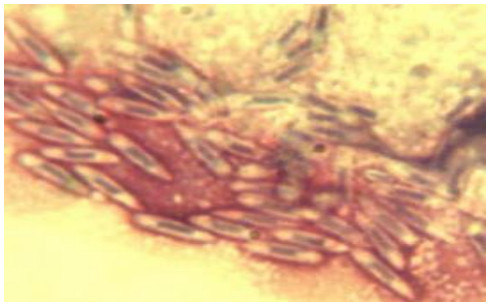




Tawny Crazy Ant (*Nylanderia fulva*) Study
Balcones Canyonlands Preserve – Jollyville Unit
Task 5: Data analysis and Final Report

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Executive Summary

Nylanderia fulva (tawny crazy ants) are an invasive ant species introduced from central South America 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* (Mn), discovered in 2015 presents a potential avenue for sustainable control of these ants. Herein we report the results of work on an effort to evaluate the impact of TCA population currently invading tracts of the Balcones Canyonlands Preserve, and to explore using this microsporidian as a control agent.

In 2021, we continued to document the decline of the TCA population on the Collins Tract of the BCP following the establishment of *M. nylanderiae.* The ants are now almost eliminated from this preserve site, and the disease is prevalent in all remaining adjoining areas where the ants persist. The major accomplishment in 2021, was the successful development and implementation of an efficient means of inoculating uninfected TCA populations with this microsporidian pathogen. The approach hinges upon fusing infected and uninfected nests in the laboratory employing strict odor controls to prevent the ants from acquiring lab derived odor profiles. Earlier tests demonstrated the emergence of aggression if TCA were held in the lab. The success of this technique appears contingent upon introducing the infected ants in late spring (April – June) into high-density TCA populations. Data on arboreal foraging by TCA, changes in the ant assemblage following TCA elimination, and monitoring of TCA at other sites are also presented.

Project Background

Nylanderia fulva (Tawny Crazy Ants), an invasive ant species introduced from central South America, is spreading throughout the Gulf Coast region of the United States [1]. These ants invade natural habitats and have devastating effects on the arthropod assemblages of those environments [2]. 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 [3]. 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* [4]. 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 [5], 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 on the results from the Collins Tract, Jollyville Unit, Balcones Canyonlands Preserve (BCP), Pace Bend Park, a Travis County Park, and other Parklands infested with TCA. Work at sites other than those on the BCP is

conducted with goal of improving our ability to control infestations within the preserve, and prevent the ants from colonizing preserve land from other infested public lands. The objectives for 2021 were to:

1) Assess tawny crazy ant (TCA) densities, distribution, and native-ant assemblage composition within the BCP
2) Quantifying spread of the pathogen *M. nylanderiae* within the Collins Tract TCA population and tracing the seasonal variation in infection intensity
3) Assess the trajectory of recovery in the native ant assemblage in previously invaded areas
4) Canopy arthropod prey availability and ant arboreal foraging study
5) Inoculative releases of the microsporidian pathogen *Myrmecomorba nylanderiae*
6) Monitoring TCA populations at other sites that may become sources of infestations on preserve lands

Objective 1: Assessment of tawny crazy ant (TCA) densities and native-ant assemblage composition within the Collins tract of the BCP

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 established in 2018 in the invaded and uninvaded areas of the preserve were run in spring, and fall of 2021. 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, and 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.

For assessing TCA density changes, three habitat areas defined by TCA occupancy were sampled: Long-term Invaded, Invaded Edge 2017, and Uninvaded. Long-term Invaded areas were in the interior of the TCA populations and have harbored TCA for several years prior to the beginning of research in fall 2017. Invaded Edge 2017 habitat were invaded areas near the edge of fall 2017 TCA distribution in the preserve. Uninvaded areas are of similar vegetation that are outside of the TCA population and never invaded. To assess the response of native ants to the decline of TCA, areas were proscribed based on their invasion history and present invasion status. Currently Invaded habitat contained TCA in the Fall of 2020. Previously Invaded habitat consisted of areas with TCA prior to 2021 but where they are now absent. Uninvaded habitat consists of areas without TCA at present or in the past.

Findings

TCA densities continued their long-term decline within the preserve. In the spring of 2021, TCA were found at only 25% of the monitoring sites where they were previously present. At these stations they averaged only 4.6 workers per pitfall trap compared to approximately 1200 workers per trap at the peak of the invasion. By the fall, TCA were not collected at any of the long-term abundance monitoring sites (Fig. 1). Tawny crazy ants have essentially been eliminated from the area long-term monitoring.

We have continued our efforts to track the current areas of the preserve and adjoining lands that remain occupied by TCA. By the spring of 2021 TCA were essentially absent from the preserve

lands. Although still prevalent in the adjacent City owned property adjoining Anderson Mill Road (Old Water Treatment Plant 4 site). By our fall survey, the TCA population in the City owned land was restricted to isolated nests in scattered locations. In the residential neighborhood to the south of the Old Water Treatment Plant 4 site, there remains an area where TCA currently occur at high enough densities to form an interconnected population. This area lies just to north of additional BCP preserve land. So far, this area of the preserve remains uninvaded (Fig. 2 and 3). The extant TCA in all areas of the preserve are infected with the microsporidian pathogen *M. nylanderiae* (see section below). As a result, we anticipate observing continued decline in 2022.

In the area of TCA monitoring, capture of native ants, ants other than TCA and imported fire ants, shows strong interannual variation. Areas with different invasion histories also differ in the dominant habitat complicating interpretation of change through time. It appears that over time both the diversity and abundance of native ants has increased in the long-term invaded, now TCA free habitat, relative to uninvaded habitat (Fig. 4).

Imported fire ants show a clear response to the elimination of TCA. There has been a rapid increase in imported fire ant densities in the long-term invaded (grassland) area of the preserve in response to the removal of TCA (Fig. 5). In contrast, imported fire ants have not increased in the short-term invaded, juniper woodland dominated habitat. The ant assemblage composition for these areas is presented in Table 1.

