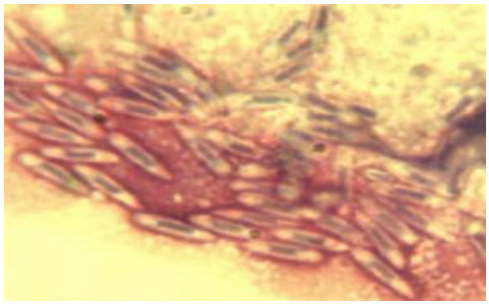
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Tawny Crazy Ant (*Nylanderia fulva*) Study
Balcones Canyonlands Preserve – Jollyville Unit
Task 5: Data analysis and Final Report

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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 supercolonies 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 the TCA supercolony currently invading tracts of the Balcones Canyonlands Preserve, and to explore using this microsporidian as a control agent.

By the fall of 2022 most of the BCP and allied TCA invaded sites had been infected with Mn. In 2023, we observed widespread declines of TCA populations at many BCP and allied sites as a result. Our inoculation efforts in 2023 were highly successful, establishing Mn at what was previously the only remaining BCP site invaded by an uninfected TCA population. Currently all extant TCA population being monitored under this contract are infected with Mn. At sites where the TCA populations have previously gone extinct, native ants are resurging including those species most closely related to TCA. The recovery of species related to TCA indicates that laboratory tests indicating the high level of specificity of this pathogen to TCA are being born out in nature.

Project Background

Nylanderia fulva (Tawny Crazy Ants), an invasive ant species introduced from central South America, are spreading throughout the Gulf Coast region of the United States (1). Current evidence indicates that all tawny crazy ants (TCA) within North America are members of the same supercolony, likely descended from a single successful introduction (2, 3). At a local scale, following introduction, the ants spread by nest budding, maintaining connections between neighboring nests, eventually forming an interconnected web of nests that can span many square kilometers. Herein we will refer to these interconnected groups of nests within a site as a supercolony although in fact they are fragments of a much larger supercolony with a highly fragmented distribution. These ants invade natural habitats and have devastating effects on the arthropod assemblages of those environments (4). 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 (5). In addition, TCA penetrate karst environments to a depth of up to 40 m from the entrance in one impacted cave. TCA prey on karst invertebrates and a trend of declining karst invertebrate abundance in impacted caves has been documented. The strength of this trend is unclear as the study was hampered by intermittent TCA site occupancy (6). However, all available data indicate that populations of endangered karst

invertebrates are threatened by this ant. To mitigate these impacts on wildlife, particularly endangered wildlife, a means to sustainably control supercolonies 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* (7). We found this pathogen infecting a minority of TCA supercolonies 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 (8). Longitudinal field studies of infected supercolonies combined with experimental introductions of this pathogen into uninfected TCA supercolonies reveal that this disease is capable of causing TCA infestations to collapse to local extinction (9). Testing of native ants naturally in contact with *M. nylanderiae* spores and laboratory efforts to artificially transmit this disease to the close relatives of TCA indicate that this pathogen is a highly specific natural enemy of TCA (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 were reporting on the results from the Collins Tract, Jollyville Unit, Balcones Canyonlands Preserve (BCP), Pace Bend 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 2023 were to:

1. Assess tawny crazy ant (TCA) densities, and distributions – currently invaded sites.
2. Assess whether tawny crazy ant (TCA) super colonies are infected with M. nylanderiae; quantify disease extent, prevalence, and intensity if infected – currently invaded sites.
3. Quantify ant assemblage and monitor for TCA at Collapsed sites where TCA have been observed within the past year – post TCA collapse sites.
4. Perform inoculative releases of the microsporidian pathogen *Myrmecomorba nylanderiae.*
5. Evaluate recovery of native ant assemblage at TCA collapse sites.
6. Evaluate TCA impacts on karst invertebrates.
7. Canopy arthropod prey availability and ant arboreal foraging study.

Objective 1&2: Within currently invaded sites: 1) Assess tawny crazy ant (TCA) densities, and distributions; 2) Assess whether tawny crazy ant (TCA) supercolony is infected with *M. nylanderiae*, and quantify disease extent, prevalence, and intensity.

Since the project began following the invasion of the Collins Tract of the BCP by TCA in 2018, additional areas of the BCP have been invaded and additional properties of importance for constraining TCA spread regionally have also become established. TCA continue to colonize sites in the Travis County area. These sites provide opportunities to accelerate our knowledge

gain and better protect BCCP sites. Some sites are also near BCCP protected areas and thus the TCA in them present a direct risk of spread into areas of the BCCP. All sites have the potential to be source populations for future BCCP infestations.

TCA supercolony densities peak in the late summer and fall. In these seasons we conducted a variety of assays described below to quantify the density of the TCA supercolony, its extent, the infection status of the TCA supercolony, and the disease intensity of the infected supercolonies.

At currently infested sites, we conducted the following sampling activities:
(For a list of currently infested sites see Table 1.)

Quantify TCA Density using Pitfall traps:

Permanent pitfall trapping stations, consisting of PVC sleeves set flush in the ground, have been established in invaded and uninvaded areas of the preserve. An open tube containing a mixture of water, soap, and propylene glycol is inserted. Propylene glycol is a non-toxic preservative (approved by the FDA as a food additive) and poses no known threat to wildlife. Tubes were left open for 24 hours to collect ants. Between trapping intervals, sleeves were plugged.

Map the extent of the TCA supercolony.

TCA supercolonies expand radially from the point of initial introduction through the process of large nests dividing into smaller adjoining nests. Throughout this process nest networks remain interconnected. As a result, TCA supercolonies tend to saturate a contiguous area of habitat. Visual inspections of the presence or absence of TCA on the ground allow for a rapid assessment of the amount of habitat occupied by a given supercolony. Year-to-year increases or decreases in the spatial extent of the supercolony provide another measure of the health of the TCA supercolony and the likelihood that it will impact nearby sensitive habitats or features.

In the Fall of 2023, areas that contained any continuous infestation of interconnected nests were mapped for TCA presence - absence. Spatial sampling designs were opportunistic and, where possible, structured around existing path networks and public rights-of-way where appropriate. Presence / absence was determined by visual inspection of the foraging ant assemblage as well as examinations of likely nesting locations (under rocks and rotten logs). Stations where non-TCA, ant workers were encountered at 3 locations, and no TCA were found or where 15 likely nest locations do not reveal TCA were considered free of crazy ants. Three negative stations in a row indicated that the observer has passed beyond the edge of TCA occupied habitat.

