ABSTRACT

PHILLIPS, SHEA LAYNE. Monitoring and Management of *Drosophila hydei* in Sweetpotato Storage Facilities. (Under the Direction of Dr. Anders Schmidt Huseth)

Drosophila hydei Sturtevant 1921 (Diptera: Drosophilidae) is a nuisance pest of rotting sweetpotato roots in North Carolina storage houses. Fly larvae cause internal damage to the roots and is a mechanical vector for pathogens, such as *Ceratocystis fimbriata Ellis* & Halst. 1890 (Microascales: Ceratocystidaceae; "black rot") that cause yield loss in storage houses. Intensive pyrethrum-based chemical control programs are commonly used to suppress *D. hydei* populations, which can lead to reduced efficacy and resistance selection. Given the rapid reproductive rate of *D. hydei*, the development of resistance is a concern and careful, limited use of insecticides is critical to slow resistance evolution. Although anecdotal reports of reduced *D. hydei* susceptibility exist, the extent of resistance is unknown. Moreover, growers have a limited understanding about monitoring populations in storage to best time effective insecticide applications. In this study, bioassays were conducted to characterize baseline susceptibility of *D. hydei* to pyrethrum among populations collected from four sweetpotato storage facilities that have different management practices. Data showed that more susceptible fly populations were found in facilities that use cultural control strategies (e.g., sanitation) and spray less insecticide. Increased resistance was observed in fly populations in storage facilities that rely on frequent insecticide use. Data showed that as the storage cycle progresses in time, pyrethrum susceptibility decreases, providing evidence that resistance develops over time within populations due to repeated pesticide exposure. Moreover, the type of management strategy, either conventional or organic, did not affect the course of resistance development. When compared to populations collected outside the storage house, we observed limited differences,

suggesting that resident populations at specific facilities all shared similar resistance levels. Bioassays documented that some populations were seven-fold more resistant when compared to a susceptible reference population. Limited information about *D. hydei* population dynamics exists in sweetpotato storage rooms, which makes it difficult to develop informed management practices that alleviate insecticide sprays. To address this knowledge gap, a behavioral choice study was conducted to assess the most attractive bait for this species, which is a first step toward improved monitoring. Laboratory bait assays were performed to assess bait preference in *D. hydei*. Field studies evaluated promising baits for *D. hydei* and other common *Drosophila* spp. The laboratory data showed that a banana yeast mixture was marginally preferred. Field bait assays showed that apple cider vinegar was the most preferred between *D. hydei* and *Drosophila funebris* (Diptera: Drosophilidae) which were the two most common species in sweetpotato storage facilities. This result contradicts previous research in similar conditions that did not detect large numbers of *D. funebris*. Further research is needed to determine the impact *D. funebris* has on sweetpotato root quality and if they are a competent vector of *C. fimbriata*. An optimal bait such as apple cider vinegar can be used to improve population monitoring in sweetpotato storage houses. Improved surveillance information will allow farmers to more effectively manage fly populations and potentially suppress *C. fimbriata* disease movement within their facilities.

Monitoring and Management of *Drosophila hydei* in Sweetpotato Storage Facilities

by
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DEDICATION

To my family, friends, and teachers/tutors/professors for their encouragement and support throughout my educational career.

BIOGRAPHY

Shea Phillips grew up in the mountains of Spruce Pine, North Carolina with a love for all things outdoors. In middle school she developed a love for entomology that was stirred by education about aquatic macroinvertebrates, for which she quickly developed a passion. Throughout middle school and high school she taught and tutored K-6 students in science which grew into a love of teaching students. With her love of all things science and outdoors she pursued a BS in Environmental Technology and Management from N.C. State University with minors in entomology and environmental toxicology. As an undergrad, she worked in three different entomological labs and was a teaching assistant for two entomology courses to gain a variety of experience. She also worked with a mining company for two years planning and creating wildlife conservation outreach projects as well as monitoring various wastewater systems. These projects were focused on community outreach through teaching students about the beauty of their local wildlife and how to conserve it. Other projects focused on the mine's effort to support and preserve local wildlife. Upon her graduation from NC State, she yearned to learn more about entomology and began pursuing a Master's degree to position herself to teach college level courses or obtain niche positions in industry. Presently, Shea enjoys hiking, gardening, painting, photography, and travel.

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CHAPTER 1

Characterizing baseline susceptibility of *Drosophila hydei* to pyrethrum in sweetpotato storage facilities

Abstract

Drosophila hydei is a destructive pest within sweetpotato storage houses in North Carolina. The fly causes internal damage to the crop and is a mechanical vector for pathogens, such as black rot, that increase yield loss in storage facilities. Extensive chemical control, primarily with pyrethrum, can lead to field-evolved resistance in fly populations. Given the rapid reproductive cycle of *D. hydei*, the development of resistance is a major concern, and careful, judicious use of insecticides is critical in preventing erosion of efficacy over time. Where resistance occurs, large resistant fly populations may lead to elevated risk for fungal pathogen transmission and subsequent root degradation. This work characterizes baseline sensitivity of *D. hydei* to pyrethrum and compares susceptibility among fly populations collected from facilities with different management practices. To understand which management factors related to elevated resistance we tested for effects of storage duration (time), management practices (organic vs conventional), and collection location (inside/outside of storage rooms). Bioassays established that some populations were seven-fold more resistant when compared to a susceptible reference population. Temporal signals of resistance increase were significant among cohorts of flies collected over three time points in the storage cycle. The type of management strategy (conventional or organic) did not affect resistance development. Differences between populations collected outside the storage house were limited, suggesting that resident populations at specific facilities all shared similar resistance levels regardless of collection locations. This work provides an increased understanding of how pyrethrum application practices affect *D. hydei* populations.

1.1 Introduction

Globally, sweetpotatoes are one of the most economically important crops. Approximately 131 million tons of sweetpotatoes are produced annually around the world (Sapakhova *et al*. 2023). In the United States, North Carolina is the leading sweetpotato producer and has been since 1971. On average, North Carolina production accounts for approximately 60% of the domestic sweetpotato supply (North Carolina Sweetpotato Commission 2021). Sweetpotatoes were ranked the third most valuable crop of NC in 2020 at $375 million—after soybeans ($674 million) and corn ($483 million), respectively (USDA NASS 2025). The per acre value of sweetpotatoes in 2020 was $3,505, while corn per acre value was $488 and soybeans were valued at $421 per acre (USDA NASS 2025). Sweetpotatos are almost 4 times more valuable than corn and beans combined. The majority of sweetpotatoes are grown and stored in the Coastal Plain of North Carolina, USA, where sandy soil characteristics are favorable for root vegetable production. Four NC counties produced more than 100 million pounds of sweetpotatoes each in 2020: Sampson (259M), Nash (187M), Wilson (181M), and Johnston (155M) (USDA NASS 2025).