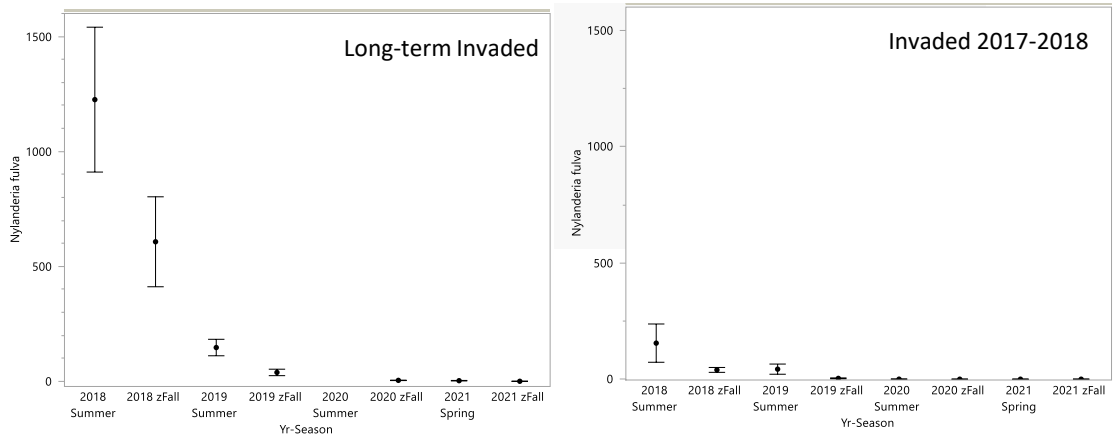


Figure 1: Abundance of N. fulva in areas of the site where the ants have been established since the beginning of the study. Trapping data is divided into traps in Long-term Invaded habitat, colonized prior to the initiation of the study, and Invaded 2017 habitat, colonized the year before formal sampling began.

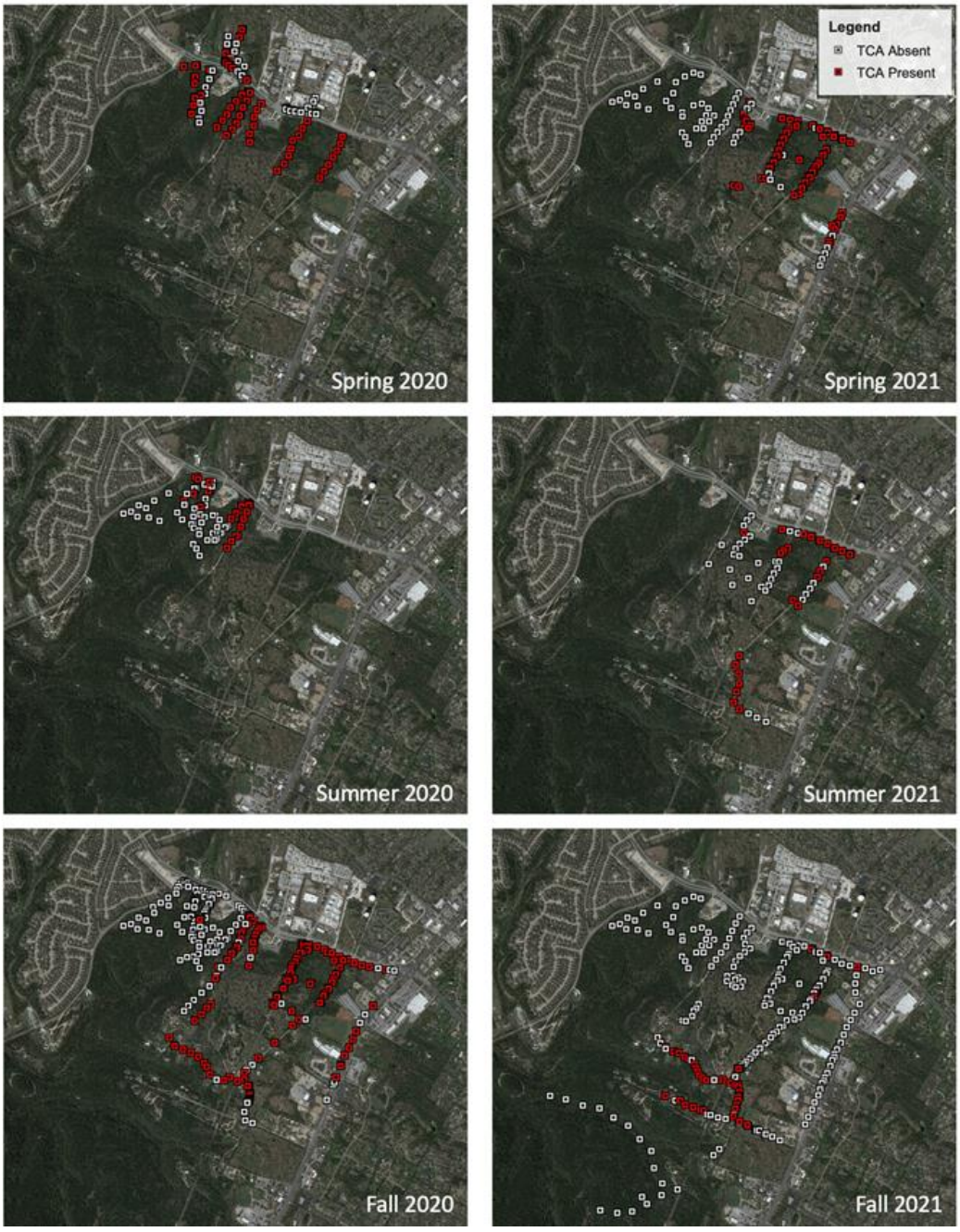


Figure 2: Intra-annual change in TCA distribution within the area of the Collins tract of the BCP. Left panels provide the same information from 2020 to provide a longer-term perspective. Right panels show 2021

