant, W., Hunter, D., and Milner, R. (2001). Habitat and environmental factors influencing the control of migratory locusts (*Locusta migratoria*) with an entomopathogenic fungus (*Metarhizium anisopliae*). *Ecological Modelling* **136**, 223-236.

Schlesinger, C. A., Christian, K. A., James, C. D., and Morton, S. R. (2011). Seven lizard species and a blind snake: activity, body condition and growth of desert herpetofauna in relation to rainfall. *Australian Journal of Zoology* **58**, 273-283.

Sparling, D., Linder, G., Bishop, C., and Krest, S. (2010). Recent advancements in amphibian and reptile ecotoxicology. In 'Ecotoxicology of Amphibians and Reptiles'. (Eds D. Sparling, G. Linder, C. Bishop, and S. Krest) pp. 1-14. (Taylor and Francis: New York.)

Story, P. and Cox, M. (2001). Review of the effects of organophosphorus and carbamate insecticides on vertebrates. Are there implications for locust management in Australia? *Wildlife Research* **28**, 179-193. doi: http://dx.doi.org/10.1071/WR99060.

Story, P. G., Walker, P. W., McRae, H., and Hamilton, J. G. (2005). A case study of the Australian Plague Locust Commission and environmental due diligence: Why mere legislative compliance is no longer sufficient for environmentally responsible locust control in Australia. *Integrated environmental assessment and management* **1**, 245-251.

Tinkle, D. W. and Dunham, A. E. (1986). Comparative life histories of two syntopic Sceloporine lizards. *Copeia*, 1 -18.

Valentine, L. E., Reaveley, A., Johnson, B., Fisher, R., and Wilson, B. A. (2012). Burning in banksia woodlands: how does the fire-free period influence reptile communities? *PloS one* **7**, e34448.

Weir, S. M., Suski, J. G., and Salice, C. J. (2010). Ecological risk of anthropogenic pollutants to reptiles: Evaluating assumptions of sensitivity and exposure. *Environmental Pollution* **158**, 3596-3606.

Zimmermann, G. (2007). Review on safety of the entomopathogenic fungus Metarhizium anisopliae. *Biocontrol Science and Technology* **17**, 879-920.