Sweetpotatoes are a vegetatively propagated crop with a relatively long growing season, often exceeding 100 days. Sweetpotato slips, sprouts cut from densely planted sweetpotato seed beds, are planted in the early summer and the resulting sweetpotatoes are harvested in the fall. After harvest, the sweetpotatoes are cured at a high temperature (29.4°C) and humidity (90% RH) for 7 days to convert the starch to sugar and to heal abrasions to prevent root rot (NC State Extension 2018). After curing, the potatoes are stored at 12.7 to 15.5°C at 80 to 85% RH (NC State Extension 2018). The storage in the general cycle lasts from fall to the following summer, with sorting and packing for sale occurring throughout this period (Kemble and Sikora 2019). During the storage cycle, sweetpotatoes are subject to moisture loss due to respiration and a variety of biotic and abiotic conditions that cause root degradation (Edmunds et al. 2003).

A key pathogen that affects sweetpotatoes during storage is *Ceratocystis fimbriata* Ellis & Halst. 1890 (Microascales: Ceratocystidaceae), a soil borne fungal pathogen that causes a disease called black rot in the field and in storage (Halsted 1890). *Ceratocystis fimbriata* has a broad host range that includes several economically important crops, including woody plants such as fig, cacao, mango, coffee, and eucalyptus (Quesada-Ocampo 2018). This pathogen can infect the roots through healthy skin tissue, but infection typically takes place at lateral roots, lenticels, and wound sites (Lauritzen 1926, Stahr and Quesada-Ocampo 2020). *Ceratocystis fimbriata* feeds on plant tissues causing it to slowly decompose. This manifests in sunken, circular lesions on the surface of the stored roots, rendering them unmarketable (Halsted 1890). *Ceratocystis fimbriata* has a sticky extracellular matrix that surrounds ascospores, which can be spread through movement of employees, tools, water, and storage bins during handling and processing, making implementation of cultural control strategies challenging (Clark and Moyer 2013). The pathogen thrives in warm humid conditions, making sweetpotato storage facilities an ideal environment for disease development. In 2015, North Carolina sweetpotato-producing counties experienced a severe outbreak of black rot, with up to 60% losses reported in fields, packing facilities, and shipping ports (Scruggs et al. 2017).

Sweetpotatoes can be damaged by transportation, insect pests in the field, rodents, and packing, increasing the risk of *C. fimbriata* infection. Since *C. fimbriata* softens the sweetpotato, the degraded root creates an optimal habitat for pests that are attracted to the decaying plant tissue. *Drosophila* spp. are a very common complex of pests found in sweetpotato storage facilities that are often attracted to rotting roots. This group of insects reproduce in the decomposing sweetpotatoes commonly found in and around storage facilities, including cull piles, mechanically damaged roots, and pathogen-infected roots that are decaying in storage bins. A recent study showed that *Drosophila hydei* (Sturtevant) is the most common *Drosophila* spp. found in North Carolina sweetpotato storage facilities (Stahr et al. 2024). They have been shown to mechanically transmit *C. fimbriata* via ascospores adhering to the exterior of the fly body (Stahr et al. 2024). As a result, greater *D. hydei* populations may elevate risk for *C. fimbriata* transmission if the disease is present during storage. Although the specific epidemiology of this insect-pathogen system is unknown, it is reasonable to assume that minimizing fly populations could reduce risk for disease transmission over time and, as a result, protect root quality throughout the storage cycle.

In addition to the problems caused by *C. fimbriata* transmission, *Drosophila* spp. are a major nuisance pest in storage facilities, forcing employees to wear masks and safety glasses to prevent exposure when flies are at high densities (Personal observation). Cultural and chemical control strategies are commonly used to reduce the fly populations in storage facilities. Cultural control tactics involve the removal of crop waste (e.g., cull piles and discarded roots) and thorough cleaning of the storage and packing facilities between storage cycles. Removal of root waste lessens the possibility of *Drosophila* spp. by removing habitat where larvae develop. Maintaining the storage facility at 12.7 to 15.5°C preserves the sweetpotato roots but also indirectly limits fly populations by slowing development time under these colder temperatures.

The only chemical control tactic available is pyrethrum (due to European standards), often in combination with the synergist piperonyl butoxide (PBO) to increase efficacy. Pyrethrum is an organic insecticide found in chrysanthemums (*Tanacetum cinerariifolium*

(Trevir.) Sch.Bip. 1844; Asterales, Asteraceae; also called the Dalmatian pellitory) that targets the insect's nervous system (Cross et al. 2017). PBO is a synthetic pesticide that acts as a synergist and increases the toxicity of pyrethrum by targeting the insect's ability to metabolize the pesticide (Cross et al. 2017). PBO is primarily used in addition to pyrethrum in crop protection products to counteract physiological mechanisms of detoxification of pyrethrum or pyrethroid insecticides (Cross et al. 2017). Pyrethrum is applied throughout the facility using either an automatic fogging system or manual sprayer. Each sweetpotato storage facility manages *Drosophila* differently; some facilities have organic rooms where flies are controlled with white vinegar traps and/or pyrethrum without PBO. In conventional storage systems, pyrethrum is typically combined with PBO as a primary management tool. These facilities are less reliant on cultural control practices, which increases risk for crop losses if resistance occurs. The frequency of sprays can also vary as the seasons change and affect *Drosophila* population dynamics. We know that higher temperatures lead to greater populations of *Drosophila* due to shorter development time and rapid population growth rates (Markow and O'Grady 2006). This has been anecdotally observed by farmers in sweetpotato storage, with the highest densities of fly populations occurring in the summer months when temperature control in storage houses becomes a challenge due to higher average temperatures outside. However, it is difficult to separate environmental drivers of populations from population growth in the facilities over the storage cycle.

Drosophila spp. remain a significant sweetpotato pest despite growers' best efforts. Farmers have reported diminishing effectiveness of pyrethrum, which could suggest evolving pyrethrum resistance. Given the short generation time of *D. hydei*, the development of resistance is a major concern, and careful, limited use of pesticides is critical in preventing erosion of efficacy over time.

This work characterizes the baseline susceptibility of *D. hydei* to pyrethrum. To do this, dose response assays were conducted to estimate sensitivity to pyrethrum in 12 storage rooms contained within four facilities distributed across the Coastal Plain of North Carolina, USA; each facility implemented different management practices. We hypothesized that populations would have unequal sensitivity to pyrethrum across locations depending on the intensity of spray application. We also hypothesized that pyrethrum exposure would result in significantly greater estimated median lethal doses (LC50s) compared to laboratory reared susceptible populations. Furthermore, it was expected that greater pyrethrum susceptibility would be present in fly populations collected from facilities that incorporate good sanitation practices and spray less, while resistance was expected to be greater in fly populations collected from storage facilities that rely on intensive chemical control programs. This work provides an improved understanding of how pyrethrum spraying impacts *D. hydei* populations.

