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Ant community and habitat limit colony establishment by the fire ant, *Solenopsis invicta*

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Summary

1. Hypotheses of community assembly include limitation through habitat physical attributes, as well as competition among species. Such hypotheses must be resolved through experimental tests.

2. Previous experiments have shown that: (i) fire ants of the monogyne social form occur mostly in highly disturbed habitat where they do not compete with mature colonies of co-occurring ants; (ii) in native pine forests of northern Florida, habitat disturbance favours fire ants while simultaneously reducing native ants; (iii) fire ants thrive in these disturbances but do not persist as these become less disturbed over time; and finally, (iv) newly mated, dispersing/colony-founding fire ant queens settle preferentially in such disturbed sites.

3. We now show that by choosing disturbed sites, newly mated, monogyne fire ant queens greatly increase their chances of successful colony establishment. Experimental plots were created in the native ground cover of a north Florida pine forest with all combinations of tilling, shading or reduction of the native ant community.

4. Newly mated fire ant queens, incipient colonies and small colonies were planted in these plots. Only five of 980 (0·5%) newly mated queen nests survived after 120 days, and only five of 400 incipient colonies (1·3%) survived after 30 days. All survivors were in plots with tilling and/or native ant reduction. Extrapolation indicated that 0·04% of newly mated queens and 0·1% of incipient colonies were likely to have survived at 1 year.

5. In contrast, planting small colonies resulted in much higher rates of survival – in plots with native ant reduction, fire ants increased on baits throughout the year but decreased in unreduced control plots. Fifteen months after planting 108 colonies, 21 mounds (19%) were found in the ant-reduced plots, but $<2\%$ of 108 colonies survived in the control plots.

6. Taken together, these results show that by landing in disturbed habitat with its reduced native ant population, newly mated fire ants queens increase their chances of successful colony establishment. In contrast to much of the previous literature, our results suggest that ant community assembly proceeds primarily by queen habitat choice and secondarily by filtering and competition.

Key-words: ant colony founding, ant community assembly, ant ecology, fire ant ecology, habitat disturbance, invasive ants

Introduction

Understanding recruitment, the process of adding new individuals to a population, provides insight into the persistence of species populations under different environmental conditions. Local community composition is determined by a number of processes, including competitive exclusion, but dispersal and recruitment limitation may be of equal or greater consequence in determining population persistence and local community structure (Caley *et al.* 1996; Tilman 1997; Clark, Macklin & Wood 1998; Siemann & Rogers 2003). For biological invasions, the environmental conditions that limit the establishment and spread of invasive species are of particular interest. Recruitment also affects how susceptible different ecosystems are to invasion, where invasive species will have greatest impact (Siemann & Rogers 2003; Puth & Post 2005) and how local communities are assembled from the

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larger, regional species pools (Ricklefs 1987). Recruitment ability may also determine whether or not exotic species appear in any given local community during the establishment and spread phases of the biological invasion (Theoharides & Dukes 2007).

Recruitment is known to limit the success of some exotic plants (e.g. Siemann & Rogers 2003) in different ecosystems (Theoharides & Dukes 2007), but factors limiting recruitment by many exotic animal species are less well understood. Exotic ants are one of the most important and widespread groups of invasive insects (Holway *et al.* 2002a), sometimes developing large, often habitat-specific populations in their introduced ranges (Holway, Suarez & Case 2002b; Tschinkel 2006). Enemy release and 'novel weapons' (e.g. larger colony size, venom, and aggressiveness) that convey competitive advantages are non-exclusive hypotheses that may help to explain the success of invasive, exotic ants in their invaded habitats (Elton 1958; Porter & Savignano 1990; Holway *et al.* 2002a). However, abiotic and biotic factors that limit successful establishment of invasive, exotic ant species are less well studied. Cold temperatures, especially frequent hard freezes or long cold periods, limit the range of many invasive ant species (Holway, Suarez & Case 2002b; Tschinkel 2006). Aridity limits invasions by Argentine ants (*Linepithema humile* Mayr) and red imported fire ants (*Solenopsis invicta* Buren) at local scales (Menke & Holway 2006; Tschinkel 2006). A key remaining question for commonly spread and pestiferous invasive ants is what factors limit their successful establishment to some, but not all ecosystems?

From its origin in Argentina and accidental introduction in Mobile, Alabama, the red imported fire ant is well on the way to becoming a cosmopolitan tramp species as it continues to be spread by human activities across the world (Tschinkel 2006; Ascunce *et al.* 2011). Within the limits of its physical tolerances, this species is strongly associated with human-altered habitats, including clearcuts (Zettler *et al.* 2004), cane fields (Cherry & Nuessly 1992), lawns (Cherry 2001), roadsides (Porter, Fowler & Mackay 1992; Porter 1993; Wojcik *et al.* 2001), pasture (Howard & Oliver 1979; Apperson & Powell 1984; Phillips & Jones 1986) and power line rights of way (Stiles & Jones 1998) (Camilo & Phillips 1990; Porter, Fowler & Mackay 1992; Tschinkel 2006). In natural areas crossed by roads, *S. invicta* is associated with the roadsides, also occurs around seasonal ponds and other local disturbed areas, is absent or rare in most undisturbed native habitat, especially those that are heavily shaded (Tschinkel 1988), but does occasionally invade at very low densities in wet prairies, in floodplains and in ecosystems underlain by clay soils (Stuble *et al.* 2011; King & Tschinkel 2013). One obvious correlate of most fire ant habitat is the general absence or low level of shade.

