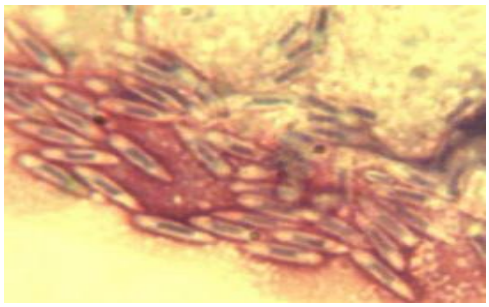




Tawny Crazy Ant (*Nylanderia fulva*) Study
Travis County's Balcones Canyonlands Preserve – Jollyville Unit
Task 5 – Technical Report

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Executive Summary

Nylanderia fulva (Tawny Crazy Ants) are an invasive ant species introduced from central South America that is spreading throughout the Gulf Coast region of the United States. These ants have devastating impacts on native arthropod assemblages and likely the wildlife that feeds on them. To mitigate these impacts on wildlife, particularly endangered wildlife, a means to sustainably control populations of tawny crazy ants (TCA) in natural habitats is highly desirable. A microsporidian pathogen (*Myrmecomorba nylanderiae*) discovered in 2015 presents a potential avenue for sustainable control of these ants. Herein we report the results of the first 6-months of work on an effort to evaluate the efficacy of inoculating the TCA population currently invading a tract in the Jollyville Unit of the Balcones Canyonlands Preserve. Important progress to date includes: 1) Quantifying the ant assemblage. We have gathered baseline data on the composition and relative abundances of the ant assemblage inhabiting invaded and uninvaded sections of the preserve. Going forward, these data will allow for the quantification of: negative impacts of the TCA invasion, any reductions in TCA abundance following establishment of the microsporidian, and any recovery of the native ant assemblage should TCA abundance decline. 2) Defining the magnitude and phenology of TCA canopy use. The abundance of TCA in the canopy increased throughout warbler nesting season and, by its end, TCA were 87 to 200 times more abundant than all other ants in uninvaded canopy combined. In 2018, TCA heavily exploited the forest canopy during the last half of golden cheeked warbler nesting season but were largely absent from the canopy in the first half. 3) Measuring TCA spread within the preserve. From the fall of 2017 to the fall of 2018, the leading edge of the TCA population moved on average 55 meters farther into the preserve. 4) Conducting the first inoculative release of *M. nylanderiae* within the preserve. *M. nylanderiae* was detected several times after the inoculation in the area of the inoculation. However, the infection remains spotty and it is unclear if it will establish in the spring. 5) Monitoring background infections of *M. nylanderiae* outside the preserve. These infections persisted throughout the growing season of 2018. In the fall and early winter, these infections increased dramatically in TCA nest infection prevalence and infection intensities. By January 2019, the infection had spread from this adventive infection acquisition into the northern edge of the preserve. *M. nylanderiae* is now prevalent in TCA nests on the northwest edge of the preserve.

Project Background

Nylanderia fulva (Tawny Crazy Ants) are an invasive ant species introduced from central South America that is spreading throughout the Gulf Coast region of the United States (Kumar et al. 2015). These ants invade natural habitats and have devastating effects on the arthropod assemblages of those environments (LeBrun et al. 2013). By doing so, these ants reduce the available prey base for foraging birds and other vertebrates. These ants also forage in large numbers in the forest canopy and directly impact bird nesting success by irritating hatchlings to the point that they fall out of the nest (J. Giese 2015). To mitigate these impacts on wildlife, particularly endangered wildlife, a means to sustainably control populations of tawny crazy ants (TCA) in natural habitats is highly desirable.

In 2015 myself and collaborators discovered and described a microsporidian pathogen of TCA, *Myrmecomorba nylanderiae* (Plowes et al. 2015). We found this pathogen infecting a minority of TCA populations surveyed in both Texas and Florida. Subsequent work demonstrated that this pathogen has impacts on the growth and mortality of TCA nests under laboratory conditions (LeBrun et al. 2017), while accumulating evidence indicates that infection can reduce abundance of TCA in field populations sometimes dramatically (LeBrun unpub. data).

The larger goal of this research program is to refine the technical expertise necessary to implement and evaluate the efficacy of using this microsporidian pathogen as a biological control agent for TCA. The data from this project is part of a larger multi-site study. Herein I will be reporting only on the results from the year 1 efforts at the Collins Tract, Jollyville Unit, Balcones Canyonlands Preserve. The goals or tasks for year 1 were to:

1) Quantify the native ant assemblage within the preserve in both the invaded and uninvaded habitat adjacent to the expanding TCA population in order to better understand impacts of this invader and be able to assess the resiliency of the ant assemblage should we succeed at controlling this TCA population.
2) To assess TCA usage of the canopy in order to gauge their likely intersection with nesting golden cheeked warblers and black capped vireos. These data will also be used to support ongoing work by Travis County staff to assess how arboreal arthropod assemblages (food resources for nesting warblers and vireos) respond to TCA invasion.
3) Map the TCA population extent within the preserve.
4) Perform an inoculative release of the microsporidian into the TCA population and follow its establishment.

Prior to the initiation of this project, naturally occurring infections of *M. nylanderiae* were encountered in TCA in areas of the residential neighborhoods surrounding the preserve. In addition to the above tasks delineated in the proposal, we conducted sampling throughout the year of these areas. The results of this work are also reported herein.

Task 1: Prepare a research plan

Completed and submitted June 5, 2018.

Task 2a: Assessment of tawny crazy ant (TCA) densities and native-ant assemblage composition within the preserve

Ant Community composition and TCA densities were assessed using pitfall traps set within the different areas of the preserve defined by the amount of time TCA had been present in the habitat.