1.2 Materials and Methods

1.2.1 Insect Collection and Colony Maintenance

To assess pyrethrum susceptibility in *D. hydei* in sweetpotato storage, populations were collected from four storage facilities across the coastal plain of North Carolina. These facilities range in size from small to very large operations. Small being that there are a few storage rooms to large facilities having 10 or more storage rooms. Some facilities participate in being contract buyers of sweetpotatoes stored on-facility elsewhere, which could present additional risk for the movement of resistance alleles. Documenting the underlying genetics and movement of fly populations was, however, beyond the scope of the present study.

Previous work has shown that multiple *Drosophila* species are present in sweetpotato storage, but *D. hydei* was the most common species detected (Stahr et al. 2024). As a result, we used this species as an model for resistance development; this increased the likelihood of collection of adequate populations for the subsequent bioassay studies. Each facility uses commercially available pyrethrum to control *Drosophila* spp. throughout the year-long storage cycle, but the frequency of applications varies among locations. Sampled facilities include segregated organic and conventional storage rooms. In conventionally managed systems, pyrethrum sprays are often combined with piperonyl butoxide to increase insecticide efficacy. Piperonyl butoxide is not registered for use in organic sweetpotatoes and is thus a distinct contrast in management practices compared to conventional rooms at the same facilities. To understand these differences, *D. hydei* populations were collected in both organic and conventional storage rooms at two facilities. The remaining two facilities do not have organic storage, and, as a result, conventionally managed populations were only collected. Populations of wild flies from outside storage rooms were collected at each location to characterize baseline pyrethrum susceptibility for insects not under strong resistance selection within storage facilities.

Flies were collected at each facility in November, March, May, and July. Individuals were collected in storage rooms using two-liter bottles with an inverted bottle top and a bait cup covered with a breathable lid to prevent flies from drowning. Traps were baited with 50 mL of apple cider vinegar and placed in storage rooms or outside for 24 hours. After that period, the traps were collected, sealed with a cotton ball, and placed in a cooler for transport back to the laboratory. Captured flies were anesthetized using a carbon dioxide flow pad system (Benchtop Flow Buddy Complete System, #59-122 BCU, Genesee Scientific, USA), and sorted to species. Flies with mites and species other than *D. hydei.* were eliminated via ethanol. *Drosophila hydei* were housed in a molasses diet cup with a label containing the management program, storage house name, room number, month sampled, and generation number (which at the time of trapping was F0) (Indiana University 2021). A susceptible comparison population (1508-1641.68 Arizona) was sourced from the Cornell *Drosophila* repository (*D. hydei* - National *Drosophila* Species Stock Center 2024).

Populations were reared in 0.95 L plastic deli cups (Heavy-Duty Deli Containers-32oz, ULINE, Atlanta, Georgia) contained in a temperature-controlled chamber set at 22°C with a 12 hours light and 12 hours dark (Markow and O'Grady 2006) diurnal cycle, in a temperature-controlled growth chamber (Model I-36VL, Percival Scientific, Perry, IA). The deli cups were pre-sprayed with approximately 0.05 mL of mold control to prevent fungal blooms in the diet (Concrobium 25326CAL Mold Control Spray, RUST-OLEUM, Vernon Hills, Illinois). The active ingredient in the mold control was sodium carbonate. Flies were fed a molasses diet consisting of cornmeal, yeast, agar, and molasses (Wheeler et al. 1993). Methylparaben was added to the molasses diet to reduce fungal and bacterial growth at a rate of 16 g per diet batch (roughly 1 g per deli cup). The deli cups were filled with 120 mL of molasses diet. Approximately 200 adult flies were moved into each cup and allowed to oviposit. Diet cups were changed every two weeks to prevent depletion of nutrients and to limit fungal growth. Fungus mites (Acaridae) were occasionally associated with wild-caught flies, however, were not predators of any fly life stage (M. Bertone personal communication). To replace the diet, deli cups infested with adult flies were placed in a freezer for three to five minutes to reduce activity and the flies were then transferred to the new diet cup. The new diet cup was labeled appropriately, and the same generation number was used. The old deli cup would be thrown out based on diet quality or kept for larvae to pupate for the next generation. On average, three generations were maintained for each colony with two to four cups per generation.

1.2.2 Pyrethrum Bioassay

Bioassay experiments were conducted across six pyrethrum doses with four replications of each dose. Three acetone-coated vials were included as a control in each bioassay. Results from bioassay experiments were not included if the control mortality was above 20% (Rosenheim and Hoy 1989). Treated assay vials were prepared in a fume hood on a cold hot dog roller to ensure the insides of the vials were evenly coated with pyrethrum. First, 500 µL of 99.5% pure acetone (4L,A18-4,UN 1090 Acetone ACS GRADE, Fisher Scientific, Waltham, Massachusetts) was pipetted into each control vial and then placed on the hot dog roller for coating. After all acetone had evaporated, the vials were recapped and stored in a zip-lock bag. Next, technical grade pyrethrum 100% concentrate was weighed on a microgram scale to measure different doses (33739-100MG Pyrethrum extract. PESTANAL® analytical standard, Millipore Sigma, St. Louis, Missouri) (Table 1.0). The set amounts of pyrethrum were mixed with 100 mL of acetone. Five hundred 500 µL of each solution was pipetted into their corresponding vials to then be coated on the cold hot dog roller. Vials were made approximately monthly and refrigerated for future bioassay use.

To conduct bioassay experiments, flies from a known generation were anesthetized using a carbon dioxide flow pad system. Fifteen (15) sedated flies were transferred into each vial using a fine paintbrush and the vial was plugged with a cotton ball to allow for air exchange but prevent escape. The bioassays were contained in a temperature-controlled chamber (Model I-

36VL, Percival Scientific, Perry, IA) set at 22°C with a 12 hours light: 12 hours dark diurnal cycle (Markow and O'Grady 2006) . After 24 hours individual flies were tabulated as either live, dead, or moribund. The data was recorded in a spreadsheet and adjusted for control mortality using Abbott's correction for each vial (Rosenheim and Hoy 1989).

1.2.3 Statistical Analysis

To estimate LC50s for each population, a probit analysis was performed using the Hill equation, $\mathrm{Mortality}=1/(1+(\mathrm{LC50}/[\mathrm{dose}])n)$ (Hill 1910) in MATLAB (MATLAB 2024). The Hill equation has been used to analyze dose response data in various entomology studies (Liu et al. 2002: McComic et al. 2021). The LC_{50} (±95% CI) and model fit statistics were calculated for each colony to compare dose response. All figures in this study were made with MATLAB (MATLAB 2024).