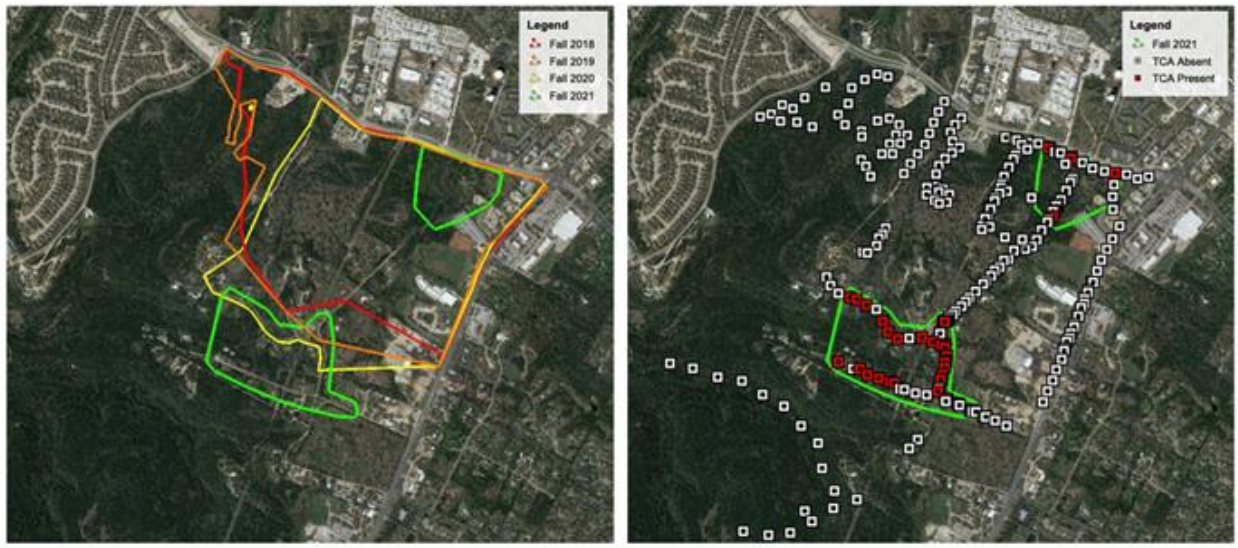


Figure 3: Limits of the TCA infestation in and around the Collins tract of the BCP and the Old Water Treatment Plant. Left: Edges of *N. fulva* population falls of 2018, 2019, ad2020. Right: Areas known to contain TCA in Fall 2021. Squares show the presence (red) and absence (white) of TCA at sampling points from November 2021.

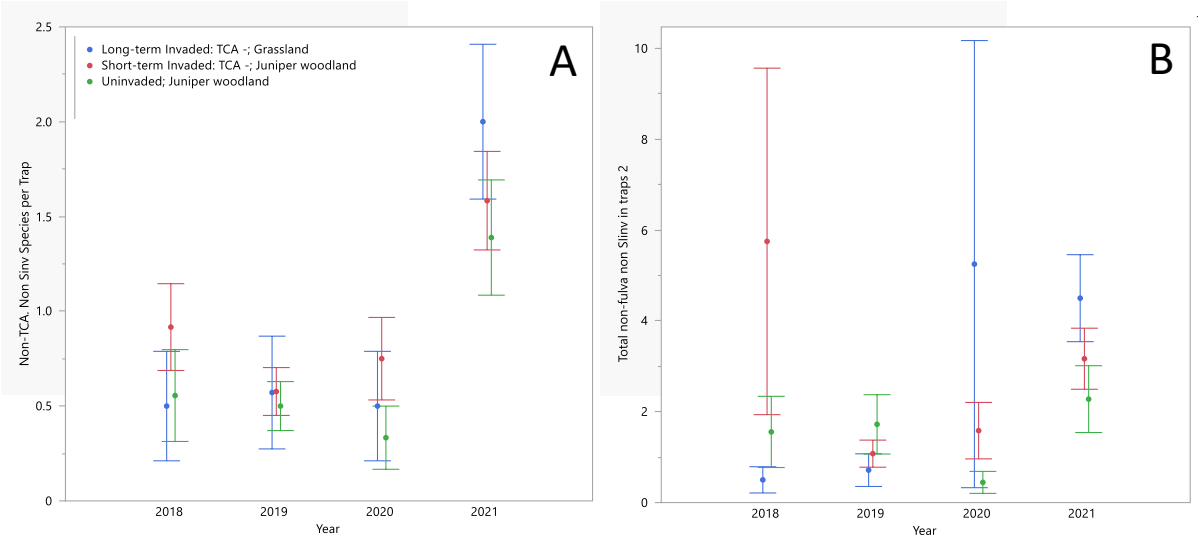


Figure 4: Native ant change through time. These data are from fall trapping intervals where we have the most consistent record. All habitats now lack TCA. (A) Average number of ant species present in traps excluding TCA and imported fire ants. (B) Total number of ant workers in traps from species other than TCA and imported fire ants. Native ants are becoming relatively more abundant in previously invaded habitats.

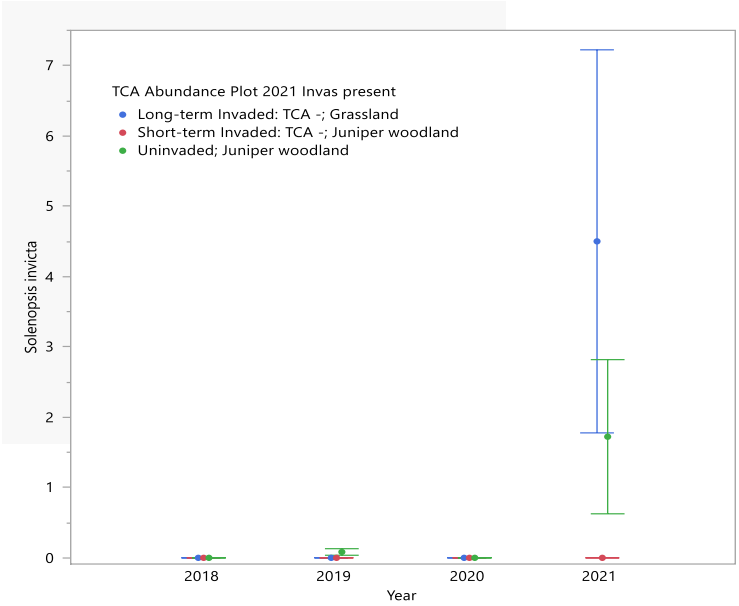


Figure 5: Change in imported fire ant abundance over time. Imported fire ants are increasing in previously invaded grassland habitats, but remain suppressed in previously invaded juniper woodlands.

Table 1: Ant assemblage composition 2021. Results from pitfall traps across three habitats sampled in summer and fall. The cells report the percent of traps that contained a given species.

Ant Species	Never Invaded		Previously Invaded		Currently Invaded	
	Spring (14)	**Fall (14)**	**Spring (30)[1]**	**Fall (20)**	**Spring (5)**	**Fall (5)[2]**
Aphaenogaster texana		7	3	15		
Brachymyrmex depilis			3			
Brachymyrmex patagonicus					20	
Camponotus sansabeanus				5		
Crematogaster laeviscula	14	7	3	5		
Forelius pruinosus		14	3			
Leptogenys elongata				5		
Monomorium minimum	43	21	67	55	100	100
Myrmecina americana			17	15		20
Nylanderia fulva (TCA)					100	
Pheidole constipata	14	7	7	5		20
Pheidole dentata	7					
Pheidole floridana				5		
Pheidole hyatti	21	14	3	5		
Pheidole pelor	7			5		
Pheidole tetra		7				
Solenopsis diplorhoptrum sp.	14	36	20	50		40
Solenopsis invicta	21	21		10		40
Temnothorax terrigena			3	10		20

[1] Supplemental pitfalls were run only during Spring 2021.

[2] Currently invaded is defined by TCA invasion in Spring 2021; no TCA were found in traps during Fall 2021.