Permanent pitfall trapping stations were established in invaded and uninvaded areas of the preserve. Permanent pitfall trapping stations consist of PVC sleeves set flush in the ground. Sleeves are plugged between trapping intervals and then an open tube containing a mixture of water, soap, and propylene glycol are inserted. Propylene glycol is a non-toxic preservative (approved by the FDA as a food additive) and poses no known threat to wildlife.

Areas

Three habitat areas defined by TCA occupancy were sampled: Long-term Invaded, Edge - Invaded and Out - Uninvaded. Long-term Invaded areas were in the interior of the TCA populations and have harbored TCA for several years. Edge - Invaded habitat were invaded areas near the edge of fall 2107 TCA distribution in the preserve. Out – Uninvaded areas are of similar vegetation that are outside of the TCA population.

Traps in the Out – Uninvaded areas were run for 1 week in the spring of 2018. These traps are intended as a measure of the existing native ant assemblage prior to the invasion of TCA and serve as a baseline for future measurements.

Traps in the Long-term Invaded areas and Edge - Uninvaded habitat were run for 24 hours in the spring, summer and again in the fall to quantify TCA density.

<u>Findings</u>

The uninvaded area of the preserve is habitat for a diverse ant assemblage. Some of the species encountered are rare for central Texas. *Temnothorax andersoni*, for example, has previously only been reported from the type locality: Big Bend National Park in west Texas. Figure 1 provides the relative abundances of all common ant species encountered in the sampling. Table 1 provides a list of the species intercepted and a measure of their relative abundance.

Traps in uninvaded habitat were run to provide a useful baseline against which to measure change in the ant assemblage over time as *N. fulva* spreads into these currently TCA free areas. As such they were run for a week to maximize interception of rare species. Traps in invaded areas were run to quantify TCA abundance. As such they were run for only 24 hours. This makes direct comparisons of ant assemblage diversity between habitat types difficult at this juncture.

In the long-term invaded habitat, the annual, seasonal increase in TCA abundance is evident with TCA abundances increasing from 41± 53 (mean±SD) workers per trap to as many as 5600 TCA workers captured in a single 24 hour pitfall trap in the fall (1803±1865 mean±SD).

In the future, these data will allow for an assessment of TCA impacts if the population continues to spread or an assessment of recovery should the introduction of *M. nylanderiae* result in TCA population control.

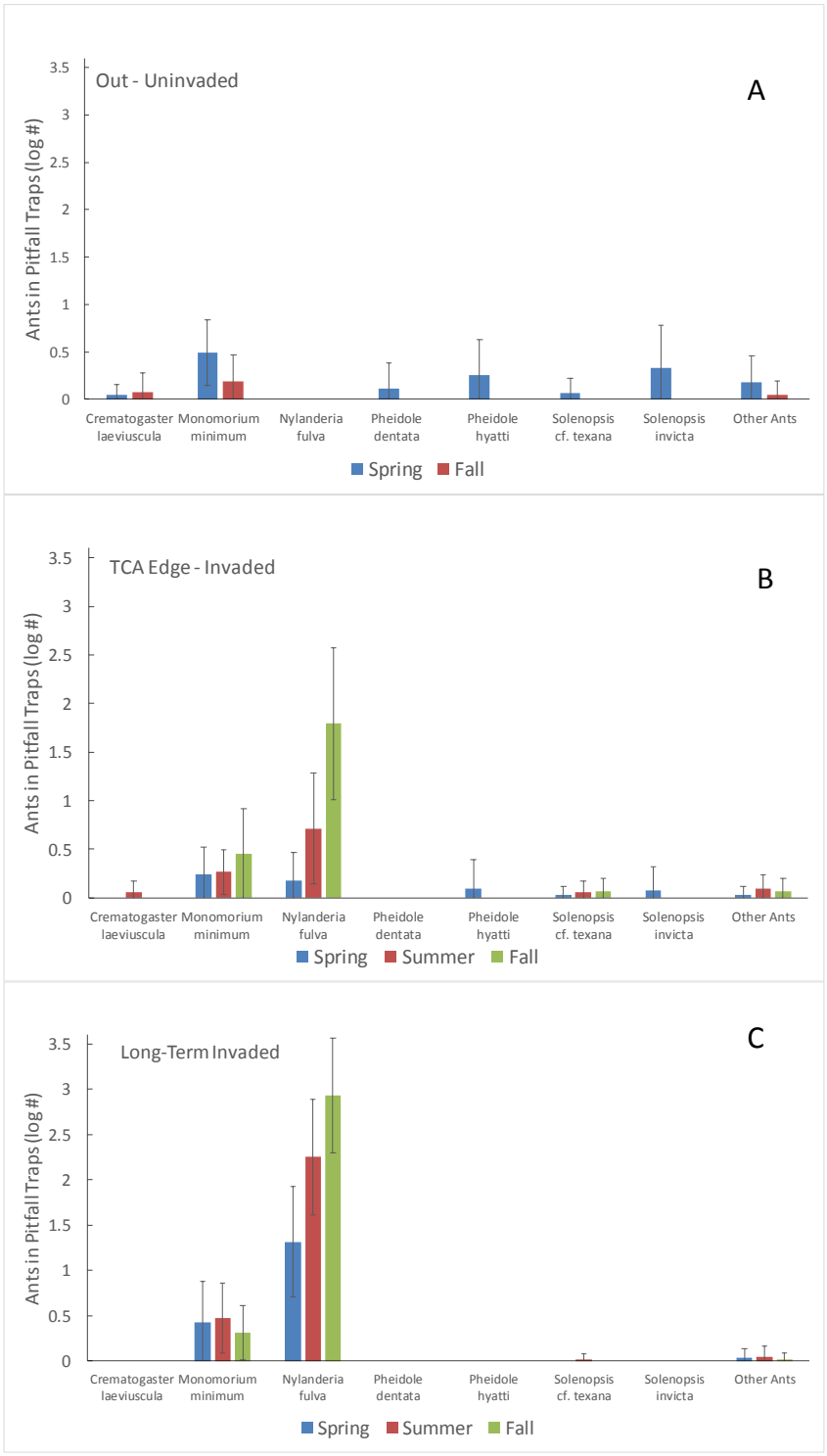


Figure 1: Abundance of common ant species in pitfall traps from the 3 sampling areas. Other Ants is the sum of workers of uncommon species. Counts were log-transformed and data are means ± 1 SD.