Assess whether tawny crazy ant (TCA) supercolony is infected with M. nylanderiae, and quantify disease extent, prevalence, and intensity.

At all extant TCA supercolonies, we tested ants to assess whether the supercolony harbors the pathogen *M. nylanderiae*. Those sites that do harbor the disease were sampled more intensively to assess the extent of the pathogens spread through the TCA supercolony and the intensity of infection present. These factors help to assess whether the TCA supercolony is on a trajectory of disease induced collapse or if an intervention via inoculation of the disease is warranted.

Approach:

Sampling followed the strategies used in 2018 to 2022. Sites were assessed for infection status by testing *N. fulva* workers from every pitfall trap for the pathogen. Further, for all TCA supercolonies, workers from three points near the edges of the TCA supercolony along the TCA mapping transects were collected and tested for infection ensuring that we were likely to detect an incipient infection if it is not yet widespread. At every point where infection status was tested, DNA was extracted from a batch of 20 workers, and pooled DNA was tested with diagnostic PCR (7).

For TCA supercolonies that previous PCR tests have revealed harbor the disease, aliquots from tissue homogenates of 20 workers were fixed on slides, stained, and *M. nylanderiae* spores counted at 1000x magnification (8). These samples provide a measure of the prevalence and intensity of *Mn* infection. In infected supercolonies, we tracked the prevalence and intensity of infection by the Mn pathogen with both Spring and Fall sampling. For all sampling efforts, worker ants were collected off baits, foraging, trails or from nest sites.

Table 1: TCA Research sites 2023. Sites within the BCP or sites of special concern with extant or recently collapsed TCA supercolonies. Previously collapsed sites are sites previously invaded by TCA where TCA declined below the level of detection prior to fall 2022.

	Infected with *Mn*	**Uninfected with *Mn***
Active TCA Population	Pace Bend Park	McNeil Tract
	Walnut Creek Park	Roy Guerrero Park
	Emma Long Park	
Previously Collapsed TCA Site	Collins Tract	
	Whirlpool Cave Tract	

Pace Bend Park

TCA History:

TCA first invaded Pace Bend Park as the leading edge of a much larger infestation spreading south from an initial introduction in the community of Briar Cliff reported in December of 2011. We successfully established the pathogen in the park following inoculative releases in 2020 and 2021.

2023 Findings:

The TCA supercolony at Pace Bend Park shrank dramatically in 2023. TCA have almost entirely disappeared from the areas of the park where we have set our pitfall trap arrays (Fig 1). More broadly, the areas of the park that exhibited high intensity Mn infection levels in 2022 largely or completely died out. In contrast the northwestern edge of the supercolony that had yet to area to pathogen in 2022 expanded approximately 245 meters. The net effect was a reduction in the invaded area from 1.28 to 0.56 km^2 (Fig 2).

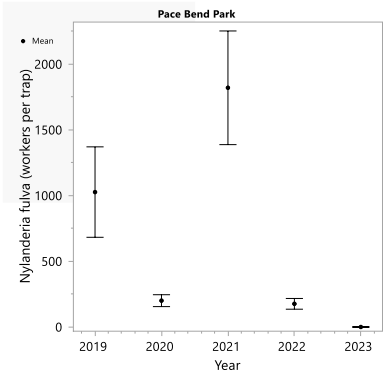


Fig. 1: TCA density Pace Bend Park. Results of pitfall trapping. Number of ants captured in traps left open for 24-hours.

Interestingly, in 2023, infected nests were extremely rare in the extant portion of the TCA supercolony (Fig. 3). We only found two infected nests in the entire remaining portion of the supercolony. Both infected nests had extremely high infection intensities but were in an area isolated from the surviving portion of the supercolony. It is unclear whether the pathogen has spread to the larger area of extant TCA. Whether the disease persists or if a follow-up inoculation is needed to eliminate the ants from the park will have to be determined in 2024.

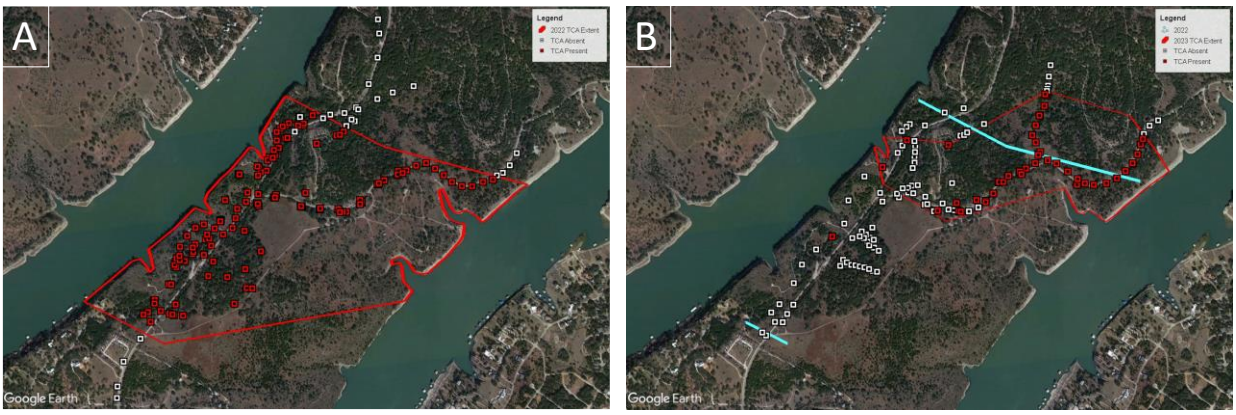


Fig. 2: Maps of TCA extent – Pace Bend Park. Red circles indicate TCA presence. White circles indicate sampled and absent. (A) 2022 - Year of maximum extent. (B) 2023 – All TCA infested areas. Blue lines indicate 2022 TCA population edges.