To understand predictors of resistance, we also conducted a logistic regression analysis that tested the effect of collection time (relative to storage cycle), facility, and an interaction between collection time and facility on the estimated median lethal dose for discrete populations. A generalized linear mixed model (GLMM) with a binomial distribution and a probit link function was used to analyze the susceptibility of *D. hydei* to pyrethrum. Three different models were developed to assess the spatial, temporal, and management-related variation in *D. hydei* susceptibility. For all models, the proportion of *D. hydei* adults that died per vial was modeled as a response variable. To account for the hierarchical structure of data, vials were nested within the relevant blocking factor for each model and included as a random effect. Model parameters were estimated using maximum likelihood estimation, and a diagnostic residuals plot was used to check the model fit. The first model includes facilities, months, and their interaction as fixed effects to evaluate temporal susceptibility across the facilities (Table 1.2). In the second model, facility management, and months interaction were included as fixed effects to assess the influence of management practices on insecticide susceptibility (Table 1.3). The third model evaluated the susceptibility of *D. hydei* at the spatial scale with collection locations and facilities as fixed effects (Table 1.4). For all models with a significant effect ($P < 0.05$), a pairwise comparison of least square mean comparisons (LS means) was performed using PDIFF and adjusted using the Tukey-Kramer method, and significance was determined at $P < 0.05$. All analysis was performed in SAS (SAS Institute Inc., Cary, NC). Another model was created to see if mortality across facilities was significant (Figure 1.3). The first model tested trends in LC_{50}s throughout the duration of storage among conventional fly populations within the different facilities. The second model was developed to see if facilities management strategy (either organic or conventional) resulted in significantly different mortality. Lastly a model was developed to compare LC_{50} s among flies inside the storage room vs. outside in the surrounding facility to determine to the uniformity of pyrethrum sensitivity.

1.3 RESULTS

1.3.1 Estimated LC50 of Populations Collected from Conventionally Managed Storage Houses.

The estimated LC_{50}s of all sampled populations are displayed in Table 1.0. To better understand predictors of varying susceptibility, we completed a full regression analysis of resistance to compare facilities, timing of collection, and insecticide management program (Table 1.2). The analysis shows that fly susceptibility varies significantly among facilities. Average percent mortality in conventional populations within facilities are not significantly different except for Facility 1 (Table 1.2; Fig. 1.2). Average percent mortality in conventional populations within facilities and the sampled month of each population are significantly different but their interaction is not (Table 1.2). Pesticide susceptibility changes over the sampled months (Table 1.2). In general, Facility 1 has the highest LC_{50}s among the facilities with a median lethal dose range of 100-179 ppm among multiple collections at Facility 1 (Table 1.1). In contrast, Facility 2 has a range of 43-172 ppm and Facility 3 has a range of 60-109 ppm. Facility 3 has a much smaller range compared to Facility 4 with a LC_{50} range of 41-139 ppm (Table 1.1). The reference population obtained from the Cornell Drosophila repository had an LC_{50} of roughly 26 ppm. Repeated observations of the same conventional storage room generally showed a decrease in susceptibility over time, suggesting that repeated selection may lead to rapidly evolving pyrethrum resistance (Table 1.2; Fig. 1.1). Differences among insecticide management programs generally revealed similar trends in median lethal doses within specific facilities (Fig. 1.2).

1.3.2 Relationship Between Facilities and Collection Months

A significant facility by time interaction indicates that the observed resistance by location is dependent on timing of collection and varies across locations (Fig. 1.3). This interaction could be driven by variable selection pressure among locations that have different intensities of pyrethrum use. The main effect of facility was significant, and the average susceptibility of *D. hydei* thus varied among sample locations (Fig. 1.2). Sampling time was also significant with a general trend toward decreasing pyrethrum susceptibility over the eight-month sampling window (Fig. 1.3).

Significant differences in susceptibility were observed between insects collected inside the conventional storage room vs. populations collected outside the storage room (Fig. 1.4). Outside and inside populations are mostly similar with a few exceptions; in Facility 1 percent mortality inside the facility was significantly lower than the other sampled colonies (Fig. 1.4).

Facility 3 inside and Facility 4 inside are statistically similar and had a greater percent mortality compared to the rest of the samples. An analysis of conventional and organic populations within Facilities 1 and 2 showed that Facility and management programs were not significantly different (Table 1.3), which suggests that recurring pyrethrum use in both organic and conventional houses resulted in similar resistance levels. Additionally, management and the interaction of facility and management was not significant. Conventional and organic colonies within Facilities 1 and 2 were not significantly different (Table 1.3). Organic populations across facilities have an average of 49% mortality (±11% SE). Conventional populations have an average of 41% mortality (±8% SE). This result suggests that conventional and organic programs should consider resistance management strategies to alleviate selection pressure since the same mode of action (pyrethrum) is used in both programs.

The sampling periods among months are significantly different (Table 1.2). November populations experienced greater mortality than July populations (Fig. 1.3), and a longer storage duration is associated with more pesticide tolerant populations. The observed susceptibility declines at unequal rates among the four facilities (Fig. 1.1). For example, Facility 1 has consistent estimates of pyrethrum susceptibility over time, which may indicate that elevated resistance could be fixed in that population (Fig. 1.1). In contrast, responses to the active ingredient clearly changed over the eight-month sampling period at the other locations. This interaction suggests that selection pressure via repeated sprays over the storage period may be associated with declining pyrethrum susceptibility over time, suggesting the onset of practical resistance over the relatively short duration of the storage cycle.

1.4 DISCUSSION

Management of *Drosophila* spp. is a major issue that impacts many agricultural systems in a diversity of locations worldwide (Dias et al. 2018). While many tactics exist to counteract this problematic group of pests, the evolution of resistance due to chronic use of pesticides is a persistent concern (Dias et al. 2018). Prior to this study, limited information of the baseline susceptibility of *D. hydei* to pyrethrum was available for the sweetpotato storage system in North Carolina. It is important to characterize baseline susceptibility of *D. hydei* to inform stakeholders about the potential for resistance and recommend appropriate insecticide resistance management (IRM) approaches that alleviate selection pressure. In other agricultural systems, similar characterization of baseline susceptibility assessment in *D. melanogaster* (Mertz et al. 2021) and *D. suzukii* (Deans and Hutchison 2022) was critical to development of better IRM strategies that reduce crop loss while alleviating the negative externalities that relate to ongoing use of ineffective insecticides.