Table 1: Per-trap averages of the number of workers found per pitfall trap for all ant species encountered in 2018. All traps were open for 24 hours with the exception of traps run in the spring in the TCA Edge - Ininvaded and Out - Uninvaded areas which were open for a week.

	Long-Term Invaded			**TCA Edge - Ininvaded**			**Out - Uninvaded**	
	Spring	Summer	Fall	Spring	Summer	Fall	Spring	Fall
Brachymyrmex patagonicus	0.0	0.1	0.0	0.0	0.0	0.0	0.4	0.0
Camponotus sansabeanus	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0
Camponotus texanus	0.0	0.0	0.0	0.1	0.0	0.1	0.0	0.0
Crematogaster laeviuscula	0.0	0.0	0.0	0.0	0.2	0.0	0.2	0.4
Forelius damiani	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0
Forelius pruinosus	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0
Monomorium minimum	3.5	3.4	1.6	1.2	1.1	4.6	3.5	1.0
Myrmecina americana	0.0	0.0	0.0	0.0	0.0	0.2	0.1	0.0
Myrmica americana	0.0	0.0	0.0	0.0	0.3	0.0	0.0	0.0
Nylanderia fulva	41.3	365.2	1803.8	1.1	10.9	216.7	0.0	0.0
Nylanderia terricola	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0
Pheidole constipata	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0
Pheidole dentata	0.0	0.0	0.0	0.0	0.0	0.0	0.7	0.0
Pheidole hyatti	0.0	0.0	0.0	0.9	0.0	0.0	1.8	0.2
Pheidole tetra	0.1	0.0	0.0	0.0	0.0	0.0	0.2	0.0
Solenopsis cf. texana	0.0	0.1	0.0	0.1	0.2	0.3	0.3	0.0
Solenopsis invicta	0.0	0.0	0.0	0.5	0.0	0.0	2.8	0.0
Temnothorax andersoni	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0
Temnothorax subdivitus	0.0	0.0	0.1	0.0	0.0	0.1	0.0	0.0

Task 2b: Arboreal foraging density assessment

In order to evaluate how TCA change the availability of prey for nesting golden cheeked warblers and black capped vireos, Travis County Staff performed arboreal arthropod sampling during the nesting season. Arthropod sampling was accomplished by standardized beating of 2 low-hanging branches per tree sampled. Up to four tree species were sampled depending upon their availability in the target areas. UT sorted, identified and counted the ants that were captured in this branch-beating arthropod-sampling effort (Fig 2).

Following canopy arthropod collections performed by Travis County staff, we conducted arboreal foraging density measurements for TCA on trees in TCA invaded habitats that were sampled by Travis County staff. As use of trees by TCA varies strongly both spatially and seasonally, density assessments follow a 2-tiered method. In low density foraging environments, tree trunks from the ground to 2 m high were searched for 3 min and all TCA observed foraging counted. In higher density TCA environments, the number of trails of TCA ascending into the canopy were counted, and, for each trail, a 3 min count made of the number of TCA climbing past a specific point. To streamline efforts, low density foraging counts were carried out primarily by Travis County staff. UT carried out all high-density foraging assessments (Fig 3).

Areas

Three habitat were sampled for the arboreal arthropod surveys: Long-term Invaded, Edge - Uninvaded and Out - Uninvaded. Long-term Invaded areas were in the interior of the TCA populations and have harbored TCA for several years. Edge - Uninvaded habitat were uninvaded areas near but outside the fall 2107 edge of the TCA distribution in the preserve. Out – Uninvaded areas are of similar vegetation that are outside of the TCA population.

<u>Findings:</u>

TCA largely eliminate the native arboreal-foraging ant fauna (Fig 2&3). The usage of tree canopies by TCA increases exponentially as the season progresses from spring to early summer (Fig 2&3). This likely reflects both the seasonal increase in TCA abundance evident from the pitfall traps and the changing nutritional needs of TCA brood produced as a cohort in early spring. By June, TCA were 87 times more abundant in tree canopies than all ants combined in the Out-Uninvaded area and 215 times more abundant than all other ants in the Edge-Uninvaded area (Fig 3). Only live oaks and junipers were available to be sampled in the long-term invaded habitat. Of these trees, live oaks were greatly preferred.

Golden cheeked warblers (GCW) typically nest on the preserve from mid-March through mid-June (T. Clark pers. comm.). In 2018, TCA were abundant in the canopy during the second of that period by largely absent in the first. If generally true, this phenology will buffer but not spare GCW from any negative impacts of TCA on nesting success.