Experimental studies have recently shown that habitat disturbance simultaneously reduces native ant diversity and abundance and increases monogyne *S. invicta* populations (Tschinkel 2006; King & Tschinkel 2008, 2013). The appearance and persistence of large populations, a measure of ecological success, of *S. invicta* in human-altered habitats has been shown to be driven primarily by habitat selection of newly disturbed habitats by colony-founding queens and not by other processes, such as random dispersal followed by environmental filtering via competitive exclusion (King & Tschinkel 2016). Habitat selection by queens during colony founding is thus a functional trait that was probably shaped by individual colony-founding success under different environmental conditions. Selection of open, disturbed habitat by founding queens probably increases lifetime colony fitness as previous work has shown. Moreover, as soil and vegetation disturbances recede, that is, as succession takes place, fire ant populations eventually decrease (Pass 1960; Tschinkel & King 2013).

For fire ants in the south-eastern USA, there is a great deal of evidence showing that there are barriers to colonizing and persisting in most undisturbed native habitat, especially during the early phases of the colony life cycle, as fully mature colonies can persist for considerable periods when transplanted into undisturbed habitat (King & Tschinkel 2008). However, even these eventually disappear (Tschinkel & King 2013). Understanding how ecological factors may select for queen habitat selection behaviour during colony founding can thus shed light on the value of this poorly understood and probably widespread and variable functional trait across ants (Hölldobler & Wilson 1990; King & Tschinkel 2016). Other examples of differences in ant communities associated with habitat disturbance have been reported (King, Andersen & Cutter 1998; Majer & Nichols 1998; Wetterer *et al.* 2004; LeBrun, Plowes & Gilbert 2012), suggesting that understanding factors that limit recruitment by invasive ant species is a key to understanding their invasion success. Functional traits such as habitat selection behaviours by queens of independently founding species may determine what ecosystems will host invasive ants. Teasing cause-and-effect out of the observed ecological patterns requires experimentation. Because fire ant populations are strongly associated with soil disturbance, reduced native ant populations and absence of shade, we manipulated these factors in a fully factorial experiment in the native coastal plains pine forests of northern Florida to identify the abiotic and biotic factors affecting recruitment of fire ants during the initial phases of their life cycle.

Materials and methods

STUDY SITE

The experiment was sited in compartment 245 of the Apalachicola National Forest in a longleaf pine plantation with natural ground cover (Fig. 1). The site ecotype was longleaf pine flatwoods (Myers & Ewel 1990) with a ground cover vegetation that consisted of gallberry (*Ilex glabra*), shiny blueberry (*Vaccinium myrsinites*), runner oak (*Quercus pumila*), wiregrass (*Aristida beyrichiana*) and palmetto (*Serenoa repens*) and was subjected to

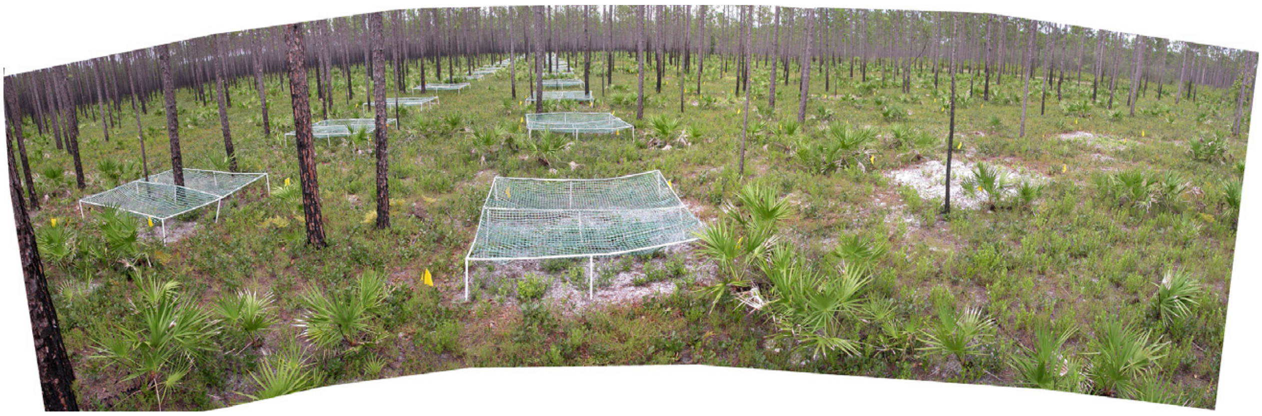


Fig. 1. The experimental array in place in the Apalachicola National Forest. The shaded treatments are to the left and the unshaded to the right. Tilled plots are apparent to the right, but untilled are not visible in this image.

prescribed burning on a return frequency of about 3–4 years. Prior to the experiment, the soil was disturbed only by a narrow old fire line. Fire ants occur naturally only along roadsides and are all of the monogyne social form.