Objective 2: Quantifying spread of the pathogen *M. nylanderiae* within the Collins Tract TCA population and tracing the seasonal variation in infection intensity

In 2021, *M. nylanderiae* became established throughout the remaining TCA population (Fig. 6). There is no further benefit to tracking the spread of this disease at this site. Seasonally, the intensity of infection did not follow the pattern of previous years with lower infection intensities in spring and higher intensities observed in fall. Instead, the highest infection intensities were recorded in the spring followed by the collapse of those areas of the TCA population with lower infection intensities recorded in the fall. The decline of the TCA population did not track 1 to 1 with the observed infection intensities. Large areas of the TCA population in the center of the Old Water Treatment plant site tested as uninfected in the spring but the ant populations in these areas had disappeared by summer in concert with the parts of the population exhibiting high infection intensities. We do not know why this occurred. We do know that our ability to detect infection even when it is present is limited (see Inoculation Results section). At this point we attribute the declines in areas that tested negative to a high detection of disease threshold in our assays.



Figure 6: Maps tracking the spread of *M. nylanderiae* (Mn) infection and intensity of infection in the preserve. All points represent sites where TCA were collected and tested for infection. Samples that were positive for Mn infection are labeled with their infection intensity (spore counts). (A) Spring (February – May 2021). (B) Summer (June – August 2021). (C) Fall (September– November 2021).

Objective 3: Native Ant Assemblage Recovery

As TCA decline at sites due to infection by *M. nylanderiae*, the native ant assemblage can rebound. It is important to document this process to see if the assemblage succession is on a trajectory to return to the pre-invasion state. This recovery will happen in the context of an environment that has been saturated with spores of *M. nylanderiae* left behind by the dying TCA population. To date, all data we have gathered indicates that this disease is specific to TCA. Following the recovery of native ant assemblages provides an additional opportunity to evaluate this hypothesis.

Approach: A combination of pitfall trapping, and malaise trapping of alate ants are being used to assess native ant community recovery. Invaded or previously invaded sites include: Whirlpool Cave Site, the Collins tract of the BCP, and Emma Long Metropolitan Park. Uninvaded contrast sites include: Dick Nichols park, the New Life Tract of the BCP, and the Blackmore tract of the BCP.

Findings

This is a longer-term project, and we will not have a comprehensive analysis of it until we have more data. There are some early results worth highlighting. Imported fire ants are rapidly recolonizing sites once the tawny crazy ant populations declines. This is not surprising as imported fire ants have 'weedy' biology, colonizing disturbances rapidly and growing quickly. Interestingly, there appears to be a shift in the social form composition of populations recolonizing the sites after TCA are eliminated. Fire ant populations post TCA appear to be dominated by monogyne (single queen) colonies. At the Whirlpool Cave site, TCA were eliminated by *M. nylanderiae* in 2019 allowing 3 years for non-TCA ants to recolonize the site. The imported fire ant population that has recolonized the site is 93% monogyne colonies. This compares to a nearby site that was not invaded, Dick Nichols Park, where the intact fire ant population is comprised of only 44% monogyne colonies (Fig 7). We will evaluate whether this is a widespread property of recolonizing fire ant populations in the coming year.

The return of imported fire ants is not desirable. Like TCA, they have known negative ecosystem impacts, including harm to endangered species and species of concern. However, a shift in social form from a largely polygyne (multi-queen colonies) to a largely monogyne system is a net benefit. Monogyne fire ant populations are of lower density than polygyne populations and are thought to be less environmentally harmful as a result.

In addition to monitoring the ants assemblage composition, we are testing the native ants we encounter in previously invaded habitat for infection by *M. nylanderiae*. We will continue to monitor this issue and look for potential non-target impacts of the pathogen.

Objective 4: Canopy arthropod prey availability and ant arboreal foraging study

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 and ant foraging density assessments during the nesting season. Arthropod sampling was accomplished by

standardized beating of 2 low-hanging branches per tree sampled, and foraging density measurements for TCA on trees in TCA invaded habitats. Up to four tree species were sampled depending upon their availability in the target areas.

Foraging density measurements for TCA on trees in TCA invaded habitats that were sampled for arboreal arthropods. 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.

Three habitats were sampled for the arboreal arthropod surveys: In - Invaded, Edge – Lightly impacted, and Out - Uninvaded. Habitat categories were defined in the Fall of 2017 and invasion status refers to their status at that time point. In – Invaded areas were in the interior of the TCA populations and harbored TCA for several years prior to 2017. Edge - Uninvaded habitat were in areas near but outside the Fall 2017 edge of the TCA distribution in the preserve. Out – Uninvaded areas are of similar vegetation type to Edge and were well outside of the TCA population in 2017. The design of this study anticipated an expansion of TCA into Edge habitat with concurrent tracking of changes in arboreal arthropod and ant foraging. In fact, TCA populations at the site collapsed prior to expansion into Edge habitats. All relevant comparisons are thus of changes in the In - Invaded habitat relative to the others. Analyses below are restricted to tree species present in all three habitat types (Ashe Juniper and Escarpment Live Oak).

This work is being led and conducted by Travis County Staff. UT is providing support by identifying and counting ants, as well as processing spider and lepidopteran larvae for biomass measurements. Arthropod samples are still being processed. Herein we report on changes in ant foraging.

Findings:

Matching the declines observed throughout the preserve, TCA have declined precipitously in the arboreal survey with only a single worker being recorded in foraging arboreally in 2021. Total native ants have responded with native ant foraging increasing in the In – Invaded habitat but remaining constant or declining in the others (Fig. 7). *Monomorium minimum,* a species resistant to displacement by TCA was responsible for the majority of arboreal foraging occurring in the In – Invaded habitat (Table 2). Red imported fire ants, a species that commonly forages arboreally, remains absent from the trees in all habitats.

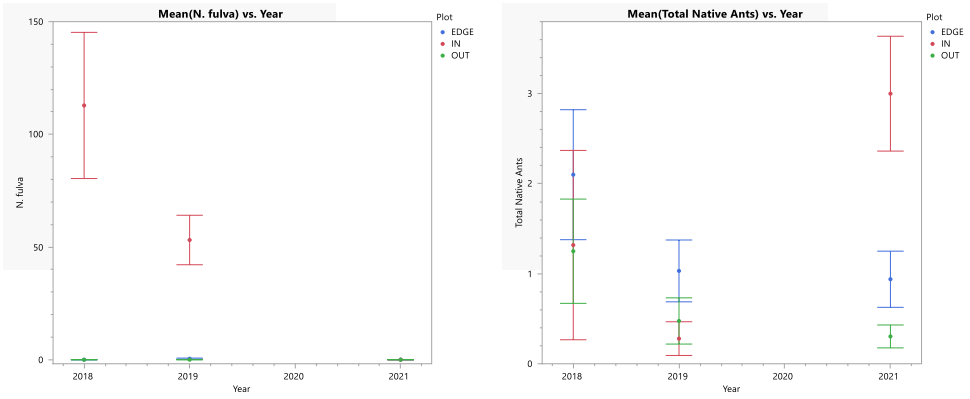


Figure 7: Arboreal foraging by ants in three habitats with differing invasion histories.