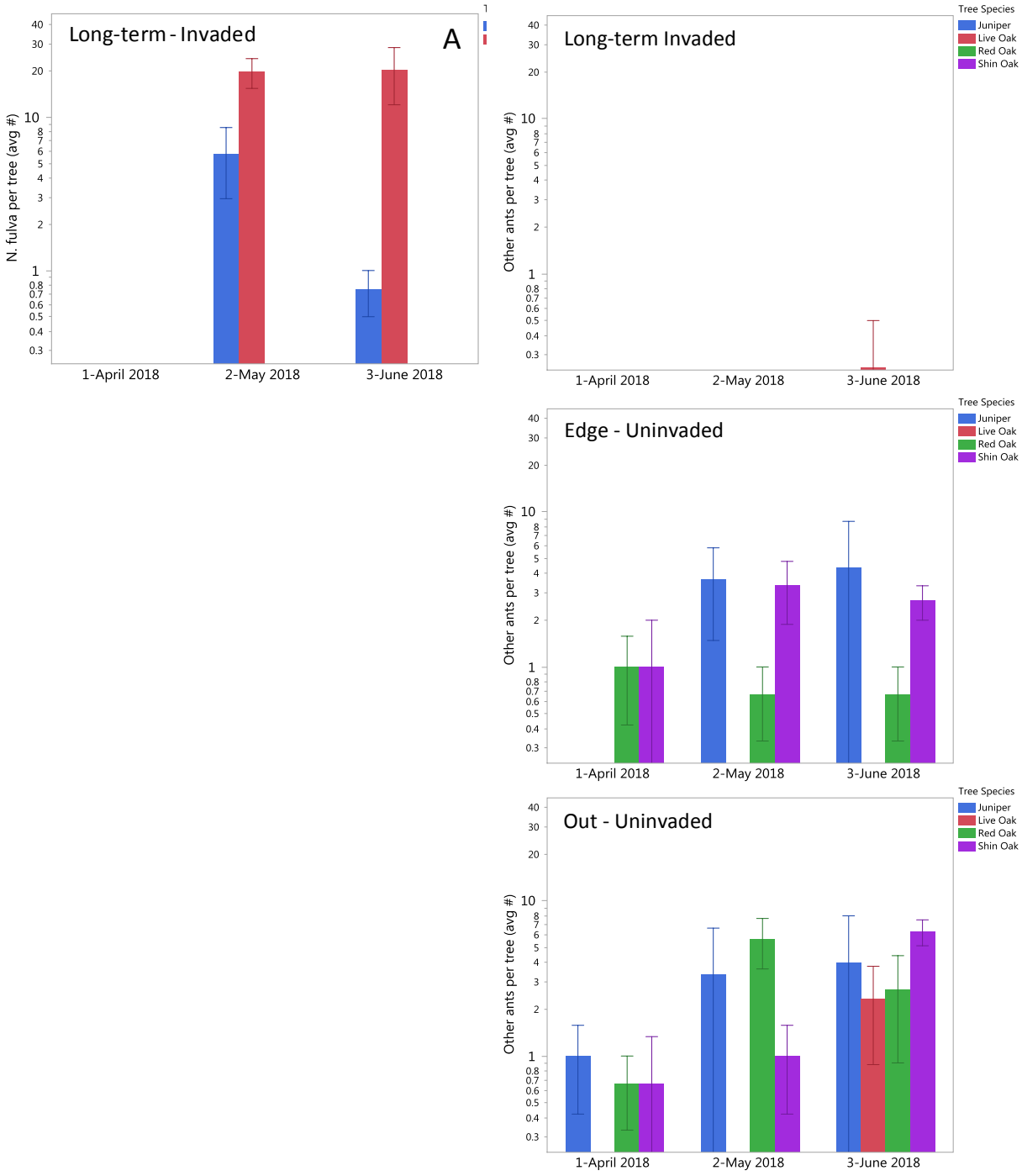


Figure 2: Ants foraging on branches. Abundance of (A) *Nylanderia fulva* and (B) Other ant species in branches of trees in the three sample areas. Average ants per tree plotted on a log scale. Error bars present standard deviations. Insufficient shin oaks or red oaks were available for sampling in the Long-term Invaded area and insufficient live oaks in the Edge-Uninvaded area.