EXPERIMENTAL DESIGN, 2012

The object of the experiment was to test the survival of three early life stages of fire ant colony reproduction (newly mated queens, incipient colonies and small colonies) under several combinations of habitat and ant community manipulations. These were soil tilling, shading and reducing the resident native ant community by killing them with insecticide. Tilling represents one of the most common and widespread human alterations of the environment, soil disturbance and plant biomass removal, associated with site development for road building, real estate or agricultural development. This approach eliminates almost all ground cover, reduces shading from vegetation and thus results in increased soil temperatures and wider daily fluctuation in soil temperature (King & Tschinkel 2016). Shading reduces average daily temperatures and temperature fluctuation in soils (King & Tschinkel 2016). Reduction of the resident ant community with poison baits was expected to eliminate one of the greatest predation threats to colony-founding queens and small colonies (Tschinkel 2006). These treatments were combined in a fully crossed design as shown in Fig. 2. An untreated control was also included. Each treatment or combination was applied to one of the eight 3 m-by-3 m plots in a replicate set of plots (Fig. 2). Plots were spaced 3 m apart. Each set of eight plots was replicated five times and subjected to one fire ant treatment (newly mated queens, incipient colonies, small colonies) resulting in 40 plots per fire ant treatment and 120 plots total (Fig. 1).

A rototiller was used to turn the top ~50 cm of soil, reducing the vegetation and exposing bare soil. The native ant community was reduced by a single application of the poison bait Amdro® spread by hand 2 weeks before planting, allowing ample time for the poison to be consumed by resident ants or to degrade before planting fire ant colonies. Shade structures 3 m square and about 30 cm above the ground were built from PVC pipe and fittings, using plastic fencing to provide about 40% shade (Fig. 1).

Newly mated queens

Approximately 1200 newly mated queens of the monogyne social form were collected after a mating flight 28 June 2012. In the evening of the next day, 18 newly mated queen founding nests (nine with two queens, nine with a single queen) were planted in each plot of five replicate sets of plots (40 plots; 980 queens). A nail hole was made in the soil, and each newly mated queen was released into a small PVC ring surrounding the hole. Queens almost all immediately entered the hole and stayed there, plugging it with excavated soil within a day. The locations of these burrows were marked with a ring of blue latex paint. Beginning after 2 weeks, each of these locations was checked monthly for nest reopening and the presence of minim workers, with the final check occurring after 120 days.

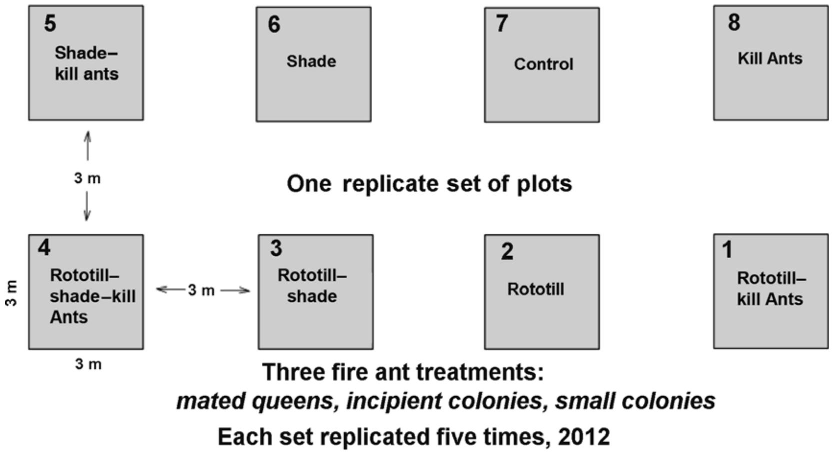


Fig. 2. Plot layout and treatments for the 2012–2013 planting experiment. Each set of eight treatments was replicated five times (see Fig. 1), so that each fire ant treatment consisted of 40 plots and the entire experiment of 120 plots.

Incipient colonies

In the laboratory, several hundred newly mated queens of the monogyne social form, either alone or in pairs, were placed into nest tubes with damp cotton and allowed to rear the first brood of workers, a process that most completed within about a month. In late July 2012, 10 incipient colonies were planted in each plot in the second group of five replicate sets of plots (40 plots). A PVC ring was placed around a nail hole in the soil, and the queen, minim workers and brood were tapped out of their nest tube and into the ring. These incipient colonies almost immediately moved into the hole. They were checked visually and by baiting with a small piece of Spam® three times from 1 August until November 2012.

Small colonies, 2012

Newly mated queens of the monogyne social form collected after a mating flight in May 2012 were placed into nest tubes in the laboratory and allowed to rear colonies. Once minim workers had appeared, the colonies were moved to sandwich boxes provided with water tubes, nest tubes and sugar water tubes. By mid-October, colonies ranged in size from about 25 workers to over 500. These were sorted into small (mean, 36 workers), medium (mean, 113 workers) and large colonies (mean, 420 workers). On 30 October 2012, one colony of each size was planted in each of 20 plots. Because the effect of killing native ants on such small plots had faded by this time, the treatments included only till, till–shade, control and control–shade with each treatment replicated 10 times. On 29 October 2012, the experimental plots were rototilled, and the shade structures repaired in preparation for planting small colonies. In mid-November, 64 nest tubes containing the small colonies were unplugged and pushed into the soil at an angle until only the mouth of the tube was aboveground. Two or three nail holes were made in the soil just outside the nest entrance. Colonies usually vacated their nest tubes and moved into the soil within a few days. By the time, these small colonies were ready to plant, the ant community in the kill plots had largely recovered, and the 2012 planting of small colonies lacked the 'kill' treatment; that is, they were planted in the presence of the native ant community. The test in the absence of the native ant community took place about a year later in 2013, as described below.

