Tick Survival Assay - Invaded vs. Uninvaded

# Invasive Plants and Ticks

*Background from proposal*

Recent studies demonstrate that invasive plants can alter TBD risk via both direct effects on tick survival and indirect effects mediated by tick hosts. For example, *Microstegium vimineum* has been shown experimentally to reduce survival of *Amblyomma americanum* and *Dermacentor variabilis* (American dog tick) relative to native vegetation (Civitello, Flory, and Clay 2008); conversely the removal of invasive *Berberis thunbergii* (Japanese barberry) significantly reduces the survival of *I. scapularis* (Williams and Ward 2010). Thus both positive and negative direct effects of invasive plants on tick survival have the potential to influence tick population dynamics. There is also growing evidence of indirect effects mediated by host responses to plant invasions, including increased infestation of *Peromyscus leucopus* (white-footed mice) by *I. scapularis* in mixed stands of invasive shrubs (Elias et al. 2006; Williams et al. 2009) and increased abundance of *A. americanum* due to attraction of WTD to dense thickets of *Lonicera maackii* (Amur honeysuckle) (Allan et al. 2010).

**No ecological studies have addressed the effects of plant invasions and potential interactions with climate change or fire regimes for TBD risk.**

We will perform one survival assay during 2018 to assess mortality of ticks due direct destruction by fire and its interactions with plant invasions (described below).

We will perform a second assay during 2019 to determine the effects of fire, and interactions with plant invasions, on microclimate suitability for tick survival.

# Experimental Design - Plant invasion & microclimate suitability (2018)

## Site Description

The study site was located in Alachua, FL, on a private landowner’s property off FL State Road 121 (29°54’05“N, 82°25’20”W). The site is a longleaf pine (*Pinus palustris*) dominated forest with distinct invaded and uninvaded habitat. There is a substanstial and dense cogongrass (*Imperata cylindrica*) invasion (X m x X m) on the property along the roadside, surrounded on all other sides by an uninvaded area. The uninvaded habitat (X m x X m) features a typical native longleaf pine ecosystem. The native plant community includes wiregrass (*Aristida stricta*), common dewberry (*Rubus flagellaris*), and hickory (*Carya sp.*), and *Andropogon sp.* grasses.

## Experimental Setup

A total of 24 plots (1 m x 1 m) were established on the property equally spaced approximately X m apart, 12 invaded and 12 uninvaded. At the center of each plot, 0.1 mm? mesh bags were deployed containing **10 nymph**, **5 female adult**, and **5 male adult** life stage *Amblyomma americanum* ticks (reared at National Tick Research and Educational Resource, Department of Entomology and Plant Pathology, Oklahoma State University, Stillwater, OK). The bags were knotted tightly at one end to minimize chance of escape by the ticks. Cylindrical wire mesh cylindrical 0.5 m in height were constructed with lids to contain the mesh bags and deter from wildlife predation and scavengers. Each mesh bag containing ticks was stood up vertically at the base of the litter layer and tied to a flag to mimic natural questing behavior. Microclimate data was collected by placing the temperature/relative humidity logger (HOBO U23 Pro v2, Onset Computer Corporation, Bourne, MA) adjacent to each cage setup. Each logger was housed in a capped 18-inch length of 1.5 inch diameter PVC pipe to protect it from rainfall and direct sunlight. Holes were drilled on four sides six inches apart along the length of the pipe to allow greater airflow and better reflect ambient temperature and relative humidity. The temperature/RH logger and tick cage were secured vertically in place by wiring them to a piece of rebar sunk six inches into the ground.

## Tick Survival

The mesh bags containing *Amblyomma americanum* ticks within the wire cages complete with tempature/relative humidity loggers were launched at all plots on 21 June 2018. Bags were examined approximately every 10-14 days to determine the number of nymphs and adults surviving. At each visit, bags were removed from the cages but not opened. The survival of each individual tick was determined by assessing their reponse to stimulation. Ticks were blown on to release CO2 to see any natural tick questing responses to an environmental stimulus. Ticks were also pressed down against a surface (pinching by fingers or clipboards) in attempt to see any responses. Any signs of movement qualified the tick as living. If there was no reponse by the tick, it was marked as dead and continued to be monitored in subsequent visits to assure the previous mortality designation was correct. After each bag was processed, they were placed vertically back into the cages attached to the flag to remain upright. Visits continued to the site until XXXX date when all ticks succumbed to desiccation.

## Vegetation and Microclimate Sampling

At each of the 24 plots, the plant communities were surveyed within 1 m x 1 m quadrats on the launch day. Data was collected on herbaceous plant height, stem density, litter percent cover, bare ground percent, litter depth, and overstory canopy cover. In addition, light data was obtained at each of the plots using a light bar (xxx type from xxx place) to assess the light availability at ground level where the ticks were placed. The temperature/relative humidity data loggers collected time points of the environmental conditions every five minutes during the full length of the study. Data was transfered off the loggers every 60 days to clear the memory and ensure proper functioning continued.

## Tick Survial Analysis

Survival results were analyzed using Cox proportional hazards regression models.

# Experimental Design - Interaction of plant invasion and fire (2018)

To examine the combined effects of invasions and fire intensity on tick mortality, **2** non-flammable enclosures each containing **5** adult life stage *A. americanum* ticks were placed on the mineral soil layer in experimental plots (4m x 4m) with invasive cogongrass (n = 10) or native vegetation control plots (n = 10) that were exposed to fire. We quantified fire temperatures at the ground level near each tick enclosure using thermocouples. We quantified tick survival after experimental fires to determine the combined effects of invasions and fires on tick mortality.

## Results

The experimental burns all covered at least 75% of the plot area with most plots having >90% area burned. The invasive dominated plots all had >95% area burned. Overall tick mortality was ~98%, with 100% mortality in invaded plots and 95% mortality in native plots. One bag of ticks was untouched by fire (95% mortality) in a plot with native vegetation and no pine tree cover contributing dried needles to the fine fuel load.

# Experimental Design - Effects of interaction of plant invasion and fire on microclimate suitability (2019)

Similar to 2018 design, but will explicitly address the time since fire (will expand on this in the future).

# Materials

* 1mm gap size nylon mesh bags, 3 per plot (72 total)
* Binder clips for each bag, 72
* Temperature/RH loggers, 1 per plot (24 available)
* PVC housings as solar shield for loggers/probes
* Rebar to suspend PVC housing allowing probe to “float”
* Flags to mark plots and mesh bag locations

# References

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