Table 2: Species composition and number of ants observed foraging in canopy. Counts are totals across all sampling intervals of the number of ants found foraging in the canopy. Insufficient shin oaks or Texas oaks were available for sampling in the In - Invaded are

Ant Species	Plot									
	EDGE				**INVADED**		**UNINVADED**			
	Sample Tree				Sample Tree		Sample Tree			
	AJ	**LO**	**SH**	**TX**	**AJ**	**LO**	**AJ**	**LO**	**SH**	**TX**
Aphaenogaster texana								1		
Brachymyrmex patagonicus					2	3				
Camponotus decipiens			1	2						
Camponotus discolor								1		
Crematogaster isolata							1			
Crematogaster laeviuscula	4			1			2	2	2	
Forelius pruinosis					13	6				
Monomorium minimum	20	8			92	91		3		
Nylanderia fulva (TCA)						1				
Pheidole sp.			5							
Pheidole dentata				3						
Pheidole hyatti	14		1				6	1	3	6
Temnothorax subditivus										1

Objective 5: Inoculative releases of the microsporidian pathogen *Myrmecomorba nylanderiae*

In 2021 we continued our efforts to identify and reliable, relatively low effort, means of introducing the microsporidian pathogen *M. nylanderiae* (Mn) into uninfected populations of TCA. Achieving this goal will enhance our ability to protect BCP preserve land that becomes infested. In addition, establishing the disease in infestations in the region of the BCP, eliminates the most likely

sources of uninfected TCA propagules that might colonize BCP lands. In August of 2020, we conducted a first round of testing a 'Laboratory Fused Nest' approach to inoculation at Pace Bend Park. In 2021, as well as following the outcome of releases made in 2020, we conducted inoculative releases at 3 sites in 2021: Pace Bend, Roy Guerrero, and Walnut Creek Parks. Pace Bend inoculations were a continuation of the 'Laboratory Nest Fusion' approach while the other parks were a test of inoculum caste using foraging trails.

Approach:

Lab Fused Nest Boxes

In the Lab Laboratory Fused Nest approach, ants were collected from an infected source site (Blue Hole Texas) rapidly extracted from their nest material, to quantify amounts, and then added to nests collected from the inoculation site (Pace Bend) that were not extracted from their nest material. Ants were handed in this manner to eliminate the acquisition of lab-based odor cues that trigger aggression between introduced and resident ants, an effect first observed in 2019 inoculations. The fused colony fragments were then returned to the nesting site form which the inoculation site nests were collected. In addition, we introduced a separate feeding treatment. Half of all inoculums received supplemental feeding every 2 weeks of 30 ml of mixed crickets and tuna fish in water and 250 ml of 50% honey water solution. In 2021, limits on the amount of infested habitat area with the park distant from prior inoculation attempts restricted us to conducting 5 additional inoculation treatments.

Caste specific foraging trail inoculations

Foraging trail introductions were attempted in August of 2021. We initiated this test to evaluate whether there was evidence for caste specific differences in transmission success and whether whole nest introductions were necessary or if introductions onto foraging trails suffice. At sites spaced at least 70 m apart 1 of 3 treatments was conducted. Either 150 infected workers or 150 uninfected workers plus ½ cc of infected brood were introduced onto a previously established foraging trail. Or a nestbox containing 150 infected workers and ½ cc of infected brood was placed near an active foraging trail and left in place.

There was insufficient habitat with high density TCA populations at Roy Guerrero Park to fully replicate the experiment. The remainder of the planned inoculations were shifted to Walnut Creek Park. The Northeast edge of the Walnut Creek Park population has a naturally acquired *M. nylanderiae* infection discovered in the fall of 2020. To avoid false positives, inoculations were conducted in the southwest section of the park. At the time of inoculation stations were at least 220 yards and on the far side of the creek from potential infected source ants. At both Walnut Creek and Roy Guerro, some inoculation stations near the edge of the population where TCA were scarce had to be abandoned due to insufficient ants. Six inoculation stations were implemented and continuously sampled at Roy Guerrero Park and 12 at Walnut Creek.

Findings:

Lab Fused Nest Boxes

In December of 2020, two out of the seven 2020 Lab Fused Nest inoculation stations had tested positive for *M. nylanderiae* (Mn) infection. By December of 2021, the disease was established and spreading out from a single 2020 Lab Fused inoculation station. The 2021 Lab Fused Nest inoculations

were conducted in April of 2021. The first of these 2021 inoculation site went positive in July and others followed in October. By December 2021, 3 out of 5 inoculation sites were persistently positive and showed signs of disease spread away from the inoculation station (Fig. 7). Ultimately, 25% of the inoculations established. However, the disease did not transmit (Survival time analysis: Wilcoxon 0.97, $N = 12$, $P = 0.77$) or establish (Wilcoxon 0.93, $N = 12$, $P = 0.77$) more quickly at fed inoculations sites than at unfed stations.

Caste specific foraging trail inoculations

As of December 2021, zero of the six of the inoculation sites at Roy Guerrero Park had shown any evidence of infection. At Walnut Creek in November and December of 2021, 4 of 12 inoculation stations tested positive for Mn infection (Fig. 8). All stations that tested positive were infected worker introduction stations. However, 3 out of 4 of the positive stations were among the closest to areas of previously known Mn infections. The absence of infection at Roy Guerrero and relative proximity of the positive stations to previously documented Mn infections suggests that these positives may not have arisen from local inoculation.

Summary

The Laboratory Nest Fusion approach provides an effective way to establish the pathogen *M. nylanderiae* in uninfected TCA populations having succeeded in 25% of the inoculation stations. Feeding the ants post inoculation provides no additional benefit reducing the effort required to inoculate in this manner. The least labor-intensive strategy, releasing infected workers onto foraging trails, may suffice to transmit the disease if care is taken to eliminate the acquisition of lab derived odor cues.