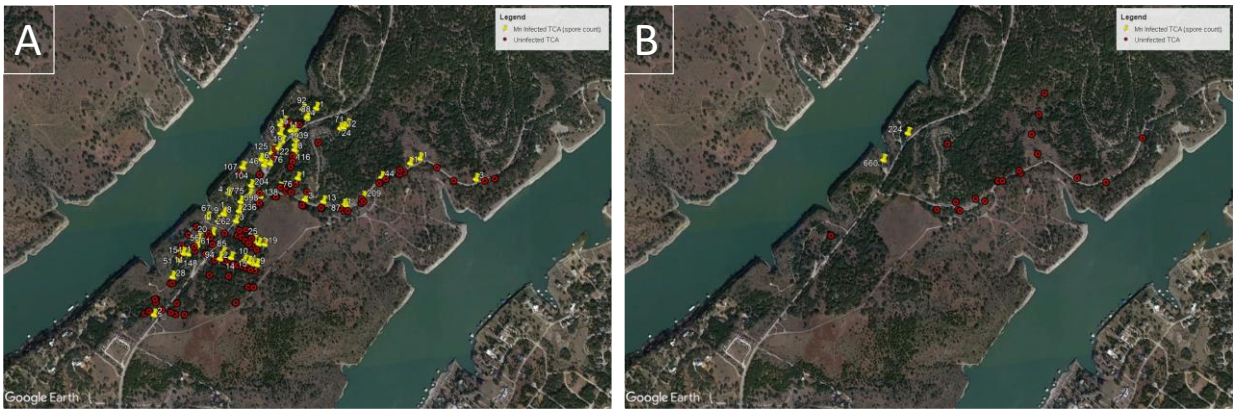


Fig. 3: Maps of *M. nylanderiae* infection extent – Pace Bend Park. Yellow pins indicate Mn presence. Number labels show infection intensity as spore count values. Red circles indicate presence of uninfected TCA. (A) 2022 -Year of maximum Mn extent. (B) 2023 – All positive samples recovered extent.

Emma Long Park

TCA History:

TCA were first found invading Emma Long Park in the spring of 2020. A low intensity, localized infection by *M.* nylanderiae was found within the supercolony, later that year.

2023 Findings:

The TCA supercolony at Emma Long Park appears to have completely collapsed. The ants were eliminated from the areas of our pitfall trap arrays in 2022 and remain absent in 2023 (Fig. 4). In the park more generally, we only found one very small area of TCA infestation in the spring of 2023 (Fig 5). These extant nests were highly infected with Mn (Fig 6), and, by the fall of 2023, we were unable to find TCA anywhere in the park. We will continue to monitor this site.

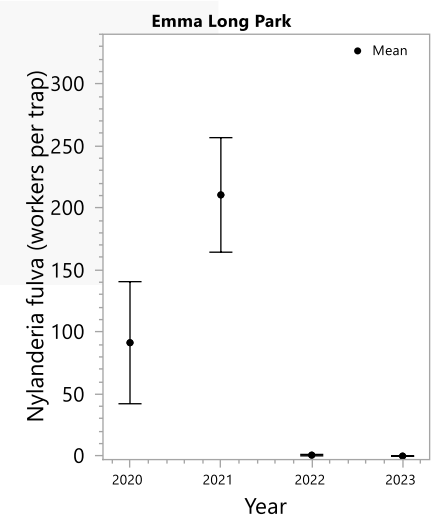


Fig. 4: TCA density Emma Long Park. Results of pitfall trapping. Number of ants captured in traps left open for 24-hours.



Fig. 5: Maps of TCA extent – Emma Long Park. Red circles indicate TCA presence. White circles indicate sampled and absent. (A) 2021 - Year of maximum extent. (B) 2023 – All TCA infested areas. Lines segments indicate population edges in years since TCA maximum extent.



Fig. 6: Maps of *M. nylanderiae* infection extent – Emma Long Park. Yellow pins indicate Mn presence. Number labels show infection intensity as spore count values. Red circles indicate presence of uninfected TCA. (A) 2020 -Year of maximum Mn extent. (B) 2023 – All Mn positive samples recovered.

Walnut Creek Park

TCA History:

TCA were first found invading Walnut Creek Park in the spring of 2018. A low intensity, localized infection by *M. nylanderiae* was found within the supercolony in summer of 2020.

Additionally, an area distant from the localized natural acquired infection was inoculated with Mn in 2021.

2023 Findings:

TCA are now largely eliminated from the main area of the park (Figs. 7). There remains an extant portion of the supercolony in the greenbelt / hike and bike trail to the north of the main portion of the park (Fig. 8). However, this portion of the supercolony is universally infected with Mn at high infection levels (Fig. 9). We anticipate that this supercolony is likely to die out entirely over the winter of 2024. We will continue to monitor this site.

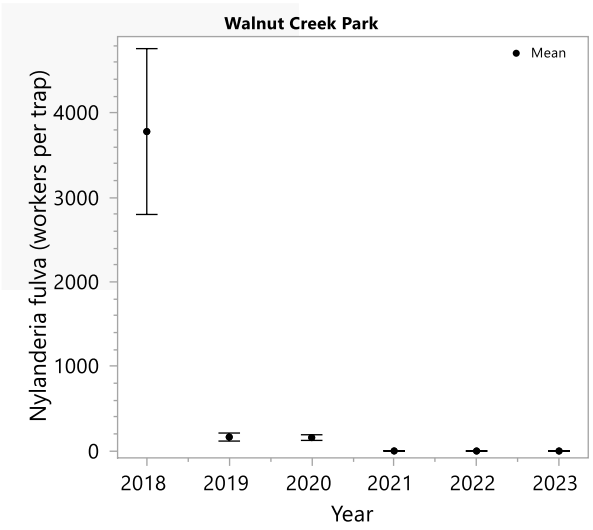


Fig. 7 TCA density Walnut Creek Park. Results of pitfall trapping. Number of ants captured in traps left open for 24-hours.

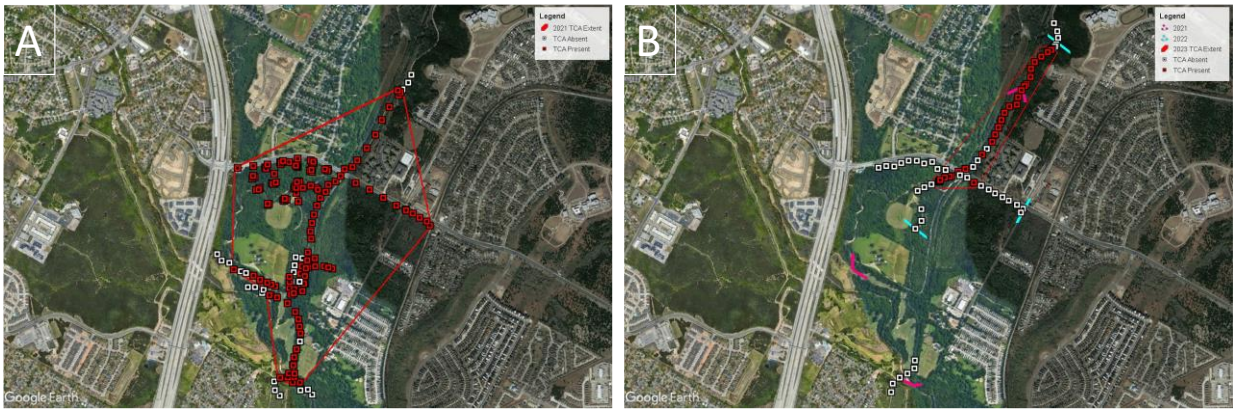


Fig. 8: Maps of TCA extent – Walnut Creek Park. Red circles indicate TCA presence. White circles indicate sampled and absent. (A) 2021 - Year of maximum extent. (B) 2023 – All TCA infested areas. Lines segments indicate population edges in years since TCA maximum extent.