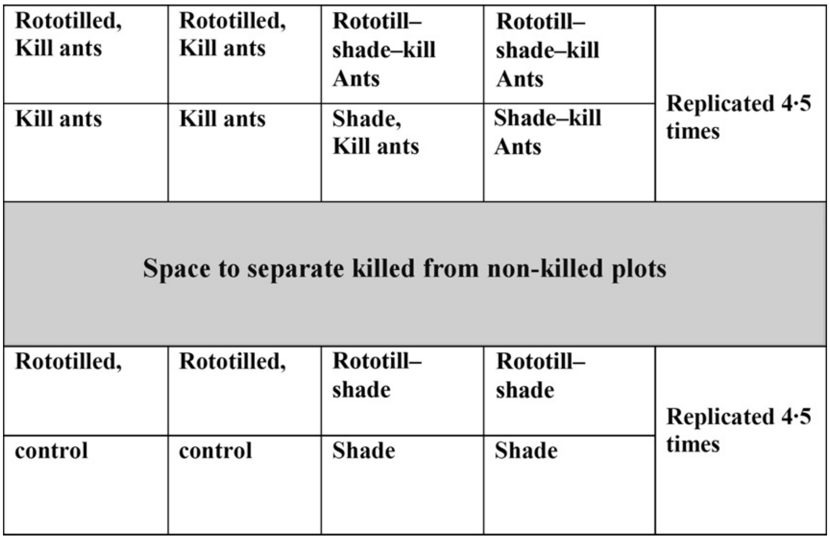
Small colonies, 2013

A large cohort of newly mated queens was collected after a mating flight in May 2013 and allowed to establish small colonies in the laboratory, as described above. When these had produced the first minims, the nest tubes were unplugged and placed into 10·1 cm by 10·1 cm sandwich boxes, provided with a water tube and a sugar tube, and fed chopped tenebrionid beetle larvae three times a week. By September 2013, there were a sufficient number of healthy, small colonies to establish the field experiment. Because we did not test newly mated queens and incipient colonies in 2013, the plots were reconfigured as shown in Fig. 3. All five replicates of the 'kill' treatments were grouped sequentially and separated from the 'control' treatments by a buffer zone 20 m wide. Tilled plots were tilled again on 1 July 2013, and palmetto was chopped and shade structures repaired. Five pitfalls were installed in each plot, for a total of 392, but were closed with lids. The entire area containing the 'kill' plots was then treated with the poison bait Amdro® 6–7 September 2013 using a hand-held fertilizer spreader. A 5-m buffer zone around the five replicate sets was also treated. This undivided 'kill' zone (Fig. 3) made it less likely that native ants did not move or wander in from the edges. In mid-September, the effectiveness of ant killing was tested first by baiting next to each closed pitfall, and then by opening the pitfalls for 5 days. Pitfalls were 15 dram, unbaited, straight-sided plastic tubes with a few ml of propylene glycol. The number and species of ants in each pitfall was recorded in the laboratory and revealed the effectiveness of the Amdro® treatment.

By mid-September, the laboratory-reared colonies ranged in size from 100 to almost 8000 workers and were grouped into small (100–2000 workers; mean 200), medium (2000–5000 workers; mean 3600) and large (5000–8000 workers; mean 7500). In late September 2013, three of these colonies were added to each plot – one small, one medium and one large. Planting proceeded as in 2012. These plantings were checked periodically, either by baiting or visually, beginning at the end of September 2013 until March 2015. Many planted colonies moved within a week or two, becoming difficult to detect visually, but confirming their presence by coming to baits. Because the colonies were small, their workers were also small and were thus not readily confused with fire ant workers from large colonies that are always larger (Tschinkel 2006).

MONITORING SURVIVAL

Survival was monitored either through visual detection of active nests, or by baiting or both. Within 2 days of planting, the newly mated queens were checked visually for evidence of nest closure. Beginning about 3 weeks after planting, nests were checked for



Rototilled, Kill ants	**Rototilled, Kill ants**	**Rototill–shade–kill Ants**	**Rototill–shade–kill Ants**	**Replicated 4·5 times**
Kill ants	**Kill ants**	**Shade, Kill ants**	**Shade–kill Ants**	
Space to separate killed from non-killed plots				
Rototilled,	**Rototilled,**	**Rototill–shade**	**Rototill–shade**	**Replicated 4·5 times**
control	**control**	**Shade**	**Shade**	

Fig. 3. Plot layout for the 2013 planting of small colonies. Each plot was planted with one small, one medium and one large colony, and the design was replicated 4·5 times. As each treatment occurred twice in each set of eight plots, the total number of replicates was nine.

re-opening (indicating successful production of minims). At this time and throughout the remainder of the experiments, baits were either small pieces of Spam or pieces of tenebrionid beetle larvae, both dyed with Rhodamine B to increase visibility. Nine baits were placed in three rows within each plot and were checked twice beginning approximately 45 min after placement. The species occupying the baits (if any) were recorded.

By mid-2014, foraging workers emanated from openings of foraging tunnels, but colonies rarely formed mounds. This changed as the winter of 2014 arrived and surviving colonies built easily spotted mounds. The penultimate check was thus a search for mounds.