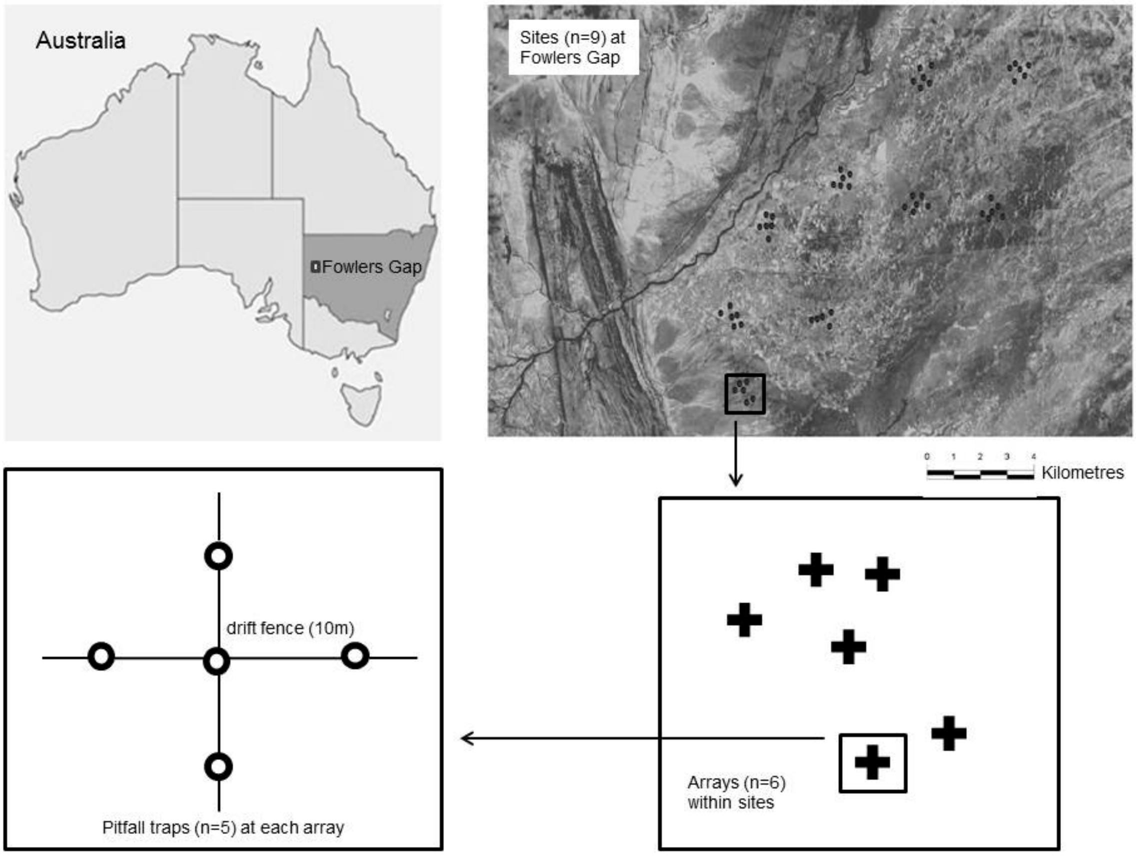

Figure 1: Location of study area within the state of NSW, Australia, site locations within Fowlers Gap Arid Zone Research Station and arrangement of pitfall traps and fences within sites.

Table 1: Spray and meteorological conditions on the day of each treatment in 2013.

Date	Pesticide	Batch number	Area treated (km^2)	Formulation applied (L)	Track spacing (m)	Latitude*		Longitude*		Wind speed (m/s)	Wind direction (degrees)	Temperature (C)
19/2	*Green Guard*®	M460 01/2011	0.61	39	50	31	53.59	141	46.52	2.0	190	36
19/2	*Green Guard*®	M460 01/2011	0.72	46	50	31	54.97	141	46.27	2.0	190	37
20/2	*Green Guard*®	M460 01/2011	0.55	39	50	31	59.71	141	53.65	4.0	130	39
23/2	Fipronil ULV	PAIE000199	0.06	4	300	31	57.41	141	49.13	3.5	75	29
23/2	Fipronil ULV	PAIE000199	0.05	3	300	31	54.82	141	48.44	3.0	130	35
24/2	Fipronil ULV	PAIE000199	0.13	4	300	31	57.05	141	50.89	2.0	210	37

*Latitude and longitude are listed as centroids for each spray target.

Table 2: Analysis of the effect of treatment (control, fipronil and *Metarhizium*) and trapping session (5 sampling periods) on reptile abundance using repeated measures MANOVA.

	degrees of freedom			
factor	numerator	denominator	F value	P value
treatment	2	6	0.66	0.55
trapping session	4	24	9.46	<0.0001*
trapping session X treatment	8	6	0.49	0.83

*signifies significant p value

Table 3: Analysis of the effect of fipronil or *Metarhizium* (n=3 sprayed and unsprayed arrays within each of the three sites within treatments) and trapping session (Dec 2012, Feb 2013, March 2013, Dec 2013 and Feb 2014) on reptile abundance using repeated measures MANOVA.

	degrees of freedom			
Factor	numerator	denominator	F value	P value
Fipronil MANOVA				
spray vs no spray	1	16	1.80	0.20
trapping session	4	13	2.06	0.14
spray X trapping session	4	13	0.75	0.57
Metarhizium MANOVA				
spray vs no spray	1	16	3.71	0.07
trapping session	4	13	2.92	0.06
spray X trapping session	4	13	0.51	0.73

Table 4: Analysis of the effect of treatments (control, fipronil and *Metarhizium*) and trapping session (5 sampling periods) on reptile community composition using PerMANOVA.

factor	degrees of freedom	Pseudo-F value	P value
treatment	2	2.55	0.005*
trapping session	4	1.37	0.10
trapping session X treatment	8	0.70	0.95

*signifies significant p value

Table 5: Pairwise tests of the effect of treatment (control, fipronil and *Metarhizium*) on reptile community composition using PerMANOVA.