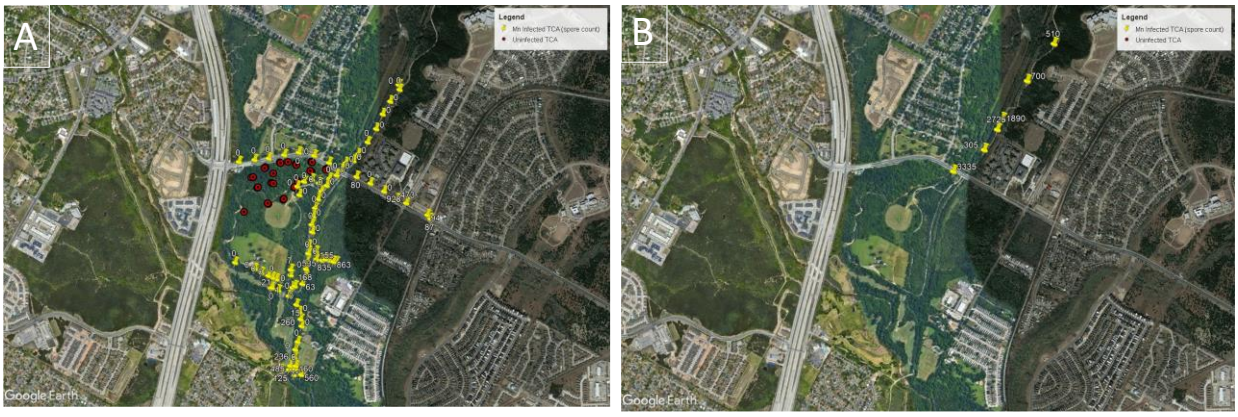


Fig. 9: Maps of *M. nylanderiae* infection extent – Walnut Creek Park. Yellow pins indicate Mn presence. Number labels show infection intensity as spore count values. Red circles indicate presence of uninfected TCA. (A) 2022 -Year of maximum Mn extent. (B) 2023 fall infections – All positive samples recovered.

McNeil Tract

TCA History:

McNeil Tract is a part of the BCP containing caves with endangered karst invertebrates. The current infestation at the McNeil site was first reported in the spring of 2022. Subsequent testing revealed it to be uninfected with *Mn*. We conducted an inoculation in August of 2022. Post inoculation testing in November, did not find evidence of disease establishment. A second inoculation was performed this past spring (2023) (See inoculation results section).

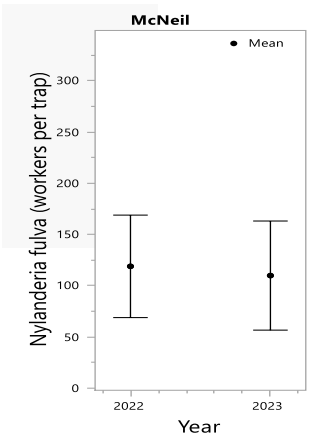


Fig.10: TCA density McNeil Caves Tract BCP. Results of pitfall trapping. Number of ants captured in traps left open for 24-hours.

2023 Findings:

TCA densities in 2023 remained high and were similar to those found in 2022 (Fig. 10). The supercolony expanded rapidly (418 meters) down the right of way adjacent to McNeil Drive (Fig. 11). We also found that the population extends across road and into the surrounding habitat. Limited access due to private property constraints prevents an accurate measurement of the extent of this population.



Fig. 11: Maps of TCA extent – McNeil Caves Tract of BCP. Red circles indicate TCA presence. White circles indicate sampled and absent. (A) 2022 - Year of maximum extent. (B) 2023 – All TCA infested areas. Blue lines indicate 2022 TCA population edges.

Roy Guerrero Park

TCA History:

The TCA supercolony at Roy Guerrero was first reported in 2020. Over the past two years, the TCA supercolony at Roy Guerero Park, never very dense, has declined due to circumstances other than infection with *Mn.* We speculate that the hard freezes over the winters of 2020, 2021, and 2022 as well as the drought in 2022 and 2023 may have contributed to this decline. The extremely sandy soil of Roy Guerrero Park may prevent the ants from penetrating into underground cavities leaving them more exposed to freezing conditions and desiccation than at other sites.

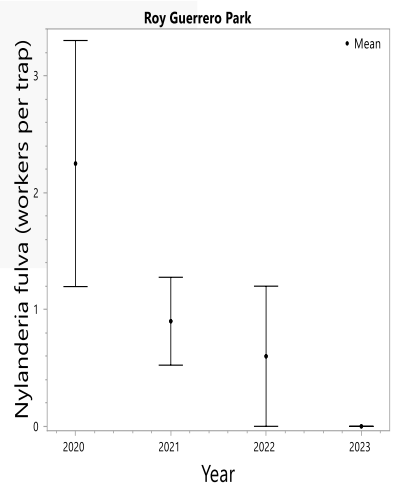


Fig. 12: TCA density Roy Guerrero Park. Results of pitfall trapping. Number of ants captured in traps left open for 24-hours.

2023 Findings:

TCA were not found within Roy Guerrero Park in 2023 (Figs 12 & 13). This disappearance is not the result of the pathogen, and a resurgence may be possible. However, given the site has proven unsuitable for the ants generally, we do not recommend further monitoring of this site.