STATISTICAL ANALYSIS

The study design was a randomized complete block design with shading, ant community reduction, tilling treatments and controls fully crossed. For incipient queens, we used a nonparametric product-limit (Kaplan–Meier) estimate to compare month by month survival of colonies among different treatments using PROC LIFETEST in SAS version 9 (SAS Institute, Cary, NC, USA). We used two tests for homogeneity among survivorship curves for each treatment: the log-rank analysis and the Wilcoxon test (SAS Institute). Newly mated queen colony survivorship was determined at 35, 82 and 120 days. All other colony survivorship data were generated by determining the number of colonies present at the conclusion of the experiment for each colony size class group, and data were analysed using ANOVA and multiple comparisons (after Tukey–Kramer adjustment) in SAS version 9 (SAS Institute) via PROC GLIMMIX. PROC GLIMMIX allows for error terms that are not normally distributed and random effects in models. As colony data were counts, they were fit to generalized linear mixed models using Poisson or negative binomial distribution as best fit the data and checked for overdispersion (Littell *et al.* 2006). Observation data (presence or absence of fire ants and other ant species) gathered from baits were analysed using ANOVA.

Results

NEWLY MATED QUEENS AND INCIPIENT COLONIES

Almost all newly mated queens died within the first 120 days: only five of 980 colonies planted on June 29, or 0·51%, survived. Of these four were in rototill–kill plots and one in a rototill–kill–shade plot. Survival of fire ant newly mated queens over 120 days thus was highest in plots that were tilled, without shade, and treated with insecticide (test for homogeneity of survival curves: log-rank test, $P = 0{\cdot}002$, Wilcoxon test, $P < 0{\cdot}001$). Thus, all five of the newly mated queen survivors were in some combination of ant reduction with another factor, four in the till–kill–no-shade treatment. The probability that this was generated by chance was very small ($\chi^2 = 22{\cdot}2$; $P < 0{\cdot}003$). At a very approximate survival rate of 95% per day, this implies that <0·04% of the newly mated queens would still be alive after 1 year.

Of the 400 incipient colonies planted in early July, five (1·3%) were alive on 1 August 2012. Of these, two were in rototill–kill plots, two in kill plots and one in shade–kill plots. By October, at the termination of the experiment, only two colonies (0·5%) were still alive, one in a rototill–kill plot and one in a shade–kill plot. There were too few checks to apply the test for homogeneity of survival curves, but there were no significant differences in final survival among treatments (ANOVA, $F_{7,32} = 1{\cdot}07$, $P = 0{\cdot}40$). Extrapolating the rate of disappearance to 1 year, only 0·1% of incipient colonies would still be alive.

Because survival was so low for both queens and incipient colonies, we combined the first check of both groups for a total survival of ten. If survival were randomly associated with treatment, the expected survival would have been 1·25 per treatment (10/8). Of the ten survivors, six were in the till–kill–no-shade treatments, two in the control–kill–no-shade and one in the control–kill–shade. The likelihood of this outcome by chance was very low (chi-square = 23·6, $P < 0{\cdot}002$). Moreover, the chi-square value for the till–kill–no-shade alone was 18·1, and all survivors were in plots that combined reduction of native ants with another treatment. Testing each factor alone showed that native ant reduction had a highly significant effect on survival ($P < 0{\cdot}002$), tilling a marginal effect ($P < 0{\cdot}06$) and shade a non-significant effect ($P = 0{\cdot}21$).

SMALL COLONY PLANTING, 2012

Visual checks for activity on 10 Nov. 2012 found a total of 28 live colonies, a survival rate of 47%. By the last check on 30 June 2013, there were 13 live colonies (22%). Extrapolating survival to 1 year, approximately six colonies (10%) would still be alive, a much higher survival than newly mated queens or incipient colonies. There were no significant differences in survival among treatments (ANOVA, $F_{3,16} = 0{\cdot}36$, $P = 0{\cdot}79$). In the light of the 2013–2015 results, this lack of treatment effect in 2012 was probably because by the time the small colonies were planted, the small treatment plots had been recolonized by the native ant fauna.

SMALL COLONY PLANTING, 2013

Treatment with Amdro® bait effectively reduced the ant populations of the ‘kill’ plots. Two weeks after treatment, 137 of the 200 pitfalls in the kill plots captured no ants, one captured *S. invicta,* and 61 captured other ants, most commonly the native species *Dolichoderus mariae, Nylanderia parvula* and *Camponotus floridanus*. In the control plots, of the 192 pitfalls, only two were without ants, 15 captured *S. invicta,* and 175 captured one or more of at least 10 common ant species.

Visual counts showed that small colony survival was affected by treatments (ANOVA $F_{5,64} = 237$, $P < 0{\cdot}0001$), with colonies failing to survive in any shade only and control plots but surviving, on average, at similar abundances in all kill and rototill treatments and combinations (Fig. 4).

Survival was also tracked by repeated baiting of the plots and revealed a more nuanced picture of survival. The number of baits dominated by fire ants was greatly increased by killing the native ants (Fig. 5; ANOVA, $F_{1,1920} = 325$;