In this study, conventionally managed populations in one facility had significantly lower sensitivity to pyrethrum when compared to the other three (Table 1.2; Fig. 1.1). Spray logs revealed that this location frequently applied pyrethrum; however, records from other locations were incomplete, which limited our ability to directly test the association between spray frequency and observed resistance. Comparison of organic and conventional management was not statistically different at the two facilities where both management systems were present. This result suggests that the persistent use of pyrethrum in both systems led to similar levels of observed resistance, and that the use of PBO in conventional houses likely did not affect the course of resistance development. Similarity among LC_{50}s may be driven by either population mixing within the facility or the fact that the same mode of action/insecticide was used in both systems at roughly the same intensity (Table 1.2; Fig. 1.2). We do know from IRM theory that frequent sprays generally increase the likelihood for resistance to develop by eliminating susceptible individuals from an insect population (Georghiou 1994). In the sweetpotato storage facility, close proximity of storage rooms and the frequency of room access during the packing cycle may enable resistant genes to spread among the different fly populations. This process of selection is well understood and provides the basis for insecticide resistance management recommendations in many agricultural and medical pest settings (Georghiou 1994; Hawkins et al. 2018). However, the rate of erosion in susceptibility was particularly striking here, likely driven by an interaction between the frequency of selection and the short lifecycle of the target pest.

The data suggests that over the duration of the storage cycle, observed LC_{50}s steadily increased (less susceptibility, more resistance) when compared to the beginning of the storage cycle in the autumn (Figure 1.4). Mortality declines as there are more applications of pesticide (Figure 1.4). Here, the significant interaction between facility and collection time is critical, with the observed susceptibility at some facilities changing over the study period and others remaining relatively static. Notably, Facility 1 had consistently low mortality throughout the entire storage cycle, whereas the susceptibility observed at Facility 4 declined significantly over time (Fig. 1.1). Based on spray logs from Facility 1 the facility utilized calendar sprays throughout the storage cycle. This could be one explanation for eroding susceptibility over time. It is important to consider how rapidly susceptibility declined at Facility 4, which suggests that this species has the capability to evolve resistance rapidly. This could directly impact related aspects of root quality, specifically the prevalence of *C. fimbriata,* when large populations of resistant flies could promote extensive disease transmission. However, the relationship between resistance evolution, *D. hydei* population dynamics, and resultant disease development is not well understood.

Temporal shifts in pesticide sensitivity have been observed in other economically important *Drosophila* pest species. For example, changing susceptibility has been observed in *D. melanogaster* collected from intensively managed vineyard production systems in New York (Mertz et al. 2021). A similar study found that field-collected *D. suzukii* populations from Minnesota raspberries had 8- to 45-fold resistance to pyrethroids relative to susceptible reference colonies (Deans and Hutchison 2022). While the levels of resistance documented in the present study are not of the magnitude of those described by other studies, we did see relatively rapid progression of resistance over time which suggests a potentially problematic transition to field-evolved resistance in *D. hydei* populations that remain under strong selection.

The storage room temperature and sanitation practices varied among facilities which may also explain the variation in fly populations and resultant spray intensity differences that lead to variation in selection. Higher temperatures and unclean facilities anecdotally corresponded to a greater fly abundance when compared to facilities that maintain cool storage conditions and adhere to strict sanitary practices (e.g., cull pile management, packing line cleanliness). This is important to note because resistance may not be the only explanation for differences in fly abundance among sweetpotato facilities.

Application technology may also contribute to selection differences among the facilities. Pyrethrum is often distributed by a cold fogger applicator in sweetpotato storage rooms that can deliver inconsistent amounts of pesticides to the flies because of varying droplet sizes and unequal coverage across large quantities of stored roots. Moreover, storage rooms also include complex internal structures that support the roof and walls. Refuges could maintain a susceptible population of flies. But, if pesticide exposure is inconsistent, individuals within a population can receive sublethal exposures while susceptible individuals decline in frequency, a process that favors resistance evolution (Roush and Tabashnik 2012, Hawkins et al. 2018).

One primary method to mitigate resistance development is alternating modes of action to reduce selection pressure (Georghiou 1994, ffrench-Constant and Roush 2012). Other ways include common cultural control practices like rotating crops to reduce the use of pesticides season after season, reducing harborage sites, adjusting the timing of pesticide applications, and monitoring before and after a spray (EPA 2018). Although many IRM principles are largely based on studies in agricultural fields, similar mitigation strategies can be applied to stored product pests in confined structures (Boyer et al. 2012). Here, the declining efficacy of pyrethrum strongly suggests that adopting more IRM strategies in sweetpotato storage facilities could stem the development of *D. hydei* resistance. Key to that process will be the identification and registration of other reduced-risk products that are acceptable for direct application to roots in sweetpotato storage. Unfortunately, the registration process for stored product pests is complicated and includes a relatively small subset of researchers focused on those issues (i.e., IR-4 and associated minor crop researchers). To counteract the pesticide deficit, adoption of cultural control practices that include removal of rotting roots and diligent cull pile management around the facility would be beneficial in reducing population densities. In the near-term, clear guidelines to break host availability within facilities would be a reasonable approach to reduce the prevalence of putatively resistant fly populations, while future research could focus on chemical and non-chemical practices to reduce reliance on pyrethrum in sweetpotato storage facilities.

References

Boyer, S., Zhang, H., and Lempérière, G. (2012). A review of control methods and resistance mechanisms in stored-product insects. Bulletin of entomological research, 102(2), 213-229.

Clark, C. A., and Moyer, J. W. (2013). Black rot. in: Clark, C. A., Ferrin, D. M., Smith, T, P., and Holm. G, J., Compendium of Sweetpotato Diseases, Pests, and Disorders. 2nd ed. The American Phytopathological Society, St. Paul, MN. Pages 29-33

Cross, A., Bond, C., Buhl, K., and Jenkins, J. (2017) *Piperonyl Butoxide (PBO) General Fact Sheet*; National Pesticide Information Center, Oregon State University Extension Services. npic.orst.edu/factsheets/pbogen.html.

Deans, C., and Hutchison, W. D. (2022). Propensity for resistance development in the invasive berry pest, spotted-wing drosophila (*Drosophila suzukii*), under laboratory selection. *Pest management science*, 78(12), 5203-5212.

Dias, N. P., Zotti, M. J., Montoya, P., Carvalho, I. R., & Nava, D. E. (2018). Fruit fly management research: A systematic review of monitoring and control tactics in the world. *Crop Protection*, 112, 187-200.

Edmunds et al. 2003 Postharvest Handling of Sweetpotatoes "LSU AgCenter" https://www.lsuagcenter.com/topics/crops/sweet_potatoes Accessed on: Jan, 20th, 2024

Ffench-Constant RH, and Roush RT (2012). Resistance Detection and Documentation: the relative roles of pesticidal and biochemical assays. In: Roush RT, and Tabashnik, B. E. (Eds.). Pesticide resistance in arthropods. Springer Science and Business Media. pp. 4-44.

Georghiou, G. P. (1994). Principles of insecticide resistance management. *Phytoprotection.* 75(4): 51-59.