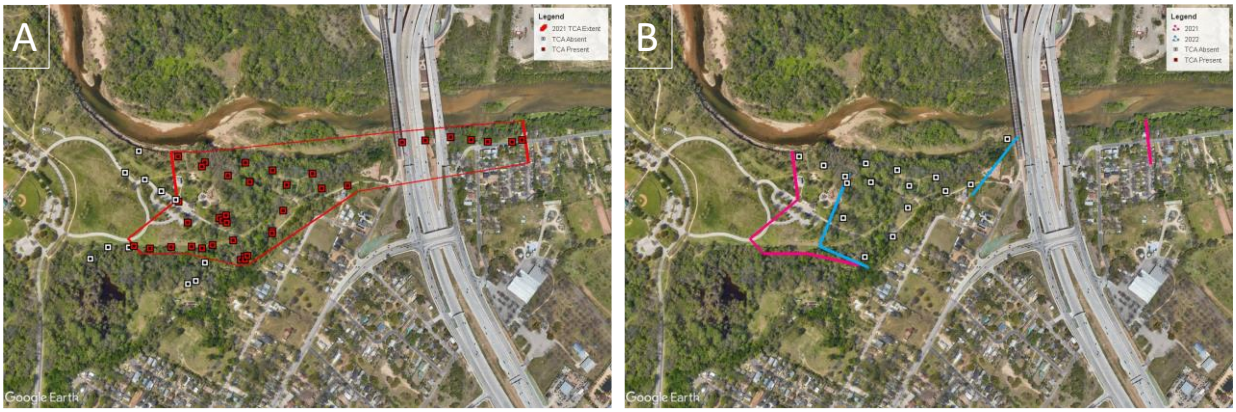


Fig. 13: Maps of TCA extent – Roy Guerrero Park. Red circles indicate TCA presence. White circles indicate sampled and absent. (A) 2021 - Year of maximum extent. (B) 2023 – All TCA infested areas. Lines segments indicate population edges in years since TCA maximum extent.

Objective 3: Quantify ant assemblage and monitor for TCA at Collapsed sites where TCA have been observed within the past year – post TCA collapse sites.

At sites where TCA supercolonies have collapsed in the recent past, there is a continuing need to assess whether a resurgence of TCA is occurring. To accomplish this, we carried out two sampling activities: continuing to run the pitfall trap array that was previously used prior to TCA collapse to assess TCA density and directly searching for TCA.

Approach:

We ran all pitfall traps at all stations that were previously used to quantify TCA abundance (see previous methods). These were run in the spring and summer season to maximize the catch of native ants to assess recovery of these ant assemblages post TCA collapse (see Native Ant Recovery).

All locations where TCA were found in the preceding year were visited at 3-month intervals beginning in May to assess whether TCA remained present. Any TCA located were collected and tested for infection status. If no TCA were found at a location for 1 year, it was dropped from the survey list.

Collins Tract of the BCP

TCA History:

In 2018, we began surveying the TCA supercolony invading the Collins tract of the BCP and Old Water Treatment Plant 4 Site. We found the disease colonized a portion of the supercolony to the north of the preserve in the fall of that year. Inoculative releases were begun in the

summer of 2018 and continued in areas distant from known established infections in super colony naturally in 2019. By 2022 TCA were found in only one small area of the preserve.

2023 Findings:

Follow up sampling of areas where TCA were found in 2022 revealed no extant infestation by TCA within the tract (Fig. 14). It has now been more than a year since we have found TCA within the Collins Tract. Further sampling for TCA will be discontinued although monitoring the recovery of the native ant assemblage will be ongoing (see below).

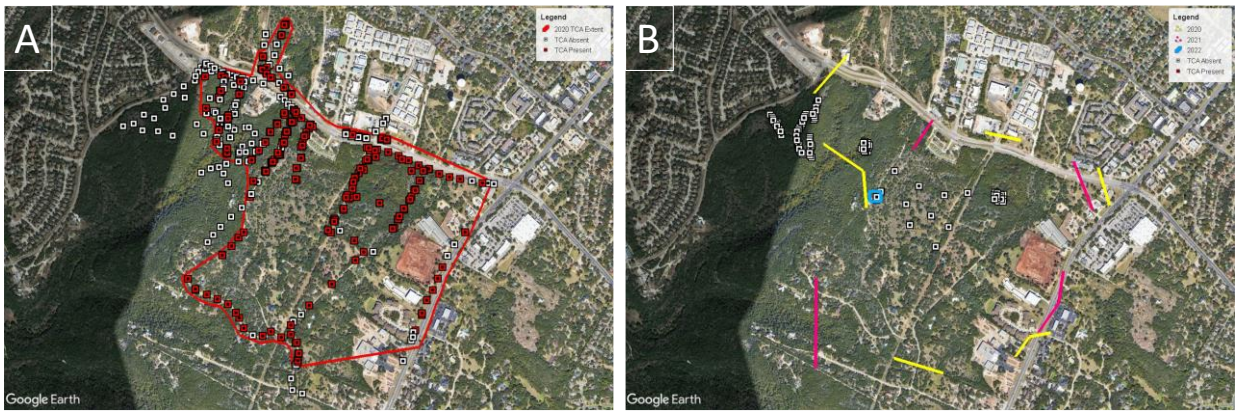


Fig. 14: Maps of TCA extent – Emma Long Park. Red circles indicate TCA presence. White circles indicate sampled and absent. (A) 2021 - Year of maximum extent. (B) 2023 – All TCA infested areas. Lines segments indicate population edges in years since TCA maximum extent.

Whirlpool Cave Tract

TCA History:

The TCA supercolony invading the Whirlpool cave tract was first reported to the lab at University of Texas in 2014. We conducted the first intentional inoculation of of a TCA population with Mn in the spring of 2017. By the fall of 2019 the local supercolony appeared to be eliminated. A resurgent TCA infestation was found in 2021 that occupied about a ¼ acre of previously invaded habitat. This resurgent infestation was infected with Mn and died out over the course of 2022. In 2022, TCA were spotted in only one area of the tract in February.

2023 Findings:

Follow up sampling of areas where TCA were found in 2022 revealed no extant infestation by TCA within the tract (Fig. 14). It has now been more than a year since we have found TCA within the Whirlpool Cave Tract. Further sampling for TCA will be discontinued although monitoring the recovery of the native ant assemblage will be ongoing (see below).

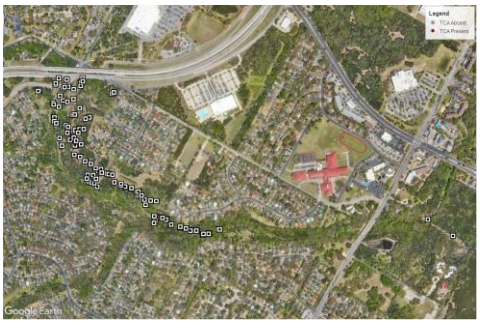


Fig. 15: Whirlpool Cave Tract sampling effort 2023. No TCA found. White circles indicate sampled and absent.