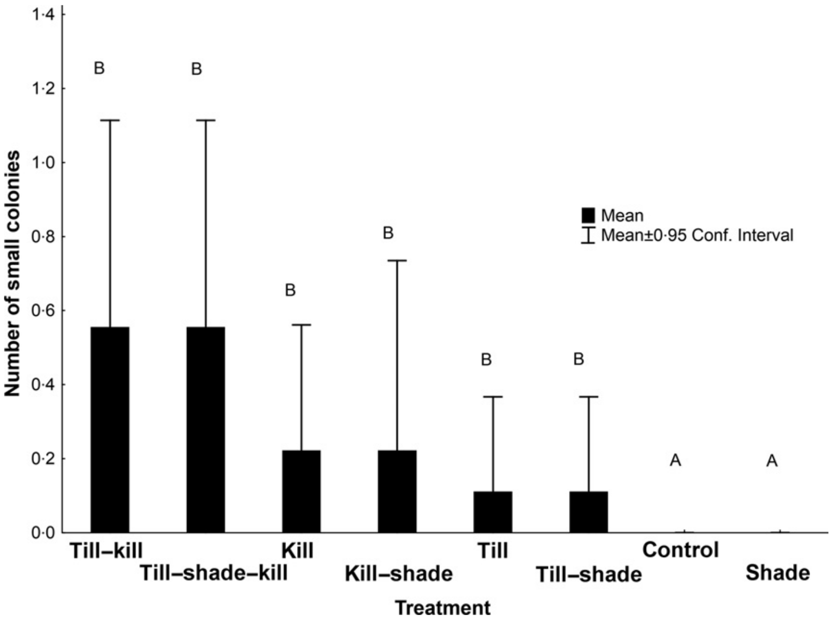


Fig. 4. Counts of surviving small colonies in the 2013 experiment in relation to treatment. Small colonies failed to survive in any shade only and control plots, but survived, on average, at similar rates in all kill and rototill treatments and their combinations (ANOVA $F_{5,64} = 237$, $P < 0{\cdot}0001$). Bars = 95% confidence intervals.

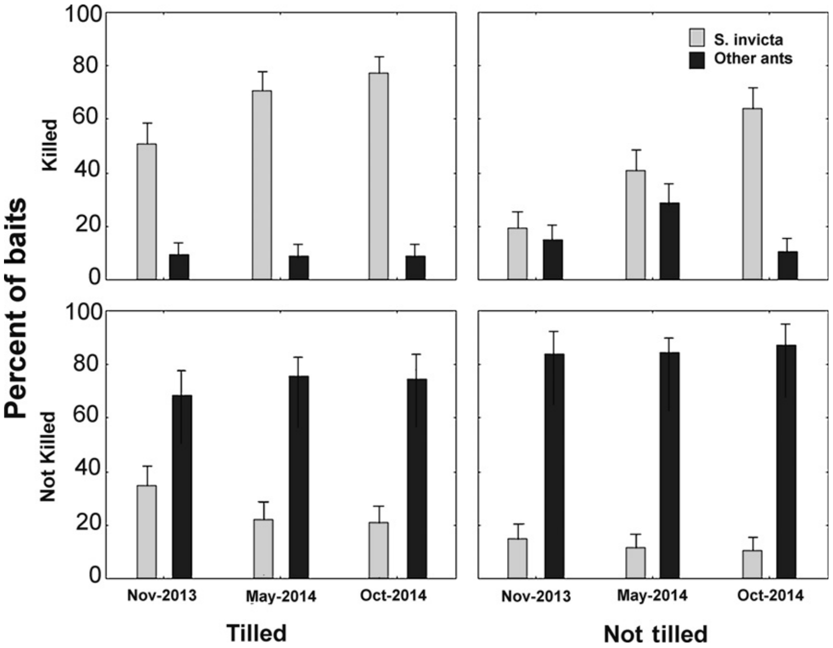


Fig. 5. Killing native ants and tilling the soil greatly increased the success of planted fire ant colonies. In the course of 1 year after planting, fire ants had increased their dominance of baits in the kill plot and decreased it in the no-kill plots. The dominance of fire ants decreased with time in the tilled plots, but changed little in the no-kill, untilled plots. The till and kill treatments both had significant effects, as well as significant interactions with shading, but shading has no significant main effect.

$P < 0{\cdot}000001$) and by tilling the soil ($F_{1,1920} = 100$; $P < 0{\cdot}000001$), but was not affected by shade ($F_{1,1920} = 0{\cdot}48$; n.s.). Over all checks, in tilled plots, 66% of baits had *S. invicta* when the native ants had been killed, and 26% when they had not. In the untilled plots, 41% had fire ants when the native ants had been killed, and 12% when they had not. The interaction of tilling and ant removal was also significant ($F_{1,1920} = 8{\cdot}66$; $P < 0{\cdot}004$). Shading did not affect fire ant frequency on baits, although there was a small interaction with the kill treatment ($P < 0{\cdot}05$).

The presence of other ants on the baits (Fig. 5) was obviously and necessarily reduced by the killing of native ants ($F_{1,1920} = 1099$; $P < 0{\cdot}000001$). Tilling also greatly reduced other ants on baits ($F_{1,1920} = 29{\cdot}9$; $P < 0{\cdot}00001$). Shading also reduced other ants on baits but much less than the previous two factors (from an overall rate of 50% to about 42%; $F_{1,1920} = 12{\cdot}9$; $P < 0{\cdot}004$). Overall, in tilled plots, only 9% of baits had other ants in the kill treatments, and 73% in the non-kill treatments. In the untilled plots, 18% had other ants in the kill plots, and 85% in the no-kill plots.

The frequency of fire ants on baits changed significantly in the course of a year (Fig. 5; main effect of date: $F_{1,1920} = 16{\cdot}3$; $P < 0{\cdot}000001$), but that of other ants did not ($F_{1,1920} = 2{\cdot}73$; n.s.). In comparison with November 2013, the dominance of fire ants on baits increased throughout the year in the kill plots and decreased in the no-kill plots (significant date by kill interaction,

$F_{1,1920} = 46{\cdot}3$; $P < 0{\cdot}00001$; Fig. 5), but changed little in the untilled, no-kill plots. Shade had no significant effect in any check, and this did not change between checks. The effect of tilling changed with check date (ANOVA, date by tilling interaction, $F_{1,1920} = 8{\cdot}66$; $P < 0{\cdot}004$.). These results showed that the planted fire ant colonies were not only still present during this entire year, but were growing, especially in the kill plots. In contrast, fire ants seem to have lost ground in the no-kill plots.

