

## Scientific Note

# Red harvester ants *Pogonomyrmex barbatus* (Smith, 1858) (Hymenoptera: Formicidae) do not distinguish between sorghum head mold symptomatic and asymptomatic seeds

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**Abstract.** Red harvester ants, *Pogonomyrmex barbatus* (Smith, 1858) (Hymenoptera: Formicidae), common in the Lower Rio Grande Valley of Texas, are known to gather seeds from areas around their nests and store the seeds inside their nests for later consumption. As these ants often nest in and near agricultural fields, some of these seeds may be from crops and may also be infected with fungal plant pathogens. These pathogens can degrade seed coats and may cause the seeds to rot within the ant nests, decreasing storage time and potentially spreading the pathogen to other stored seeds. We studied how head mold, a common sorghum disease, changed ant preferences for sorghum seeds. Using seed depots, we evaluated foraging preferences for sorghum seeds with and without head mold and then monitored how many seeds of each type were collected by the colonies after 1, 2, 4, and 24 hours. We found that red harvester ants did not have any significant preference for infected or uninfected seeds, taking both equally over time. Given this non-preference, ants were assumed to be storing infected seeds next to uninfected seeds within their colonies. However, the risk that stored pathogen-infected seeds poses as a source of future seed infection to seeds within the nest and plants in the surrounding field needs to be further examined.

**Keywords:** Seed preference, fungal infection, ant-seed interactions.

Part of maintaining healthy colonies in social insects, or social immunity, is preventing and mitigating microbial infection from entering and spreading within the colony (Cremer 2019). Fungal pathogens and associated toxins are often cues that induce social immunity behaviors and physiological responses in social insects such as ants (Liu et al. 2019). One crucial part of preventing pathogens from spreading within a colony is the detection and management of infected food items (Meunier 2015). This may entail being highly selective of food items to prevent pathogens from entering the nest. For instance, leaf cutter ants preferentially forage on plant materials without certain microbes and then "weed out" unwanted fungal isolates from their fungal garden (Kyle et al. 2023; Rocha et al. 2014). Alternatively, management can include processing or cleaning food items to make them more suitable. In the *Atta sexdens rubropilosa* (L., 1758) (Hymenoptera: Formicidae) leaf cutter ants, workers produce mandibular gland secretions that are able to inhibit multiple pathogens, including known plant pathogens, such as *Candida albicans* (Mendonça et al. 2009), *Botrytis cinerea* (Marsaro Junior et al. 2001), and *Fusarium solani* (Rodrigues et al. 2008). Further, seeds that have been handled by ants and placed in the nest differ in their surface microbial communities (Lash et al. 2020), and some seeds may experience reduced levels of fungal infection after handling by ant workers (Ohkawara & Akino 2005).

Harvester ants are social insects that gather seeds from the surrounding environments to be stored in the nest for future consumption (MacMahon et al. 2000), and their preferential seed collection may be related to plant species and seed characteristics such as fungal infection status (MacMahon et al. 2000; Penn & Crist 2018; Elliott-Vidaurri et al. 2022). For instance, a study of *Pogonomyrmex occidentalis* (Cresson, 1865) (Hymenoptera: Formicidae) found that foragers collected control seeds from a depot at twice the rate as seeds with external fungal symptoms (Crist & Friese 1993). Similarly, *P.*

*occidentalis* and *Pogonomyrmex rugosus* (Emery, 1895) (Hymenoptera: Formicidae) disproportionately discarded otherwise preferred fescue seeds when those seeds contained endophytic fungi (Knoch et al. 1993). However, seed microbial inoculation status does not always impact selection rates, as previous research on red harvester ants, *Pogonomyrmex barbatus* (Smith, 1858) (Hymenoptera: Formicidae), did not find any significant difference in foraging rates for wheatgrass and radish seeds that had been inoculated with a nitrogen-fixing bacteria (Elliott-Vidaurri et al. 2022).

Harvester ants can occur in crop production areas, nesting and foraging near and within the boundaries of commercial crop fields (Uhey & Hofstetter 2022). Many crops are affected by fungal plant pathogens, particularly pathogens of seed heads that can impact plant health and postharvest storage longevity due to mold (Fleurat-Lessard 2017). Fungal plant pathogens that affect seeds can sporulate and may spread to other nearby seeds, rapidly contaminating seed stores (Hernandez Nopsa et al. 2015). Some of these fungal pathogens produce secondary metabolites like mycotoxins that can be toxic to insects and other animals even at low doses (Fleurat-Lessard 2017; Mannaa & Kim 2017). A major threat to sorghum production in the Lower Rio Grande Valley of Texas is sorghum head mold, caused by multiple causative agents including *Fusarium* spp., *Aspergillus* spp., *Curvularia* spp., *Colletotrichum* spp., and *Alternaria* spp. (Ackerman et al. 2021). Red harvester ants, *P. barbatus*, have been observed locally in grain sorghum fields, a major crop that is susceptible to sorghum head mold. Given the proximity of harvester ants in crop fields with infected seed heads, foraging ants may forage on seeds that have been infected with plant pathogens. However, it is poorly understood if sorghum seed infection with head mold affects the preferences of red harvester ants when selecting seeds. Our objective was to determine if harvester ants preferentially forage on symptomatic or asymptomatic crop seeds

using seed depots.