Goindin, D., Delannay, C., Gelasse, A., Ramdini, C., Gaude, T., Faucon, F., David, J.-P., Gustave, J., Vega-Rua, A., and Fouque, F. (2017). Levels of insecticide resistance to deltamethrin, malathion, and temephos, and associated mechanisms in Aedes aegypti mosquitoes from the Guadeloupe and Saint Martin islands (French West Indies). *Infectious Diseases of Poverty*, vol. 6, no. 1, Feb. 2017, https://doi.org/10.1186/s40249-017-0254-x.

Halsted, B. D. (1890). Some fungous diseases of the sweet potato. Bull. N.J. Agric. Exp. Sta. 76:7-14. Accessed on: Feb, 2th, 2024

Hawkins, Nichola J., Chris Bass, Andrea Dixon, Paul Neve. (2018)The Evolutionary Origins of Pesticide Resistance. *Biological Reviews*, vol. 94, no. 1, pp. 135–155, https://doi.org/10.1111/brv.12440.

Huseth, A. S., Chappell, T. M., et al. (2016). *Frankliniella fusca* resistance to neonicotinoid insecticides: an emerging challenge for cotton pest management in the eastern United States. *Pest management science*, *72*(10), 1934-1945.

NC State Extension (2018) "Chapter 8: Crop Production Management - Sweetpotatoes | Publications." Available at: content.ces.ncsu.edu/north-carolina-organic-commodities-production-guide/chapter-8-crop-production-management-sweetpotatoes.

North Carolina Sweetpotato Commission. "Industry (2021)." Available at: ncsweetpotatoes.com/sweet-potato-industry/ Accessed on: Dec, 20th, 2024

Kemble, J., and Sikora, E. (2019). "Harvesting and Curing Sweet Potatoes." *Alabama Cooperative E*xtension System ANR-1111 https://www.aces.edu/blog/topics/lawn-garden/harvesting-and-curing-sweet-potatoes/

Liu, Z. Tan J., Valles S., and Dong K. (2002) Synergistic interaction between two cockroach sodium channel mutations and a tobacco budworm sodium channel mutation in reducing channel sensitivity to a pyrethroid insecticide." *Insect Biochem. and Molecular Bio.*, vol. 32, no. 4, pp. 397–404, https://doi.org/10.1016/s0965-1748(01)00116-3.

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Markow, Therese A., and O'Grady, Patrick M., (2006). "Life History Variation." *Drosophila A Guide to Species Identification and Use*, I, In: Elsevier Academic Press, pp. 189–198.

McComic, Sarah E., Kumudini Meepagala. M., and Daniel Swale. R., (2021) "Characterization of Toxicological and Neurophysiological Effects of Natural Product Based Chromenes to Fall Armyworm, *Spodoptera Frugiperda.*" *J. of Econ. Entomol.* vol. 114, no. 6, pp. 2485–2492,

Mertz, Robert W., Hesler, Stephen, Pfannenstiel, J. Luke, Norris, H. Rachel, Loeb Gregory, Scott .G. Jeffrey (2021). "Insecticide Resistance in Drosophila Melanogaster in Vineyards and Evaluation of Alternative Insecticides." *Pest Management Science*, 19 Dec. 2021, https://doi.org/10.1002/ps.6745.

Indiana University (2021). *Bloomington Drosophila Stock Center*. Molasses Fly diet . https://bdsc.indiana.edu/information/recipes/molassesfood.html
Accessed on: June, 1st, 2024

Rosenheim, Jay A., and Marjorie A. Hoy (1989). "Confidence Intervals for the Abbott's Formula Correction of Bioassay Data for Control Response." J. Econ. Entomol. vol. 82, no. 2, 1 Apr. 1989, pp. 331–335, https://doi.org/10.1093/jee/82.2.331. Accessed 1 June. 2025.

Sapakhova Z., Raissova N., Daurov D., Zhapar K., Daurova A., Zhigailov A., Zhambakin K., Shamekova M. (2023). Sweet Potato as a Key Crop for Food Security under the Conditions of

Global Climate Change: A Review," *Plants*, vol. 12, no. 13 : p. 2516, doi: 10.3390/plants12132516.

Scruggs, A. C., Basaiah, T., Adams, M. L., and Quesada-Ocampo, L. M. (2017). Genetic Diversity, Fungicide Sensitivity, and Host Resistance to *Ceratocystis fimbriata* Infecting Sweetpotato in North Carolina. *Plant Disease*, vol. 101, no. 6, pp. 994–1001, https://doi.org/10.1094/pdis-11-16-1583-re. Accessed 22 March. 2024.

Somvanshi, Pramod R., and Kareenhalli V. Venkatesh (2013). Hill, A.V. (1910) "Hill Equation." *Encyclopedia of Systems Biology*, 2013, pp. 892–895, link.springer.com/referenceworkentry/10.1007%2F978-1-4419-9863-7_946, https://doi.org/10.1007/978-1-4419-9863-7_946.

Stahr, M., and Quesada-Ocampo, L. M. (2020). Assessing the role of temperature, inoculum density, and wounding on disease progression of the fungal pathogen *Ceratocystis fimbriata* causing black rot in sweetpotato. Plant Dis. 104. https://doi.org/10.1094/PDIS-12-18-2224-RE Web of Science

Stahr, M. N. (2021, June 11). *Black Rot of Sweetpotato: Epidemiology, Dispersal, and Detection of Ceratocystis fimbriata.* Ncsu.edu. https://www.lib.ncsu.edu/resolver/1840.20/38903

Stahr, M., Lytle, A., Avila, K., Huseth, A. S., Bertone, M., and Quesada-Ocampo, L. M. (2024). Drosophila hydei as a potential vector of *Ceratocystis fimbriata*, the causal agent of sweetpotato black rot, in storage facilities. *Phytopathology*, 114(7), 1664-1671. https://doi.org/10.1094/phyto-09-23-0328-r

(USDA NASS) United States Department of Agriculture - National Agricultural Statistics Service (2025). Quick Stats Database. Available at: https://www.nass.usda.gov/Quick_Stats/ Accessed: 19 May, 2025.

(US EPA) United States Environmental Protection Agency (2018). Slowing and Combating Pest Resistance to Pesticides. Available at: www.epa.gov/pesticide-registration/slowing-and-combating-pest-resistance-pesticides.
Accessed: 19 May, 2025.

Wheeler, D. A., Hamblen-Coyle, M. J., Dushay, M. S., and Hall, J. C. (1993). Behavior in Light-Dark Cycles of Drosophila Mutants That Are Arrhythmic, Blind, or Both. *Journal of Biological Rhythms*, vol. 8, no. 1, pp. 67–94, https://doi.org/10.1177/074873049300800106.