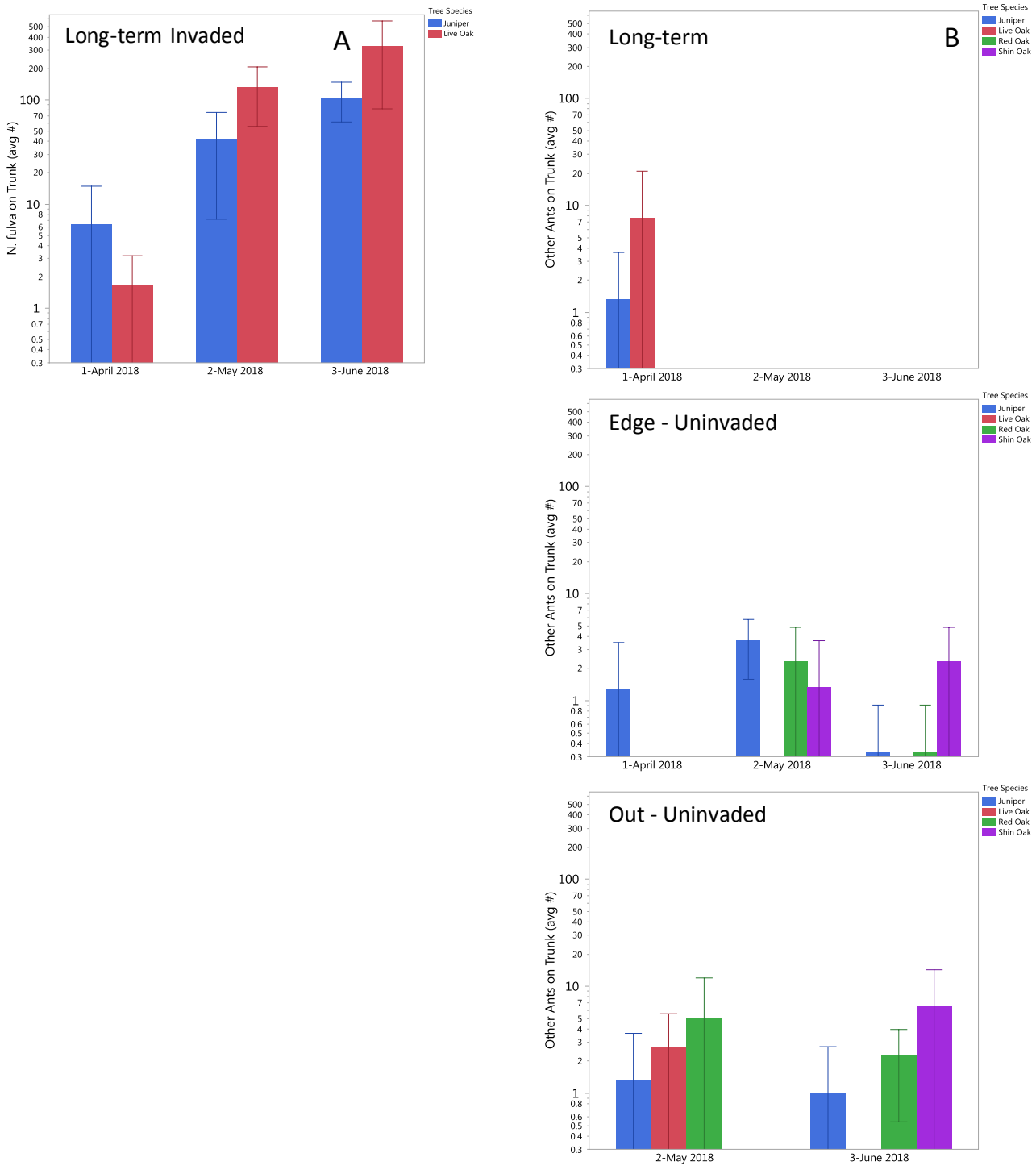


Figure 3: Ants foraging on trunks. Abundance of (A) *Nylanderia fulva* and (B) Other ant species on trunks of trees in the three sample areas. Average ants per tree plotted on a log scale. Error bars present standard deviations. Insufficient shin oaks or red oaks were available for sampling in the Long-term Invaded area and insufficient live oaks in the Edge-Uninvaded area.

Table 2: Non-*Nylanderia fulva* ant species counts by area and tree species. Counts are totals across the three sampling dates. Insufficient shin oaks or red oaks were available for sampling in the Long-term Invaded area and insufficient live oaks in the Edge-Uninvaded area.

	Juniper			**Live Oak**			**Red Oak**			**Shin Oak**		
	In	Edge	Out	In	Edge	Out	In	Edge	Out	In	Edge	Out
Camponotus decipiens	0	0	0	0	N/A	4	N/A	4	9	N/A	7	3
Crematogaster laeviuscula	0	0	0	0	N/A	2	N/A	2	10	N/A	7	1
Monomorium minimum	0	22	10	0	N/A	0	N/A	0	0	N/A	2	0
Pheidole hyatti	0	2	15	0	N/A	1	N/A	0	7	N/A	4	20
Pseudomyrmex brunneus	0	0	0	0	N/A	0	N/A	1	1	N/A	1	0
Pseudomyrmex pallidus	0	0	0	1	N/A	0	N/A	0	0	N/A	0	0

Task 2c: Mapping the extent of the TCA Population within the preserve

TCA lack alate flights with nests reproducing though budding. These ants are also supercolonial with all nests within a population belonging to the same colony. As a result, as populations expand they form a discrete boundary with one-side occupied by a dense network of interconnected TCA nests and the other side lacking TCA entirely. Mapping this boundary provides a way to track the spread of the invasion.

In the Fall of 2017, data on the extent of the TCA population within the preserve was collected in collaboration with Travis County Staff. UT continued this effort in the Fall of 2018 to assess the extent of preserve occupied and the amount of spread that has occurred in the intervening year.

In October 2018 along transects spaced at approximately 150 m intervals throughout the 750 m section of the TCA population front that occurs in the preserve, we assessed the presence or absence of TCA. Presence / absence was determined by visual inspection of the ant assemblage foraging near likely nesting locations and at the bases of trees. Observers identified the ants present at locations spaced every 20 m along the transects. Stations where 3 non-TCA ant workers were encountered, and no TCA were found were considered free of TCA. Three negative stations in a row were defined as the threshold indicating the observer has passed beyond the edge of the TCA population.

<u>Findings</u>

From the fall of 2017 to the fall of 2018, the rate of expansion of the TCA population was highly variable. Measuring from edges located along the 2018 transects to the nearest point on the 2017 TCA population edge, the edge moved from 10 to 122 meters into uninvaded habitat in 2018 with a median expansion of 55 meters.

Previously measured TCA population expansion rates have ranged from 0 to 200 meters a year with the median expansion rate tending to be closer to 200 meters. These edges have typically been tracked in urban or grassland systems (Meyers 2008, LeBrun et al. 2013). The slower spread rate documented here likely reflects the