Sorghum seeds (*Sorghum bicolor* (L.) Moench) with and without sorghum head mold symptoms were sourced from a commercial grain sorghum field in Monte Alto, TX, USA. Neighboring sorghum fields on the same and two neighboring farms were also briefly observed for red harvester ant presence to ensure natural co-occurrence with infected sorghum seeds. Seeds were then visually sorted into symptomatic and asymptomatic classes then stored at -20 °C until use to prevent further seed degradation. All symptomatic seeds had similar levels of degradation, featuring discoloration of the entire seed.

To determine harvester ant preferences for sorghum seeds with and without symptomatic sorghum head mold, seed depots were deployed according to methods developed in (Elliott-Vidaurri et al. 2022) at 20 active colonies located within the boundaries (~1.5 km<sup>2</sup>) of the UTRGV Edinburg, TX campus (26°18'33.1" N 98°10'26.8" W) (Elliott-Vidaurri et al. 2023). This peri-urban location allowed us to evaluate colonies that were naïve to sorghum head mold. All preference tests were completed between March and June 2021 when wind speeds were ≤ 32 km/h on 8-10 colonies at a time that were separated by a minimum of 10 m. Each depot consisted of a sanded I-plate Petri dish, with 3 U-shaped entrance holes in the side for easy seed removal (Elliott-Vidaurri et al. 2022). One side of the plate was marked with a red square to differentiate the treatments. Ten seeds of a single treatment (symptomatic and asymptomatic sorghum) were placed on a randomized side of the Petri dish, with ten seeds of the other treatment on the alternate side. Depots were placed ~1.8 m along the colony's main truck trail. Bird and mammal predation was prevented by securing 23×23 cm wire cages made of 1×1 cm hardware cloth around each dish using 4 U-shaped nails. The number of seeds removed was determined after 1, 2, 4 and 24 h; at each timepoint, the temperature, wind speed, and cloud cover percentage were measured. In total, 29 seed depots were used over the course of the study.

All statistics were completed in RStudio Version 1.4.1717 (R Core Team 2023). The Kaplan-Meier survival estimator (function "survfit" in the "survival" package was used to calculate seed removal event likelihood over time and differences between treatments (Therneau 2024). Seeds that were right-censored due to external events (e.g., flipped dishes due to high wind speeds, removal of the cage prior to the 24 hours period, etc.) and those not removed after 24 h were censored.

Sorghum head mold was observed primarily in several non-irrigated fields throughout Hidalgo and Cameron Counties in Texas, and frequently co-occurred in fields with red harvester ant nests (Fig. 1). When cultured on potato dextrose agar, all tested sorghum seeds that were symptomatic with sorghum head mold were contaminated with fungal species from the genera *Fusarium*, *Aspergillus*, *Cladosporium*, *Epicoccum*, and *Alternaria*, all fungi that are commonly associated with sorghum head mold.

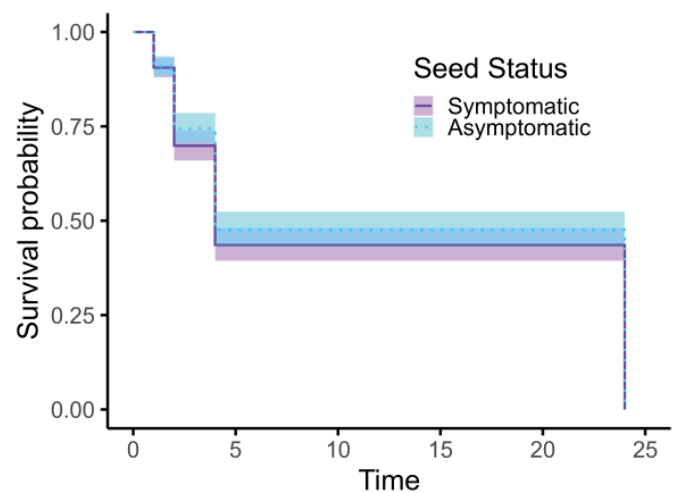
The seed depot trials were run between March and April 2021, with an average temperature of 27.5 °C, an average wind speed of 22.5 kph, and an average cloud cover of 49.3%. Overall, there was no significant effect of seed-health status when assessing the time to removal ( $p = 0.306$ ), with harvester ants removing both symptomatic versus asymptomatic seeds at relatively similar rates (Fig. 2). Both treatments had roughly half of their seeds removed within the first 4 h and all seeds from both treatments were removed after 24 h in all tested sites.