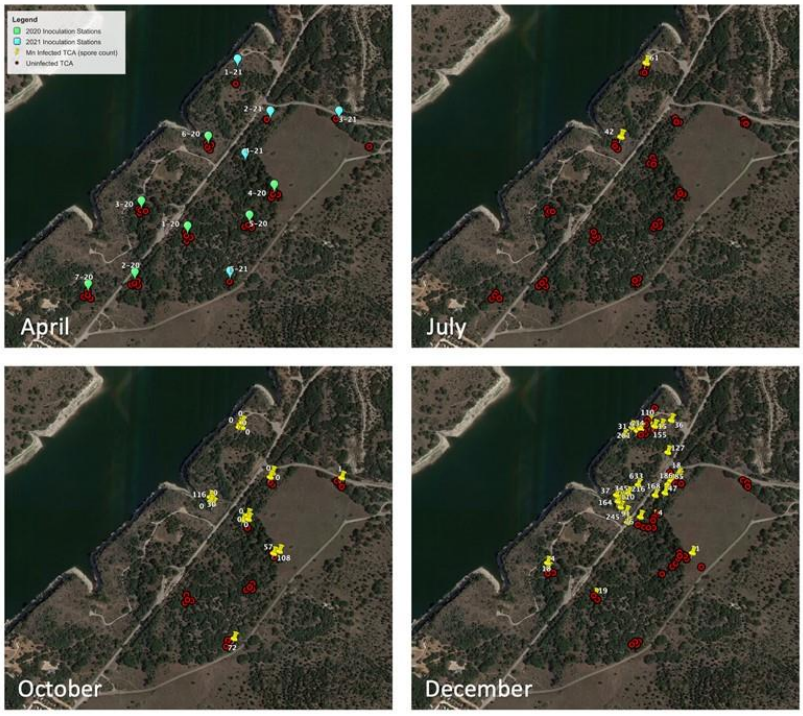


Figure 8: Maps tracking *M. nylanderiae* (Mn) infection at Lab Fused Inoculation stations in Pace Bend Park from April-December 2021. Green pins indicate inoculations started in August 2020 and blue pins

indicate inoculations started in April 2021. Red circles indicate an Mn- negative sample and yellow pins indicate an Mn-positive sample. Positive samples are labeled with their infection intensity (spore counts). Zero spore counts indicate samples that were positive based upon molecular testing but no spores were evident. Typically, this represents very low levels of infection as PCR is the more sensitive assay.

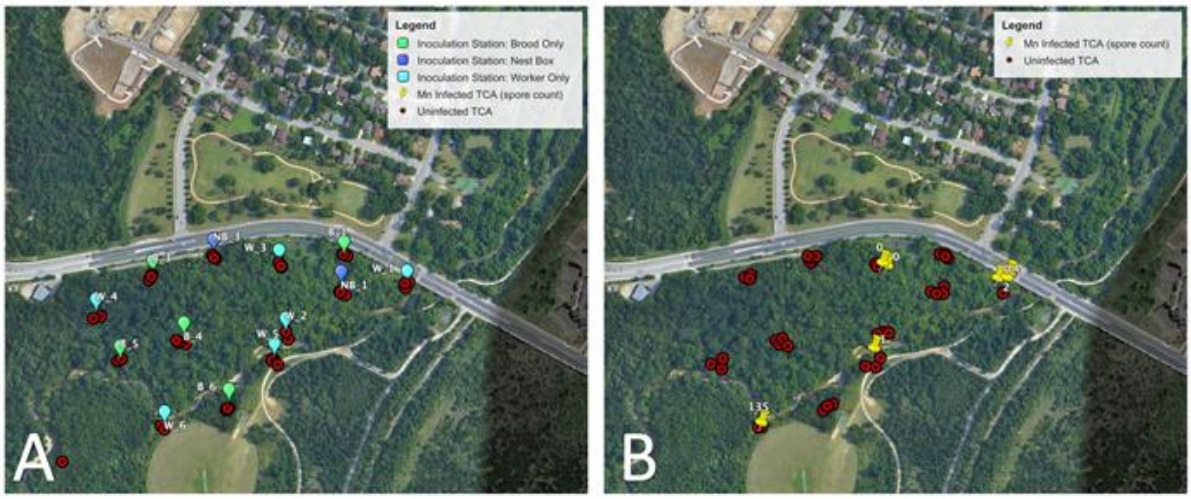


Figure 9: Map of inoculation stations at Walnut Creek from A) August 2021 and B) November 2021. Inoculation stations were split between brood-only, worker-only, and nest box treatments. Red circles indicate an Mn- negative sample and yellow pins indicate an Mn-positive sample. Positive samples are labeled with their infection intensity (spore counts).

Objective 6: Monitoring TCA populations at other sites that may become sources of infestations on preserve lands

Approach:

The abundance of TCA at selected sites invaded by TCA is tracked annually using pitfall traps. Pitfall traps are the same as described previously and run for 24 hours in the fall. In addition, the limits of the infestations are tracked and the infection ntensity of sites

Findings:

Changes in abundance of TCA at other sites was highly variable in 2021. TCA abundances as measured by pitfall traps increased at Emma Long Park and Pace Bend Parks. However, TCA abundances decreased at both Walnut Creek and Roy Guerrero Parks. It is unclear what drives this difference. However, both parks where TCA increased were located in the Edwards Plateau ecoregion (the ecoregion of the BCP), and both parks where they decreased were in the Blackland Prairie ecoregion. In the Edwards Plateau, the increase in TCA abundance at Emma Long Park (mean increased by a factor of 3.8) where *M. nylanderiae* infection is highly prevalent was considerably less than that observed in Pace Bend Park (mean increased by a factor of 8.7) (Fig. 9). We successfully established M. nylanderiae at Pace Bend Park this past fall, but the disease is not yet prevalent in the area of the pitfall traps.

In a new development, the Whirlpool Cave Greenbelt area has areas where TCA appear to be resurging. This TCA infestation at this site was inoculated with *M. nylanderiae* in 2017 and TCA abundance had declined below the level of detection by 2019. Areas where TCA are currently being found are all distant from the long-standing pitfall trap