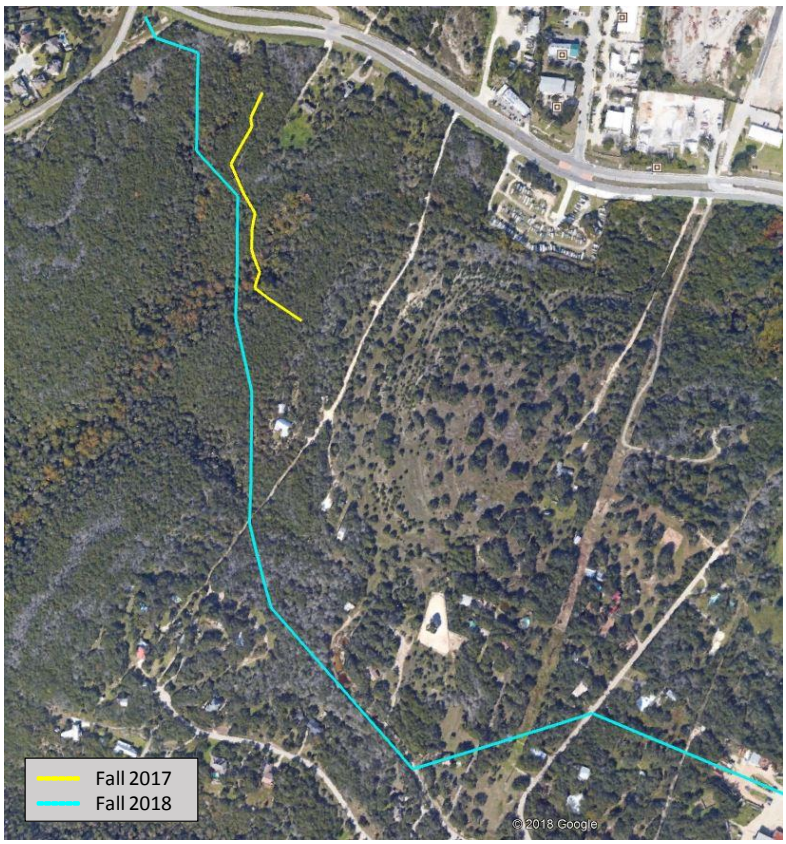


Figure 4: Edge of *Nylanderia fulva*-invaded area in Fall 2017 (yellow line) and Fall 2018 (blue line).

higher three-dimensional complexity of this closed canopy forest.

Task 3: Inoculative releases of the microsporidian pathogen *Myrmecomorba nylanderiae* at a site within a tawny crazy ant infested area of the preserve and tracking its establishment

Inoculation of field populations of TCA with *M. nylanderiae* is greatly facilitated by the unicolonial nature of TCA in Texas. Within Texas TCA from any population recognize TCA from any other population as colony mates and rapidly join an alien nest when introduced both in the lab and in the field (E. LeBrun In Prep).

The inoculative release took place in early August 01, 2018. To perform the inoculation, nests of infected ants were collected from a population in south Austin, Texas. Ants were separated from nest materials in the lab at the Brackenridge Field Laboratory. Five samples of 20 workers were taken and spore counted following our batch counting protocol to assess the spore load of the inoculum. Approximately 4800 infected workers and 0.25 cc of associated infected brood were split amongst 16 small containers. At the inoculation site, baits were placed in a 5x5 grid with 10 m separation between baits. The infected ants were released into the largest 16 recruitment trails that TCA formed to these baits (Fig 4). The introduced ants were observed to rapidly join the trail of recruiting ants.

Tracking acquisition of infection and spread from inoculation site

Three weeks following the inoculation we began sampling to assess whether the TCA population acquired the infection. Sampling to assess acquisition and spread was done approximately every 3 weeks from inoculation until January. In addition to sampling the bait stations where the infected ants were released, we also sampled three 300 to 500 m transects of baits at 50 m intervals radiating out from the inoculation site to attempt detect any non-local establishment induced by infected ants migrating through the local nest network. In late November through January, TCA had ceased to forage for the winter complicating sampling. On these dates sample number was restricted by the ability

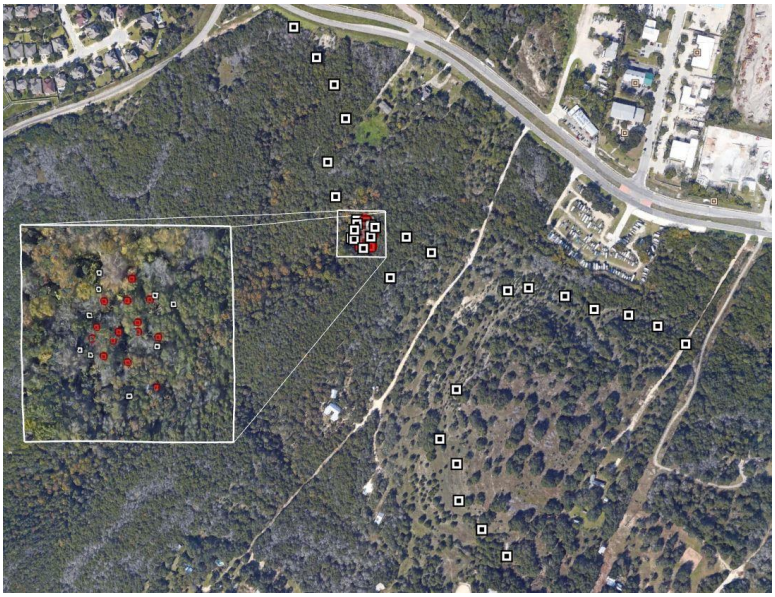


Figure 5: Google Earth map depicting inoculation points and subsequent sampling points for microsporidian testing. The 16 inoculation release points are represented by red circles and are located in a 1600 m^2 grid. Sampling stations are represented by white squares. Twenty-five sampling stations are located in the grid. Additional sampling stations extend outward every 50m along three transects. The transects to the east and the south run 500m, and the transect to the north runs 300m.

to find TCA nests near the target location.

Findings

Within the inoculation grid, we recovered *M. nylanderiae* positive samples on 3 different sampling intervals, at 4 stations. The positive detections from the inoculation grid were interspersed with sampling efforts in which no positive samples were recovered (Table 3). At this time, there is no evidence that infection is becoming more prevalent at the inoculation site.

Along the East and Southeast spread transects radiating out from the inoculation site, no positive detections of *M. nylanderiae* were found (Table 3). The Northwest spread transect was excluded from this analysis (See results from Background Infection Sampling below).

We will not be able to determine the ultimate success or failure of this initial inoculation effort until the ants become fully active in the spring.