Objective 4: Perform inoculative releases of the microsporidian pathogen *Myrmecomorba nylanderiae*.

At sites with uninfected TCA supercolonies, we have been introducing the pathogen *M. nylanderiae.* This pathogen is now a proven and highly effective biological control agent for TCA. Its establishment at high rates of nest prevalence leads to complete collapse of the TCA supercolony (9). In 2023 we conducted inoculations at the McNeil site. The infestation at the McNeil site was first reported in the spring of 2022. Subsequent testing revealed it to be uninfected with *Mn*. We conducted an initial inoculation in August of 2022. Drought conditions limited the number of infected ants we were able to introduce. In post inoculation testing in November, did not find evidence of disease establishment.

Approach:

In August 2022, we conducted a late season inoculation at the McNeil Tract. We conducted follow-up testing of these inoculation stations in April of 2023. The Threshold of Detection study conducted in 2022 demonstrated the need for deeper sampling of inoculation stations to assess disease establishment. At 3-month intervals from April to December, we sampled and tested 150 ants per inoculation station to evaluate establishment. Ants were collected from multiple locations within 10 meters of the inoculation station and pooled into batches of 50 ants for testing. The sensitivity to Mn detection of the current PCR protocols using these larger DNA volumes were evaluated in the coming months.

In May of 2023 we conducted an additional inoculative release. Inoculations were conducted using the direct nest inoculation technique. Ants were collected from Emma Long Park, removed from their nest material without exposing them to laboratory odors, split into individual inocula containing 600 ants and mixed stage brood, and then released into disturbed nests at the inoculation site. Pathogen establishment was tracked in the same manner as above.

2023 Findings:

We recovered our first Mn positive disease establishment testing sample in August of 2023. By December of 2023 pathogen establishment had been detected in all areas of inoculation including those done in 2022 (Fig. 16). Infection intensities have increased up quickly relative to previous successful inoculations we have conducted. In 2024 we transition to monitoring the spread of this pathogen across the preserve tract.

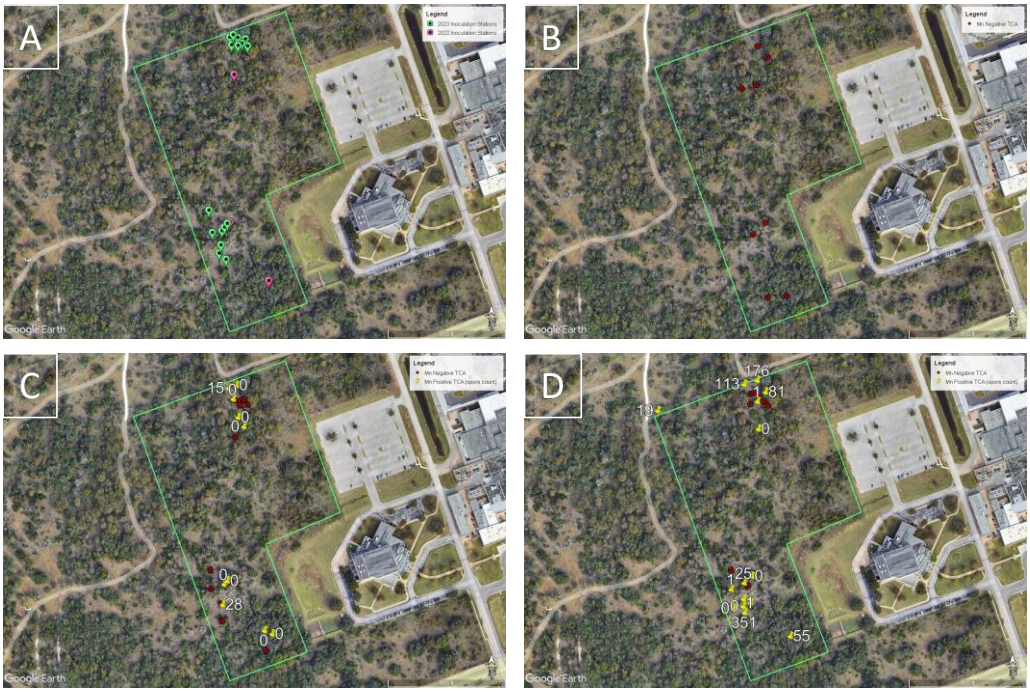


Fig. 16: McNeil Caves Tract inoculation effort. Maps of *M. nylanderiae* infection establishment sampling. Yellow pins indicate Mn presence. Number labels show infection intensity as spore count values. Red circles indicate presence of uninfected TCA. (A) 2023 inoculation stations. (B) April 2023 establishment testing. (C) August 2023 establishment testing. (D) November + December 2023 establishment testing.

Objective 5: Evaluate recovery of native ant assemblage at TCA collapse sites.

As TCA declines 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 happens in the context of an environment that has been saturated with spores of *Mn* left behind by the dying TCA supercolony. To date, all data we have gathered indicates that this pathogen is specific to TCA. Quantifying the recovery of native ant assemblage is an important act of due diligence as it ensures that we know that our laboratory-based prediction that the introduction of *Mn* will not cause unintended consequences is borne out in nature.

Approach:

Initial data on recovery of sites post TCA collapse indicates a period of rapid change in the first 3 years followed by a slower process in the following years. With specialist pathogens like Mn, species closely related to the host are always at greatest risk of negative impact from spill-over infections. Evidence for impacts of Mn on the recovery of native ant taxa were assessed by contrasting the rates of recovery of species related to TCA with species more distantly related to TCA. Both recovery trajectories were put into context by contrasting them with the equilibrium assemblage at uninvaded sites.

Pitfall traps were used to assess native ant community recovery. Pitfall trapping followed the methods outlined in the above section on tracking TCA densities.

2023 Findings:

Recovery of the native ant community at sites where TCA have declined to extinction is marked and rapid. These ants were almost uniformly eliminated by TCA when they were abundant. Native ant species density (number of species found per pitfall trap) increases to the levels seen in uninvaded control sites within about 3 years after TCA population collapse (Fig. 17A). At some sites this recovery appears characterized by an overshoot where recovering sites briefly become more diverse that uninvaded sites before returning to a level quite similar to the uninvaded control. Sites this pattern is what would be expected in a competitively structured assemblage that is being rapidly recolonized following a disturbance. At all sites, imported fire ants have reinvaded rapidly following the elimination of TCA. By two years after TCA collapse, imported fire ant abundance is comparable to that found in the uninvaded control sites (Fig. 17 B).