Quesada-Ocampo, Lina (2018). "Black Rot of Sweetpotato | NC State Extension Publications 2018." *Content.ces.ncsu.edu*, content.ces.ncsu.edu/black-rot-of-sweetpotato Accessed on: Dec, 20th, 2024

TABLES

Table 1.0 Bioassay concentrations used to evaluate *Drosophila hydei* resistance to pyrethrum**.**

Technical Grade Pyrethrum (mg)	Acetone (mL)	Percent of Technical Grade Pyrethrum	ppm
20	100	2%	200
16	100	1.6%	160
11	100	1.1%	110
6	100	0.6%	60
2	100	0.2%	20
0	100	0	0

Table 1.1 Estimated median LC50 values (ppm) for populations collected from four facilities. Please note, the susceptible colony (1508-1641.68 Arizona) was obtained from the Cornell Drosophila repository.

Facility	Management	Month	LC50 (95% CI)	DF	RR50	N Flies
Facility 1	Conventional	November	135.58 (121.22-149.55)	28	5.42	405
		March	124.96 (91.07-158.85)	21	4.56	405
		March	114.61 (94.62-134.60)	21	5.70	405
		May	148.56 (136.01-161.11)	21	5.60	405
		July	179.33 (158.78-199.87)	21	4.97	405
	Organic	May	108.46 (85.96-130.96)	21	4.34	405
	Outside	March	99.67 (85.52-113.81)	21	3.99	405
		May	101.17 (88.16-114.18)	21	4.05	405
		July	132.05 (117.96-146.14)	21	3.61	405
Facility 2	Conventional	November	56.09 (48.55-63.63)	28	2.54	405
		March	93.71 (66.86-120.57)	21	3.75	405
		May	84.34 (58.48-110.19)	21	3.37	405
		July	105.77 (94.46-117.07)	21	3.86	405
	Organic	March	76.28 (66.76-85.80)	21	3.05	405
		May	66.26 (47.89-84.62)	21	2.65	405

Table 1.1 (continued).

Facility	Management	Month	LC50 (95% CI)	DF	RR50	N Flies
	Outside	March	42.47 (31.26-53.68)	21	2.32	405
		July	171.61 (148.51-194.70)	21	6.30	405
Facility 3	Conventional	November	77.39 (64.77-90.01)	28	3.10	405
		March	60.36 (50.04-70.67)	21	2.41	405
		May	89.45 (61.95-116.95)	21	3.58	405
		July	63.99 (52.50-75.38)	21	2.82	405
	Outside	March	108.99 (84.42-133.55)	21	4.50	405
		May	87.66 (70.06-105.27)	21	3.51	405
		July	104.35 (88.62-120.07)	21	4.35	405
Facility 4	Conventional	November	42.53 (37.70- 47.34)	28	1.70	405
		May	41.05 (30.42-51.68)	21	2.60	405
		July	138.52 (127.31-149.74)	21	5.41	405
	Outside	March	101.94 (88.09-115.79)	21	3.64	405
		May	90.76 (53.19-128.31)	21	3.63	405
		July	103.03 (79.74-126.31)	21	4.54	405
Lab Population	-		25.94	21	1.04	405

Table 1.2 Two-way ANOVA statistical values testing the relationship between average *D. hydei* mortality and the predictor variables Facility and Month (month in which sample was collected).

Predictor	**df**	***F*-value**	***P*-value**
Dose	1,43	246.03	< 0.0001
Facility	3,32	49.27	< 0.0001
Month	2,32	14.63	< 0.0001
Facility × Month	6,32	5.88	0.0003

Table 1.3 Two-way ANOVA statistical values testing the relationship between average *D. hydei* mortality and the predictor variables Facility and Management Strategy (i.e., conventional insecticide vs organic insecticide program).

Predictor	**df**	***F*-value**	***P*-value**
Dose	1,27	216.74	< 0.0001
Management	1,24	1.77	0.1960
Facility	1,24	8.99	0.0062
Facility × Management	1,24	0.43	0.5186

Table 1.4 Two-way ANOVA statistical values testing the relationship between average *D. hydei* mortality and the predictor variables facility and collection location, either within conventionally managed storage rooms or outside surrounding the facility.

Predictor	**df**	***F*-value**	***P*-value**
Dose	1,83	1614.32	< 0.0001
Collection Location	1,76	0.64	0.4267
Facility	3,76	4.09	0.0095
Facility × Collection Location	3,76	3.86	0.0126

FIGURES

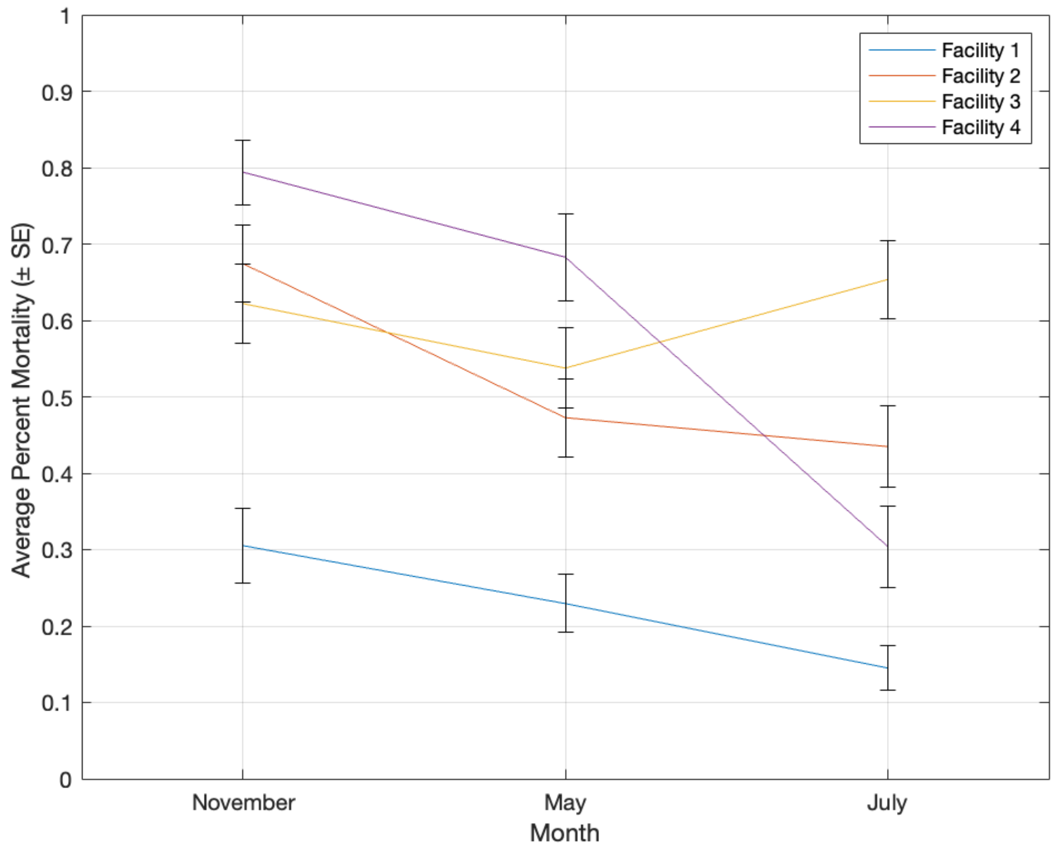

Figure 1.1 SAS least squares means for facility and sample window with means separations. Shows trends in mortality over the eight month storage duration. Means separation was conducted using Tukey HSD (α = 0.05).