Despite the despite how readily alien workers will join resident nests, across all attempts our lab at UT has made to date, only about 40% of our inoculation attempts at other sites have succeeded. The high inoculation failure rate may arise because adoptive ants are isolated from brood care duties and unable to transfer the infection to developing brood; worker to larva transfer is the only efficient means of passing the infection. Ants working in outside tasks at the periphery of the colony (eg. foraging or removal of dead workers) commonly do not interact directly with the brood or queen. Ultimately more research is required to determine why inoculations fail and how to best overcome this hurdle.

Table 3: Summary of all testing (inoculation grid):

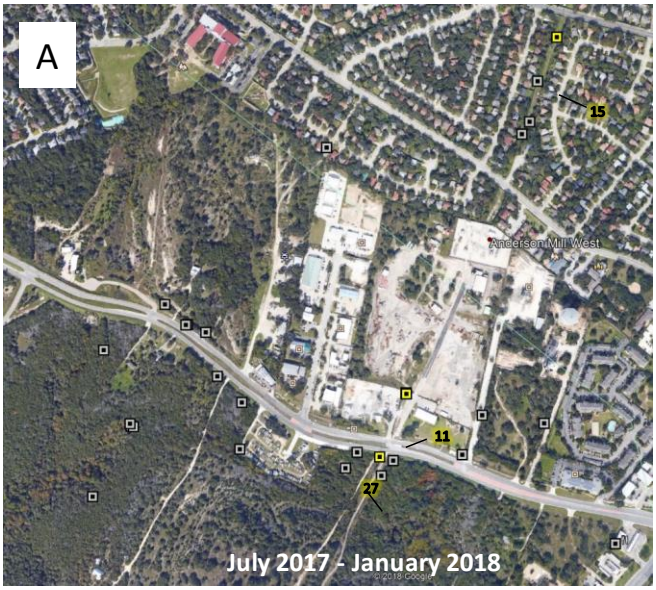
	Inoculation Grid			**Spread Transects**	
Collection Date	**Total Samples**	**Mn Positive Samples**	**Infection Intensity (Avg Spore Count)[1]**	**Total Samples**	**Mn Positive Samples**
8/23/2018	29	0	0	0	NA
9/11/2018	22	1	80	0	NA
9/25/2018	25	1	148	0	NA
10/30/2018	20	0	0	16	0
11/29/2018	5	0	0	16	0
12/12/2018	5	2	11.5	16	0
1/8/2019	9	0	0	16	0

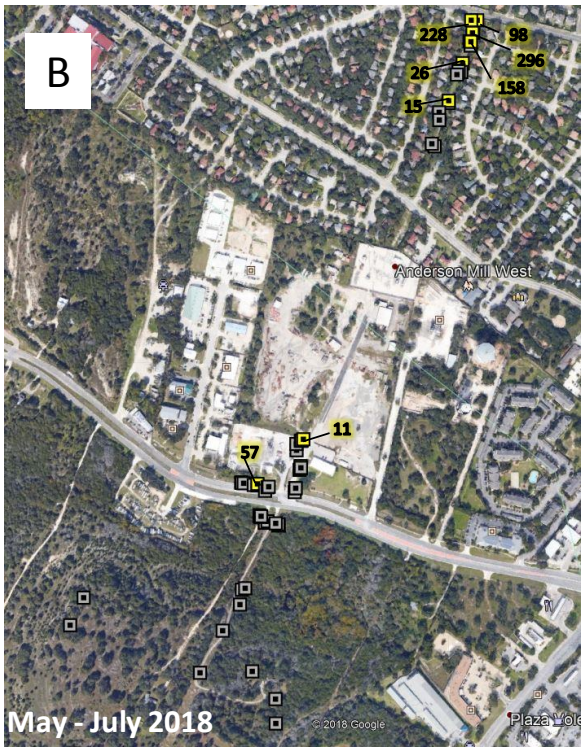
[1] The average spore count for all spore positive samples.

Supplemental Data Collection: Background infection mapping and testing

In January of 2018, prior to the initiation of this project, we detected and reported 2 areas with *M. nylanderiae* positive nests within the Anderson Mill x 2222 TCA population. We have continued to track these infection sites throughout. Both areas were outside the preserve boundary, so tracking these infections was not included as a deliverable in the original contract proposal. We are providing a report of the results of these tracking efforts below.

Areas of previously documented infection were visited every 2-3 weeks from May through January. Samples were collected and spore counted in the lab.





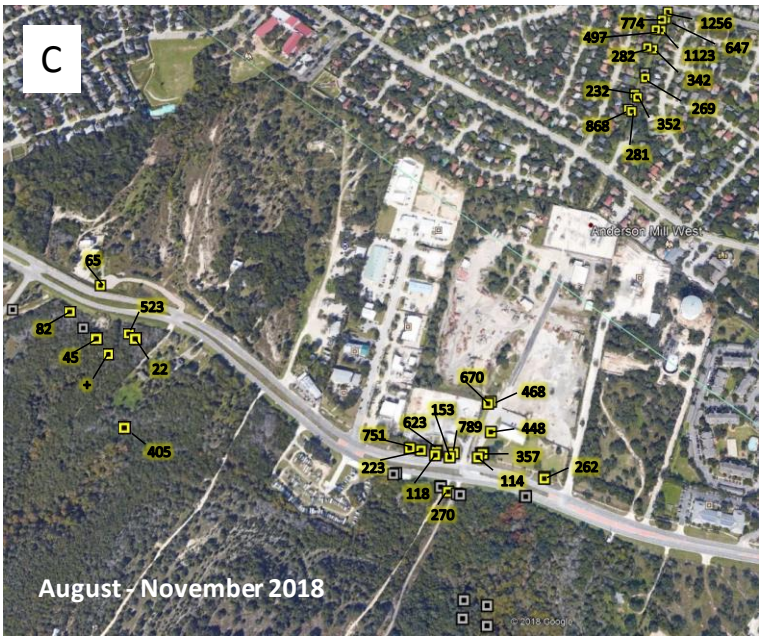
<u>Findings</u>

Both of the original sites of infection have spread in the intervening time. However, until November of 2018, no *M. nylanderiae* positive detections were made south of Anderson Mill road after the initial sampling in January (Fig 6 A-D).