Treatment pairs	t	P (perm)
M, C	1.15	0.26
M, F	1.83	0.002*
C, F	1.81	0.008*

Treatment abbreviations: M = *Metarhizium*, C = Control, F = Fipronil

*signifies significant p value

Table 6: Community analysis using SIMPER shows determinant species for dissimilarities between before and after spray monitoring (December and February trapping sessions pooled to represent before and after time periods). Average abundance represents numbers of animals trapped per site (n=3 sites per treatment), averaged across two trapping sessions for each time period.

Time period:	Before Spray	After Spray	
Reptile Species	Average abundance	Average abundance	Contribution of species (%)
Ctenotus strauchii	4.11	1.67	30.69
Ctenotus leonhardii	1.83	0.78	17.98
Tympanocryptis tetraporophora	0.89	0.56	10.05
Ctenotus olympicus	0.44	0.22	6.93
Menetia greyii	0.00	0.67	6.90
Ctenotus schomburgkii	0.33	0.39	5.26
Rhynchoedura spp	0.33	0.06	3.14
Heteronotia binoei	0.06	0.28	3.00
Diplodactylus tessellatus	0.17	0.17	2.94
Pogona vitticeps	0.17	0.06	2.33
Delma tincta	0.22	0.00	1.59

Table 7: Analysis of the effect of fipronil or *Metarhizium* (sprayed or unsprayed arrays within the three sites) and trapping session (5 sampling periods) on reptile community composition using PerMANOVA.

factor	degrees of freedom	Pseudo-F value	P value
Fipronil perMANOVA			
spray vs no spray	1	2.81	0.045*
trapping session	4	1.29	0.19
trapping session X spray	4	0.68	0.80
Metarhizium perMANOVA			
spray vs no spray	1	2.15	0.02*
trapping session	4	1.57	0.02*
trapping session X spray	4	0.82	0.75

*signifies significant p value

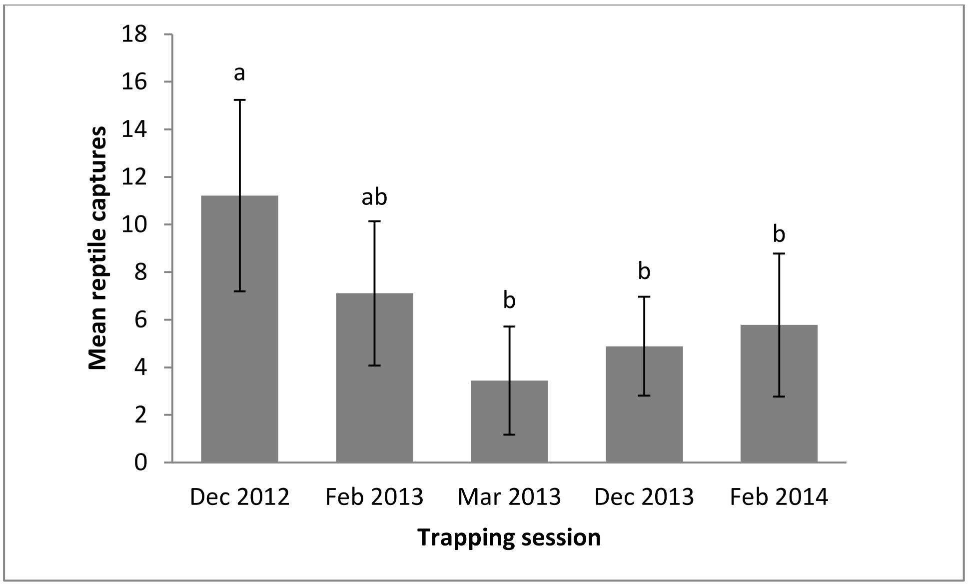

Figure 2: Reptile abundance during different trapping sessions. Bars represent the mean number of reptiles captured (± SD) at sites (n=9), and letters suggest significant differences among trapping sessions determined by Tukey-Kramer HSD.