Although prior work has indicated that several species of harvester ants often reduce foraging or increase removal of seeds containing fungal infection (Crist & Friese 1993; Knoch et al. 1993), we did not observe any differences in the foraging by *P. barbatus* on sorghum seeds with and without head mold symptoms. Approximately half of the seeds in the seed depots from both symptomatic and asymptomatic sorghum were foraged by about four hours into the assay, similar to the foraging rate found in other studies on the same species (Elliott-Vidaurri et al. 2022). However, we were unable to determine the fate of collected seeds. In some cases, harvester ants may initially collect seeds and then drop them before storage in the nest, or take the seeds into the nest before later removing them to an external refuse pile (MacMahon et al. 2000; Mull 2003; Arnan et al. 2010). Further, we only observed these interactions during a portion of the year, and changing

environmental conditions may alter foraging selectivity by limiting food availability and/or increasing environmental stress (Whitford & Ettershank 1975). Our use of naïve ant colonies helped to prevent bias in our experiments, however ants that had previously interacted with symptomatic seeds may experience seed learning and memory (Greene et al. 2013), altering the likelihood of future interactions. Future studies should explore how past experiences with symptomatic seeds could influence the likelihood of seed selection.



**Figure 1.** (A) Red harvester ant (*Pogonomyrmex barbatus*) nest disk in a sorghum field in Hidalgo Co., Texas and (B) grain sorghum head displaying symptoms of head mold.



**Figure 2.** Kaplan-Meier curves ( $n = 20$ ) indicating the likelihood of seed removal [survival] by red harvester ants (*Pogonomyrmex barbatus*) over the course of the seed preference trial (observations at 1, 2, 4, and 24 h) for sorghum seeds symptomatic or asymptomatic for sorghum head mold.

Regardless of final seed fate, ant dispersal of infected seed even partially across a crop field may facilitate plant disease spread. However, if the infected seeds were stored for any length of time within the nest, then potentially large numbers of diseased seeds may be held underground, allowing pathogens to persist between cropping years. This becomes a greater concern to growers if the nest granary is shallow enough to be disrupted when the field is tilled during standard agricultural management. Prior observations of this species indicate that granaries can be fairly close to the soil surface (4-75 cm deep), with whole seeds in the upper galleries and shucked seeds in lower galleries and some galleries being filled with seed covered in a "glutinous material" (McCook 1880). Tillage can reach a depth of 25 cm, meaning that the granaries of *P. barbatus* may be potential sources of both seed and disease inoculum in agricultural fields. However, the viability of plant pathogens through this dispersal pathway needs to be further evaluated.

Although plant disease symptoms did not alter the removal rate in our study, many ant species can detect and alter the microbial communities in their immediate surrounding environments (Cremer et al. 2018; Cremer 2019; Lindström et al. 2019). For instance, active *P. occidentalis* nests were associated with an increased density of mycorrhizal fungi in the surrounding soil (Friese & Allen 1993). This may be directly through their manipulation of seeds (Ohkawara &

Akino 2005) and other plant materials (Cremer 2019) and secretion of antimicrobial materials (Marsaro Junior et al. 2001; Mendonça et al. 2009; Rodrigues et al. 2008). Or these changes may be mediated more indirectly by changing soil properties and chemistry (Farji-Brener & Werenkraut 2017) or plant communities (Whitford & Ettershank 1975). While some ants avoid soil with higher densities of entomopathogenic fungi (Huang et al. 2020), other ant genera have also been shown to not avoid foraging in areas even if entomopathogenic fungi are present in the environment (Pereira et al. 2021). Ultimately, understanding how ants interact with a broader range of microbial partners can expand our understanding of their role in agroecological processes in understudied regions. Without a foraging preference for symptomatic or asymptomatic seeds, red harvester ants will continue to move and store diseased seeds, possibly leading to degradation of seed depots within their nests and outbreaks in future crop cycles.

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## Authors' Contributions

LVE-V: Conceptualization, Methodology, Writing - review & editing, Formal analysis; HJP: Conceptualization, Writing - review & editing; RAC: Conceptualization, Writing - review & editing, Formal analysis.

## Conflict of Interest Statment

The authors have no competing interests to declare that are relevant to the content of this article.

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