The frequency of non-fire ants on baits was not significantly affected by date of the check (Fig. 5). In spite of some recovery in the untilled–kill plots, the pattern in the other treatments was similar across dates. A three-way interaction among date, soil treatment and ant treatment was significant ($F_{1,1920} = 3{\cdot}32$; $P < 0{\cdot}04$) because the frequency in the untilled–kill plots was higher, but this effect was small.

During most checks, visual searches for the actual fire ant nests did not reliably detect the sources of the foragers on baits, because small fire ant colonies do not build mounds. In some cases, foraging trails could be traced back to the openings of foraging tunnels, but the mound remained invisible in the complex soil surface, litter and vegetation of the study site. However, fire ant colonies reach their annual maximum size during the winter, so that a search for fire ant mounds was productive during the winter of 2014–2015. Figure 6 provides a map of the locations of nest mounds for the kill and control plots. Fifteen months after planting, a total of 21 mounds were found in the kill plots, a survival rate of about 19% of the 108 planted colonies. In contrast, less than 2% of the 108 colonies planted in the no-kill plots survived, and even these two colonies may already have been present when the experiment was set up, as they inundated baits even during the first check on 5 November 2013.

Because fire ant colonies readily move, the origin of some of the mounds was not clear. Based on proximity, 12 mounds were associated with till plots, and eight with no-till plots. One could not be readily assigned. These differences were not significant (chi-square test), in spite of the early effect of tilling on fire ant survival and colony growth. This suggests that the early effects of tilling fade with time, overwhelmed by the effects of the rebounding native ants.

Discussion

Through a series of experiments, we have now shown that mature colonies of fire ants of the monogyne social form do not compete with co-occurring ants in highly disturbed ecosystems (King & Tschinkel 2006), that habitat disturbance favours fire ant proliferation and simultaneously reduces native ant populations (King & Tschinkel 2008), that fire ants thrive in disturbed areas and do not persist when these revert through habitat succession to less disturbed condition (Tschinkel & King 2013) and, finally, that newly mated, dispersing fire ant queens settle preferentially in disturbed sites to found new colonies (King & Tschinkel 2016). To this, we have now added evidence that the choices that newly mated fire ant queens make greatly increase the probability of successful colony establishment. Collectively, these results suggest an alternate mechanism of ant community assembly driven primarily by queen habitat choice and secondarily by environmental filtering following dispersal and interspecific competition among mature ant colonies (Livingston, Philpott & de la Mora Rodriguez 2013; Livingston & Jackson 2014; King & Tschinkel 2016). Thus, a behavioural trait functions to shape community assembly.

The earliest stages of the fire ant colony life cycle are a time of high mortality resulting from the presence of an intact ant community, along with habitat conditions that affect soil temperatures and possibly structure. Regardless of conditions, it is likely that a majority of mated fire ant queens will die during the first few weeks of the colony-founding event. Results suggest that landing in unvegetated, disturbed sites with few or no mature colonies of other ants improves the survival, however slightly, of queens during the claustral and incipient periods (King & Tschinkel 2016). The positive effect of soil disturbance on early survival may act either through higher soil temperatures or reduction of the native ant community, or both. The lack of effect of our shading treatment suggests that the ants may be more important, but it is also possible that our shading was too light. Once colonies grow to at least several hundred workers, their survival probability increases but the presence of an ant community and undisturbed soil still strongly limit colony survival (Fig. 5). In spite of the modest number of colonies used, survival of small colonies was much greater after 7 months (1·5%) than that of newly mated queens or incipient colonies after only 4 months (1·3%). This suggested that the bottleneck to population establishment was somewhere between the incipient stage and small colony stage. The particular threshold size could not be determined, as we could not distinguish the initial size of surviving colonies.

The ecological conditions that challenged newly mated queen and incipient colonies in these experiments suggest that predation by other ants and soil conditions are primary selective forces facing founding queens. Thus, fire ant queens have evolved to be highly selective during their mating flights, choosing to land almost exclusively in sites devoid of vegetation and with soil disturbance, such as tilled plots and roadsides (King & Tschinkel 2016). These traits evolved in its South American home where *S. invicta* is also strongly favoured by disturbance (Tschinkel 2006). Similar behaviours have been noted in other species, including a number of native ant species in this ecosystem (King & Tschinkel 2016).