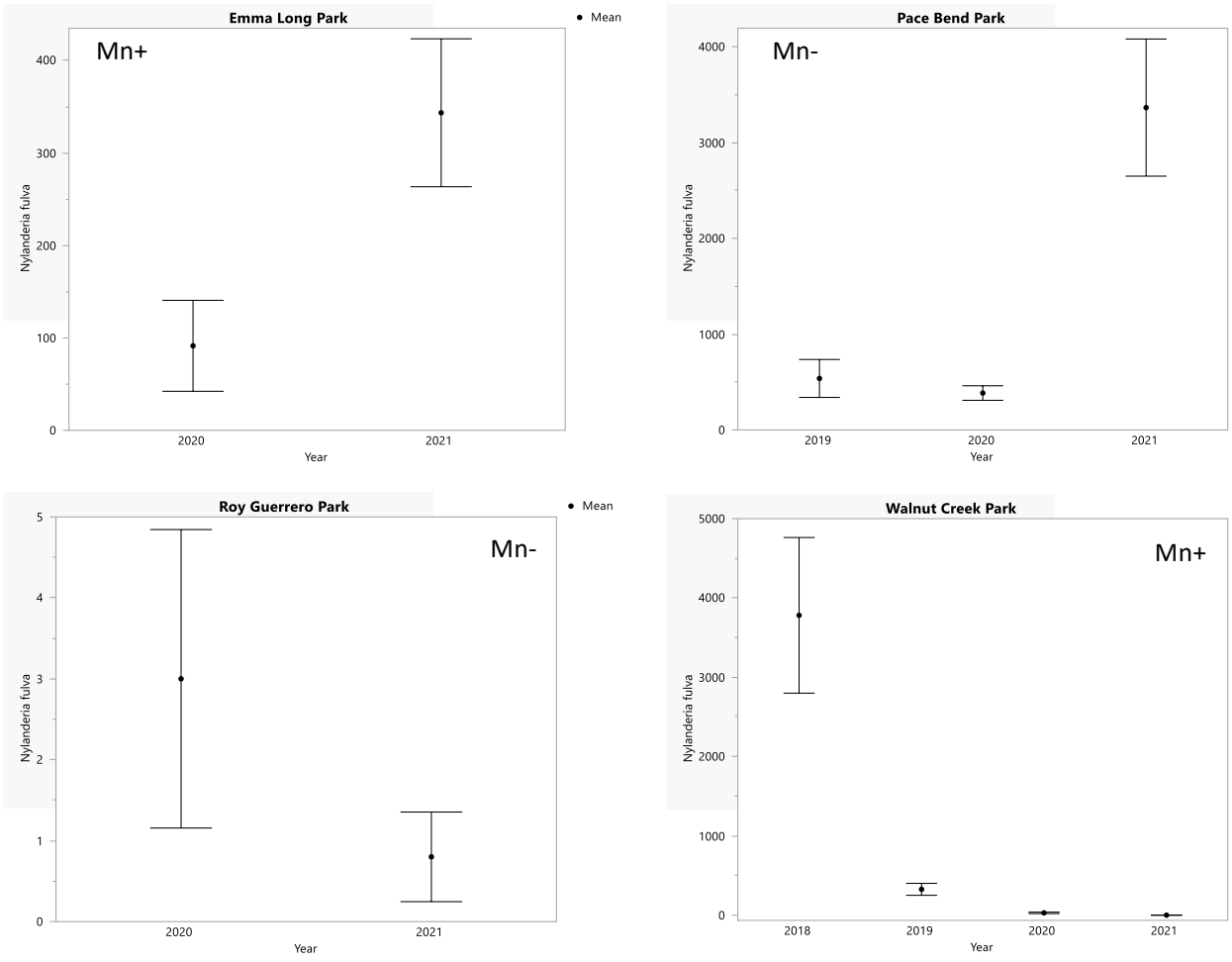


Figure 10: Interannual changes in TCA abundance at selected invaded sites. Abundance is measured with pitfall traps run for 24 hours in the fall. Top panels are sites in the Edwards Plateau ecoregion. Bottom panels are in the Blackland Prairie ecoregion. Mn+: There is a high prevalence of *M. nylanderiae* infection in the area of the pitfall traps. Mn-: There is no evidence of *M. nylanderiae* infection in the area of the pitfall traps. Bars present standard errors.

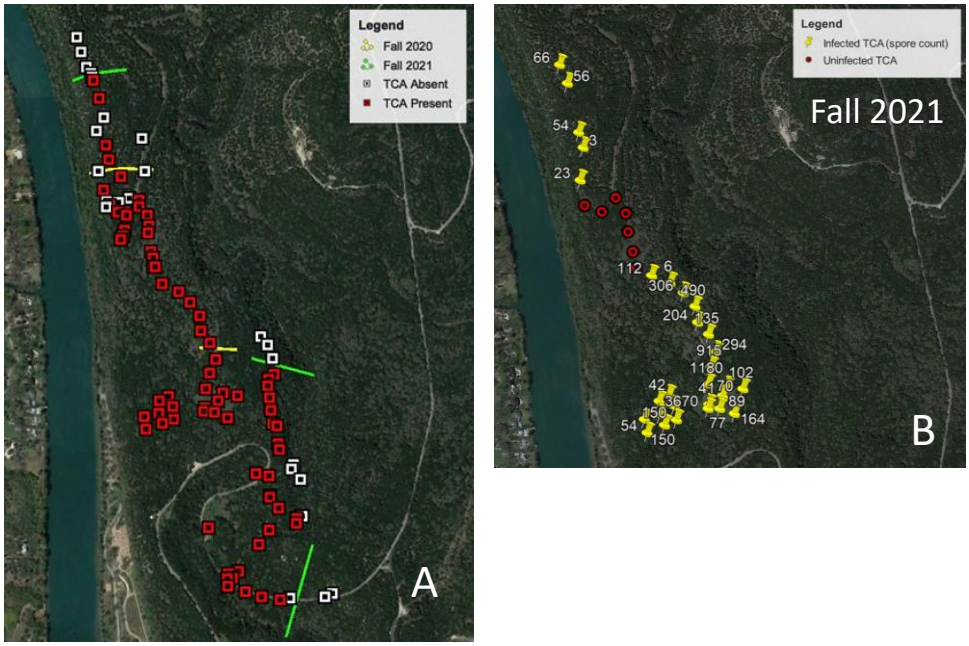


Figure 11: Distribution of TCA population at Emma Long Park and Distribution of *M. nylanderiae* infection from Fall 2021 (August-December 2021). (A) TCA distribution. Red squares indicate TCA present. White Squares indicate TCA absent. Lines indicate population edges in 2020 and 2021. (B) *M. nylanderiae* distribution. Red circles indicate TCA present but the tested negative for *M. nylanderiae* and yellow pins indicate TCA present and positive for *M.* nylanderiae. Positive samples are labeled with their infection intensity (spore counts).