In October of 2018 we found spore positive samples along our Northwest spread transect radiating from the inoculation site. The positives were closer to the Anderson Mill Rd edge of the preserve than they were to the inoculation site, which stimulated us to do further sampling in that area (Fig 6C).

Sampling through December and January revealed extensive detection of *M. nylanderiae* along both sides of Anderson Mill Road. Positive samples were also recovered on the northern edge of the private property that is across from the preserve on the north side of Anderson Mill road. During this interval, this infection also fused with the area of infection we had been monitoring on the north side of Anderson Mill Road (Fig 6D). In the fall and early winter sample intervals (October through January), both infection intensity (spores per sample) and the prevalence of infected nests increased dramatically (Fig 6 C&D).

M. nylanderiae infection has spread from the part of the TCA population occupying private property on the north side of Anderson Mill road into the TCA nests on the Northwest corner of the preserve. It is now highly prevalent in nests along the north edge of the preserve (Fig 6D).



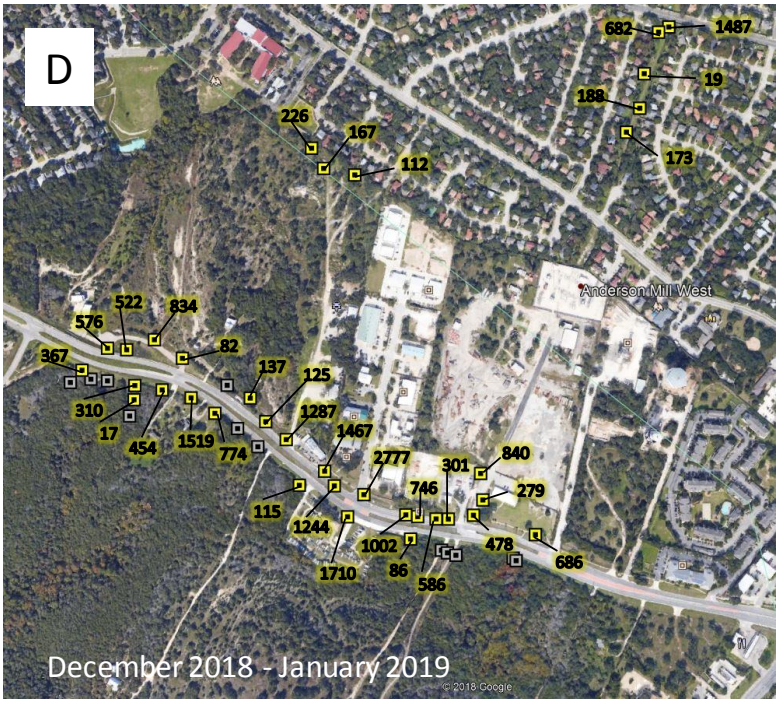


Figure 6: Maps of sampling of naturally occurring *M. nylanderiae* (Mn) infection off and on the preserve. Grey squares indicate an *Mn*-negative sample and yellow squares indicate an *Mn*-positive sample. Positive samples are labeled with their infection intensity (spore counts). (A) July 2017-January 2018 sampling period. All positive samples were discovered in January 2018 sampling. (B) May – July 2018. Infection dies out on the south side of Anderson Mill Rd but spreads in the other two known foci. (C) August – November 2018. First detection of infection within the preserve. Infection intensity increases dramatically in all areas. (D) December 2018 – January 2019. Infection become widespread on the north edge of the preserve. Infection intensities increase again throughout.

Acknowledgments:

Samantha Gallagher contributed to the data collection and the generation of this report. Alejandro Santillana contributed to the collection of the data herein. Todd Bayless and Travis Clark baseline data on TCA extent in the preserve, collected low density arboreal foraging counts, and facilitated the project in a variety of other ways.

Literature Cited:

Giese, J. 2015. White-wing dove nest, Estero Llano Grande State Park. YouTube. www.youtube.com/watch?v=JseAq01-OO4

Kumar, S., E. G. LeBrun, T. J. Stohlgren, J. A. Stabach, D. L. McDonald, D. H. Oi, and J. S. LaPolla. 2015. Evidence of niche shift and global invasion potential of the tawny crazy ant, *Nylanderia fulva*. Ecology and Evolution **5**:4628-4641.

LeBrun, E. G., J. Abbott, and L. E. Gilbert. 2013. Imported crazy ant displaces imported fire ant, reduces and homogenizes grassland ant and arthropod assemblages. Biological Invasions **15**:2429-2442.

LeBrun, E. G., K. Ottens, and L. E. Gilbert. 2017. Intracolony transmission of the microsporidian pathogen (Myrmecomorba nylanderiae) and its impact upon the growth of tawny crazy ant (Nylanderia fulva) colonies. Journal of Applied Entomology **142**:114-124.

LeBrun, E.G. In prep. The organization of tawny crazy ant (Nylanderia fulva) societies in their native and introduced range: nature of intercolonial aggression.

Meyers, J. M. 2008. Identification, distribution and control of an invasive pest ant, *Paratrechina* sp. (Hymenoptera: Formicidae), in Texas. Doctor of Philosophy. Texas A&M University, College Station, Texas.

Plowes, R. M., J. J. Becnel, E. G. LeBrun, D. H. Oi, S. M. Valles, N. T. Jones, and L. E. Gilbert. 2015. *Myrmecomorba nylanderiae* gen. et sp. nov., a microsporidian parasite of the Tawny Crazy Ant *Nylanderia fulva*. Journal of Invertebrate Pathology **129**:45-56.