The exact nature of the interactions among fire ants, soil disturbance and native ants is unknown. Founding *S. invicta* queens in their burrows are preyed upon by other species of ants such as thief ants, but probably other ant species as well

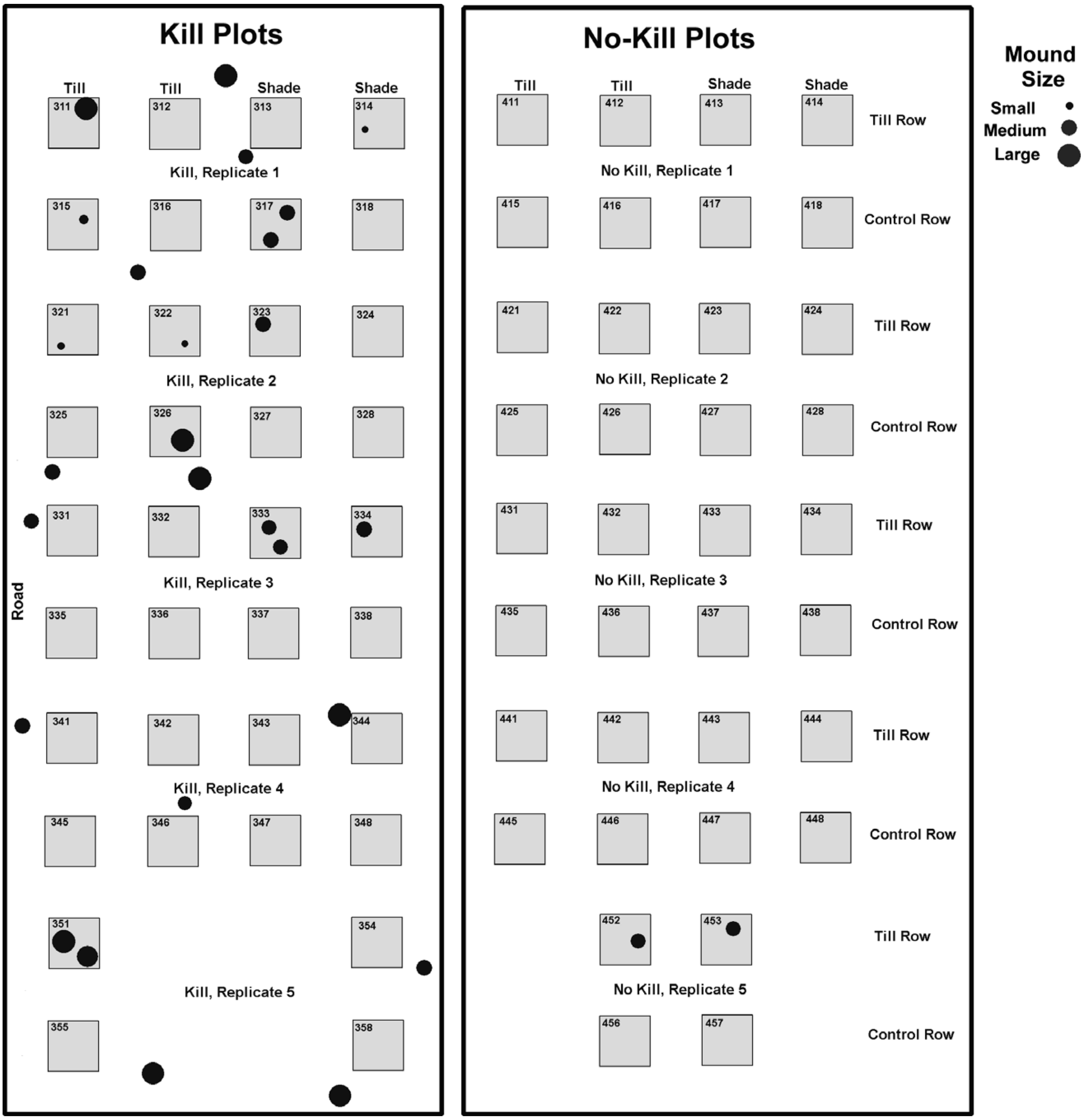


Fig. 6. Of the 108 small fire ant colonies planted in the plots in which the native ants had been previously killed with Amdro, 21 (19%) had survived to produce visible mounds by the winter of 2014–2015. This contrasts with less than 2% (two colonies) survival in the plots with intact native ant communities (map not shown). Even these may have predated the experiment. It was not possible to determine the effect of initial colony size on survival because all three sizes were planted in each plot.

(Whitcomb, Bhatkar & Nickerson 1973; Nichols & Sites 1991). Whatever this interaction, as the young fire ant colony grows, it escapes this source of mortality, and mature *S. invicta* colonies are capable of tolerating habitat conditions that are lethal to earlier stages (King & Tschinkel 2008, 2013). For example, mature colonies persist to varying degrees in a variety of conditions, depending on the particular nature of the habitat, but eventually they vanish, likely because queens do not recruit into non-ideal habitat (Tschinkel & King 2013). In sum, results suggest that fire ant impacts will continue to be greatest in human-modified environments and along the edges of human-modified natural areas. An important step towards a better understanding of how ant communities are assembled and affected by invasive species lies in understanding the functional trait of habitat selection by queens after mating flights.

Our work emphasizes the general importance of testing community assembly and recruitment hypotheses with field experiments, rather than relying on interpretation of patterns. Billick, Wiernasz & Cole (2001) removed harvester ants from plots and found an effect on harvester ant recruitment in some plots but not others, but it should be noted that these were intraspecific effects, not interspecific as in our study. An experimental study by Stuble *et al.* (2011) found that re-establishment of fire ant and native ant populations after reduction with insecticide did not differ, but there were major site and design differences between their study and ours. Whereas we planted fire ants, Stuble *et al.* (2011) depended upon natural recruitment. Whereas our plots supported no fire ants initially, theirs had moderate initial populations and lower overall species diversity, indicating that their habitat was already capable of supporting fire ants before the experimental manipulations.

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Data accessibility

Data available from the Dryad Digital Repository http://dx.doi.org/10.5061/dryad.84q2t (Tschinkel & King 2016).

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