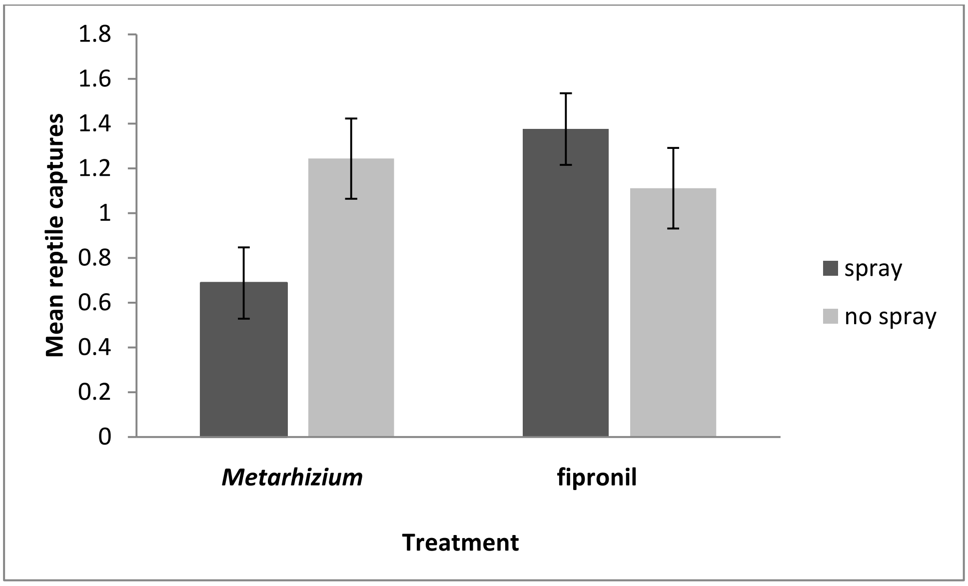

Figure 3: Reptile abundance at sprayed and unsprayed arrays within treatment sites. Bars represent the mean number of reptiles captured (± SE) at sites (n=9), and no significant differences among arrays was determined using repeated measures MANOVA (see table 3).

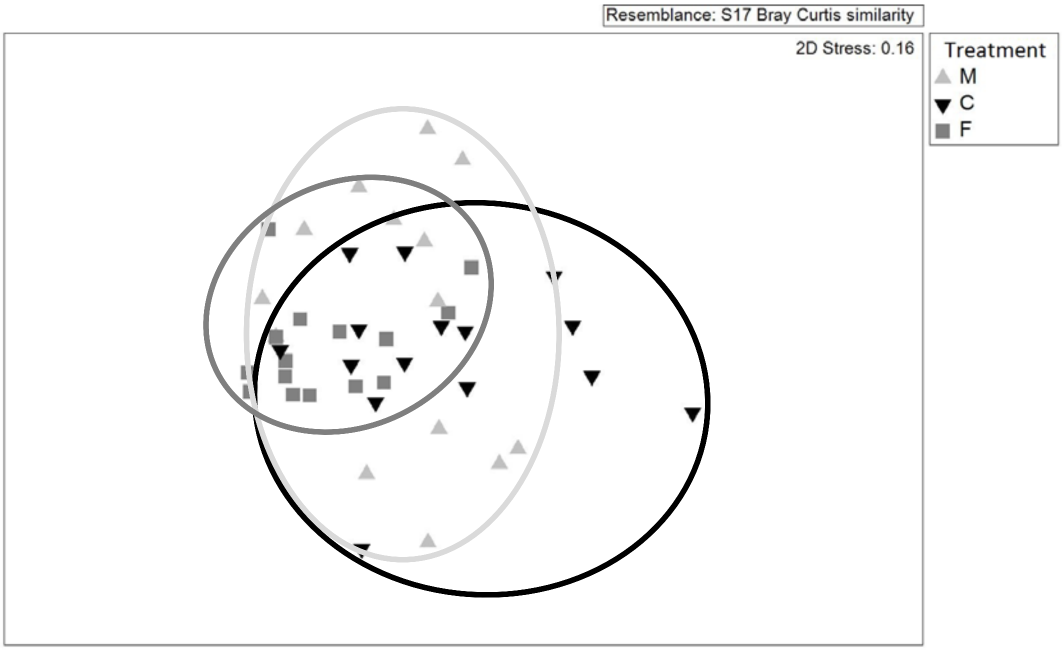

Figure 4: Community analysis (all 5 trapping sessions pooled) of the effect of treatment application using MDS. Treatment abbreviations: M = *Metarhizium*, C = Control, F = Fipronil. Control and *Metarhizium* sites are similar, while fipronil sites are significantly different from other sites (based on perMANOVA results in Table 4).