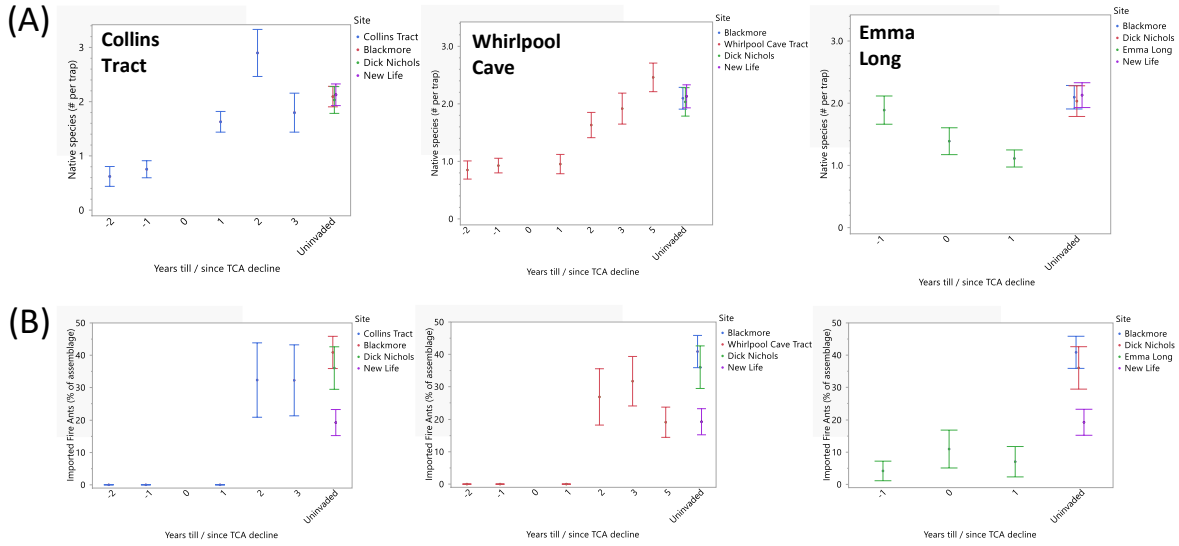


Fig. 17 – Results of native ant assemblage recovery study to date. All sites are areas previously invaded by TCA populations that have now collapsed and disappeared post the establishment and spread of *M. nylanderiae*. Response variables plotted versus the number of years since or till the collapse of the TCA population. The year of collapse (year 0) determined as the first year that TCA prevalence in pitfall traps dropped below 50%. The last year displayed in each plot is 2023 data. Average values across all years sampled provided in final column for uninvaded control sites. (A) Species density of native ants. Ant species other than TCA and imported fire ants. (B) Fraction of the assemblage comprised of imported fire ants. Calculated as percent of all ants that were imported fire ants.

To see if there is evidence that the Mn spore load present in the soil of recovering sites might be influencing the pattern of recovery amongst the native ants, we contrasted the responses of ant species related to TCA with ant species unrelated to TCA. Related species are more likely to be susceptible to infection by shared pathogens. Both Formicine ants and other ants recover to levels like those seen at uninvaded control sites within five years (Fig. 18). The response of Formicine ants is dominated by the most abundant native species, *Nylanderia terricola*. There is an indication that the recovery of this group takes longer than the group of unrelated ants. This response could be the result of low-level susceptibility of this ant to the pathogen. However, if this is the case, this ant is still able to recover its previously extirpated populations in the face of this potential but currently unproven disease burden. Table 1 provides data on all ant species found in pitfall trap surveys in 2023.

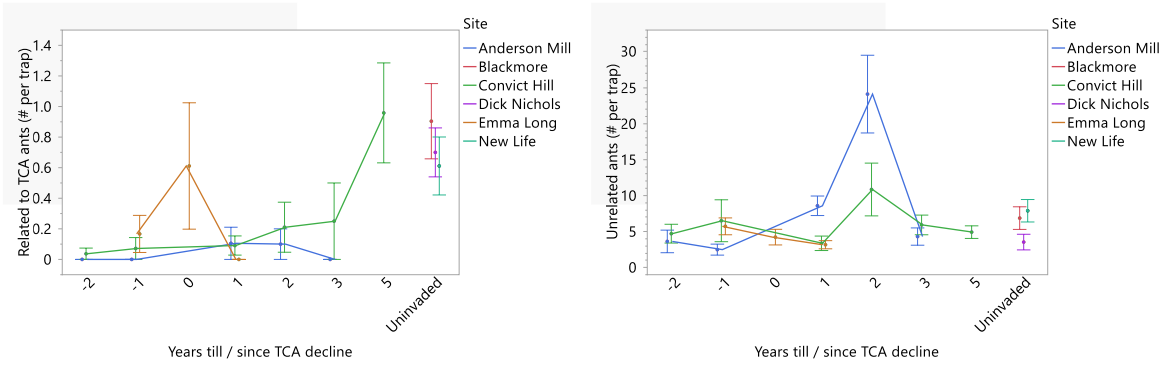


Fig. 18 – Recovery of ants related to and unrelated to TCA. All sites are areas previously invaded by TCA populations that have now collapsed and disappeared post the establishment and spread of *M. nylanderiae*. Response variables plotted versus the number of years since or till the collapse of the TCA population. The year of collapse (year 0) determined as the first year that TCA prevalence in pitfall traps dropped below 50%. The last year displayed in each plot is 2023 data. Average values across all years sampled provided in final column for uninvaded control sites. (A) Ant species in the same subfamily as TCA Formicinae. (B) Non-formicine ants.

Table 1: Ant species found in pitfall traps at all sites sampled in 2023.