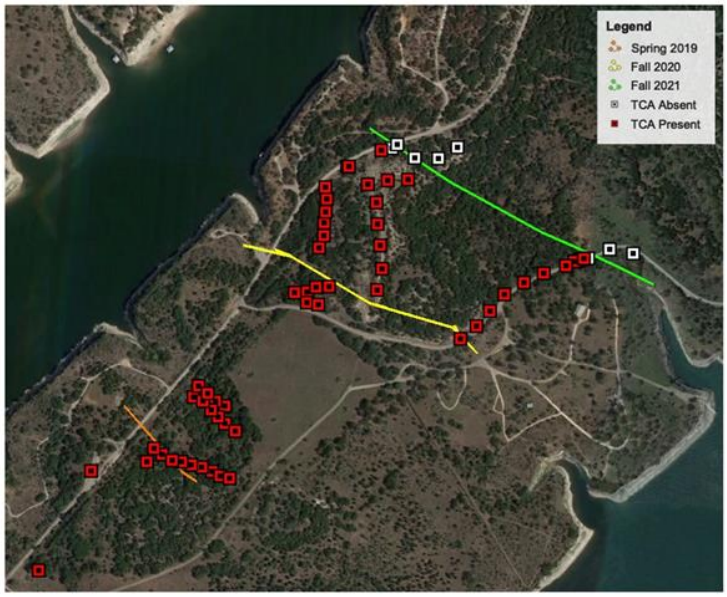


Figure 12: Progression of edges of TCA-invaded area from Spring 2019-Fall 2021. Squares show the presence (red) and absence (white) of TCA at sampling points from Fall 2021.

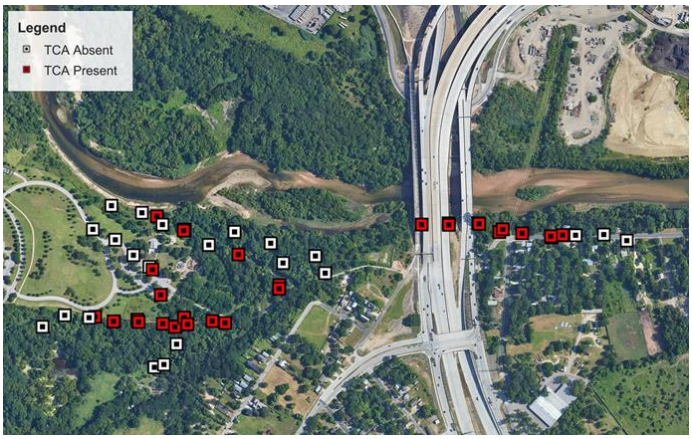


Figure 13: Distribution of TCA population at Roy Guerrero Park in Fall 2021 (August-November). White squares are areas sampled where TCA were absent. Red squares are sites with TCA.

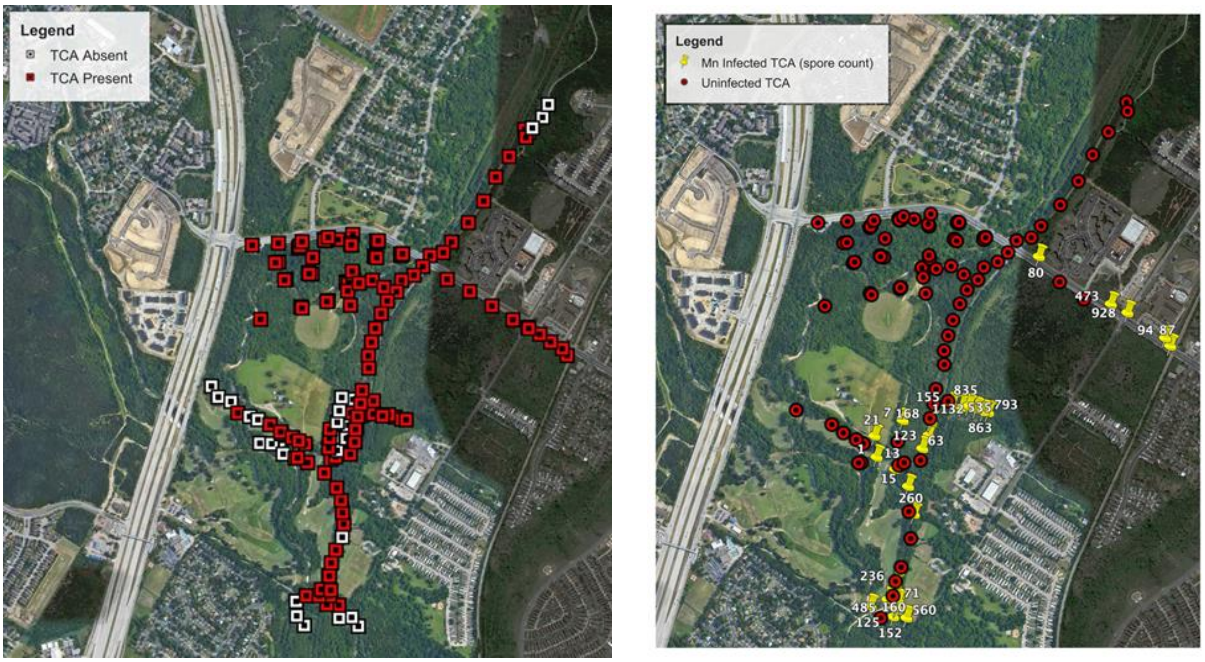


Figure 14: Distribution of TCA population and intensity of infection with Mn at Walnut Creek in 2021 (March-November). Left Panel: White squares are areas sampled where TCA were absent. Red squares are sites with TCA. Right Panel: Red circles indicate an Mn- negative sample and yellow pins indicate an Mn-positive sample. Positive samples are labeled with their infection intensity (spore counts).

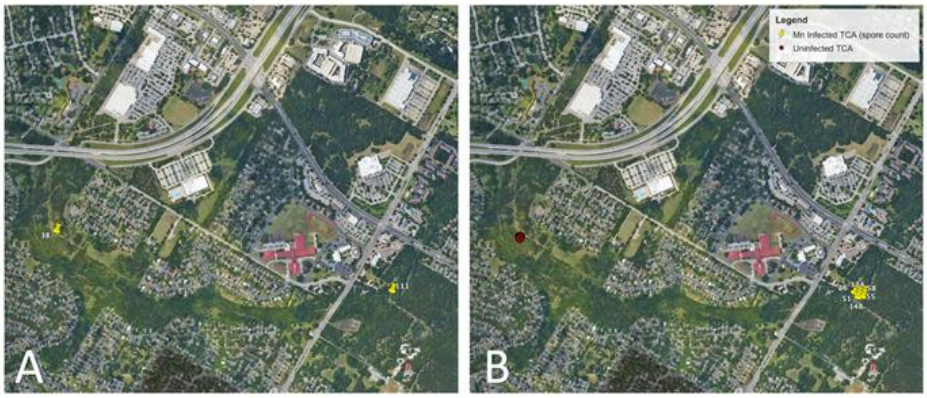


Figure 15: Resurgent areas of TCA infestation, Whirlpool Cave Greenbelt. Distribution of TCA population at Whirlpool Cave Greenbelt from (A) Spring (February – May 2021) (B) Fall (August-October 2021). Red circles indicate an Mn- negative sample and yellow pins indicate an Mn-positive sample. Positive samples are labeled with their infection intensity (spore counts).