Site	*Nylanderia fulva* (TCA)	*Solenopsis invicta* (RIFA)	*Brachymyrmex patagonicus*	*Camponotus decipiens*	*Camponotus texanus*	*Carebara longii*	*Crematogaster laeviscula*	*Crematogaster lineolata*	*Cyphomyrmex rimosus*	*Dorymyrmex flavus*	*Forelius damiani*	*Forelius mccooki*	*Forelius pruinosus*	*Monomorium minimum*	*Myrmecina americana*	*Neivamyrmex opacithorax*	*Nylanderia terricola*	*Pheidole constipata*	*Pheidole dentata*	*Pheidole floridana*	*Pheidole hyatti*	*Pheidole lamia*	*Pheidole pelor*	*Pheidole tetra*	*Solenopsis cf. texana*	*Temnothorax subditivus*	*Temnothorax terrigena*
Blackmore		100			1			3					84	12	1		6	5	31					4	9		
Collins Tract		41				1	3						4	10				1	9			3		10	2		
Dick Nichols		35				1	1		14		14	1	1		6	6	11		13	3			1		3		
Emma Long		8									1		16	32	1			6					1		43		
McNeil	1099		1								14			14											1		
New Life		31			1		7				2		2	11	1		9	15	20	2	6			1	6		
Pace Bend		6	1											95				2	2						17		
Roy Guerrero		68								13	11			1					10	4			4		13		
Walnut Creek		23									17			2			1	7	41			1	1		30		2
Whirlpool Cave Tract		45		1			1				4			29	8		22	8	45	1			2		19	1	
Total	1099	357	2	1	2	2	12	3	14	13	63	1	107	206	17	6	49	44	171	10	6	4	10	15	143	1	2

Objective 6: Evaluate TCA impacts on karst invertebrates.

The invasion of No Rent and Weldon Caves at the McNeil Tract of the BCP creates the opportunity to enhance our understanding of the impacts of TCA on karst invertebrates. The University of Texas, Travis County, and City of Austin staff collaborated on a study of the impacts of TCA on karst invertebrates and mitigation measures (6). However, at that time, there were insufficient caves invaded by this ant to be able to draw general conclusions from

the work. We used the methods of in this previous study to collect additional data from No Rent and Weldon caves to increase our understanding of what TCA do to karst invertebrate assemblages.

Approach:

Travis County staff quantified TCA occurrence and cave use by setting up bait stations outside of cave entrances and at set distances inside the caves. For the larger Weldon cave, they deployed bait stations every 10 m beginning above ground near the entrance (-10 m) and from just inside the entrance ("cave drop zone";0 m) to the far end of the survey area (Weldon: 0 m to 30 m). Because of No Rent Cave's relatively small size, they deployed bait stations every 5 m to the back of the cave (0 m to 10 m). To assure TCA attraction to bait stations, they provided both protein bait (hot dog slice) and sugar bait (cotton ball soaked in 30% sucrose solution), deployed in 15 ml falcon tubes. Each bait station consisted of two sets of paired bait tubes, placed >2 m apart from each other. Bait stations were left open for one hour, then collected and label each bait tube, first removing all non-ant species that may be present.

UT Austin staff then counted, identified, and recorded all ants collected in each tube, and averaged the totals across the protein and sugar tubes for a final TCA count per bait station.

2023 Findings:

TCA were found invading both No Rent and Weldon Caves in 2023. In both caves TCA fully occupied the cave penetrating at high densities to the final bait stations (10 m for No Rent and 40 m for Weldon). TCA densities in both caves were highest in the summer survey when conditions above ground were very dry and hot, and cave usage peaked again in the late November survey. In the spring, very few TCA were present in the caves.

Objective 7: Canopy arthropod prey availability and ant arboreal foraging study.

Since 2018 UT staff have been supporting an ongoing golden-cheeked warbler impact study conducted by Travis County staff. The original design of the study was to examine changes in arboreal invertebrate prey availability as TCA invaded previously unoccupied habitat. The rapid spread and impact of the *M. nylanderiae* pathogen mean that TCA never strongly impacted the areas that were sampled ahead of the TCA front. The current sampling intent is to evaluate how arboreal prey availability responds to the disappearance of TCA from the habitat.

Approach:

Travis county staff conducted all canopy arthropod prey availability sampling. They employed a method of standardized beating of select tree branches over a collection sheet

stratified by tree species. Travis county is in the process of carry out general arthropod identification. UT has identified all ants encountered during sheet beat sampling.

The arboreal foraging density assessment involve timed counts of numbers of ants (both native and TCA foragers) entering the tree canopy in currently infested, previously infested, and never invaded areas during the golden-cheeked warbler's peak nesting season. Ants were counted and identified to species. Arboreal invertebrate prey sampling and arboreal foraging ant assessments were conducted on the same trees.

Ant foraging density assessments followed a 2-tiered method. In low ant density foraging environments, entire tree trunks were searched for 3 min and all ants observed foraging were counted. Voucher specimens of each species counted were taken. In situations of higher density ant foraging, the number of trails of ants ascending into the canopy were counted, and, for each trail, a 3 min count were made of the number of ants climbing past a specific point.

2023 Findings:

Neither native ants nor imported fire ants are foraging in the canopy with a similar intensity to what TCA was doing prior to its decline. Canopy arthropod densities should be largely unimpacted by these levels of canopy foraging. It remains to be seen if samples of canopy arthropods being sorted by Travis County contractors will show an increase in diversity or abundance following the decline of TCA.

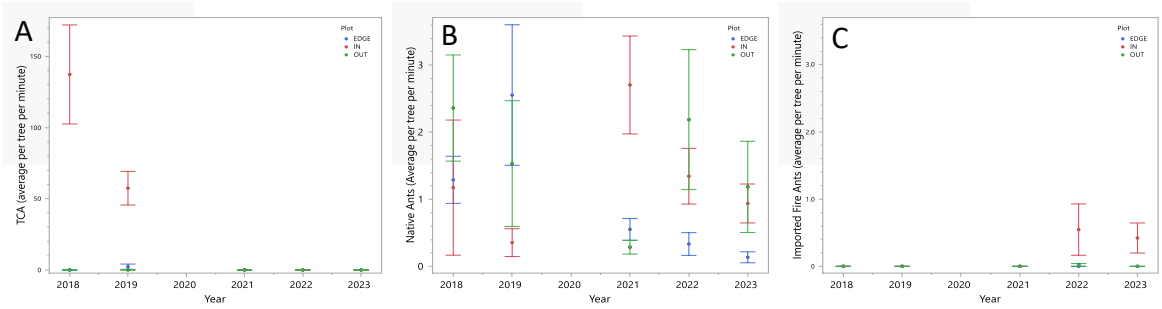


Fig. 19 – Arboreal foraging activity across habitats with different invasion histories. All habitats currently free of TCA (see Collins Tract findings). In – Invaded prior to 2018. Edge – At the edge of historical TCA infested habitat. Out – Far from prior TCA infestation. A) *Nylanderia fulva* (TCA), B) Native ants across three habitats with different invasion histories from 2018-2022 at the BCP Collins Tract, and C) *Solenopsis invicta* (imported fire ant).

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