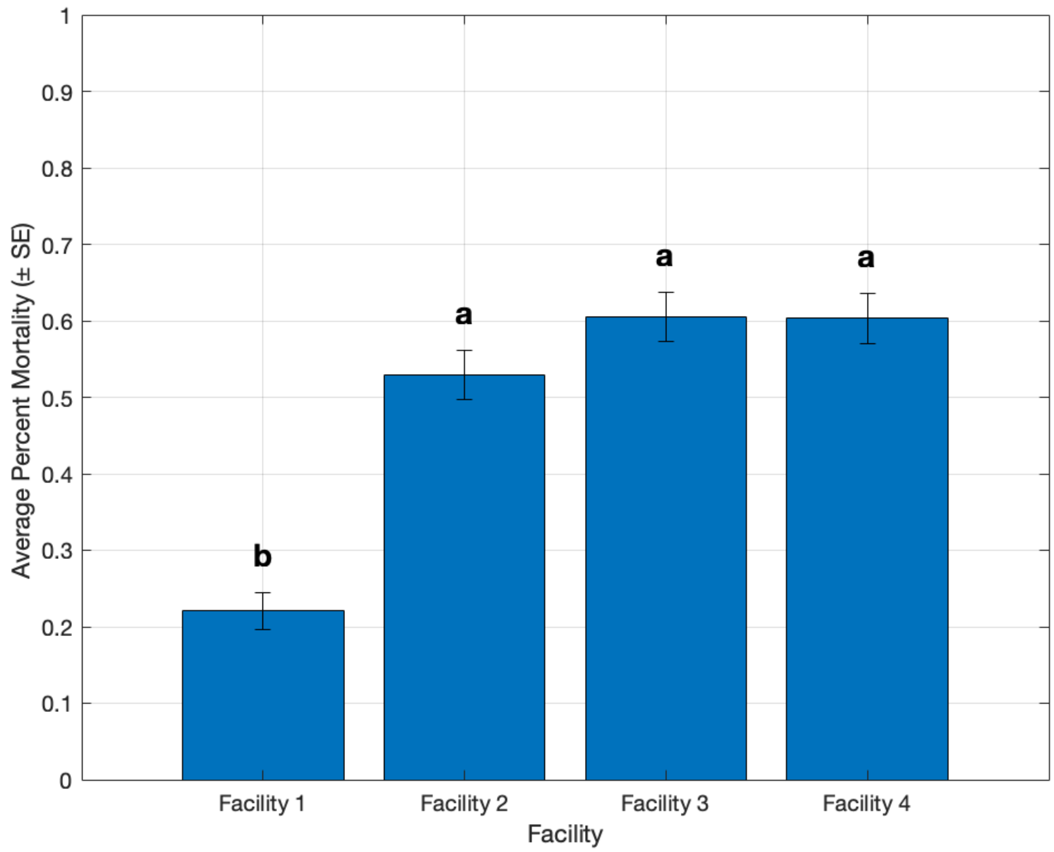

Figure 1.2 Average percent *D. hydei* mortality among four sweetpotato facilities where roots are stored. Means separation conducted with Tukey HSD (α = 0.05).

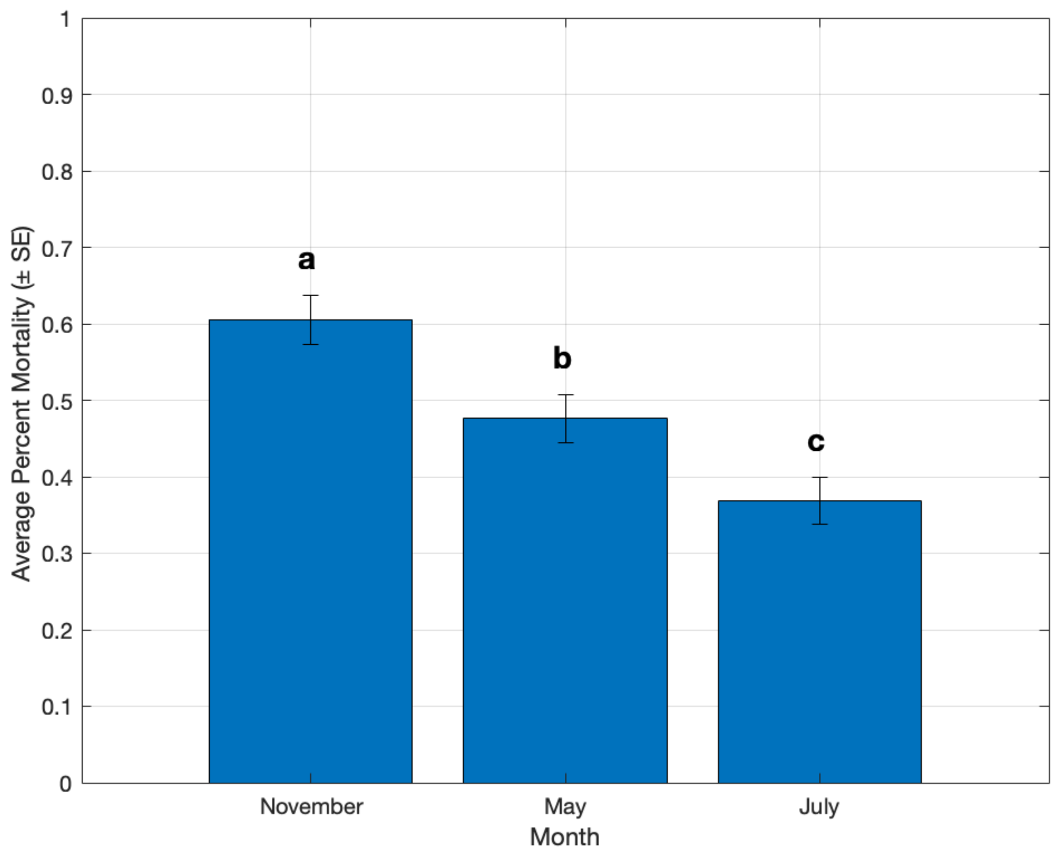

Figure 1.3. SAS Facility x Window least squares means with letters from Tukey analysis. Changing susceptibility to pyrethrum over sampling windows occurring during the sweetpotato storage cycle. Means separation conducted with Tukey HSD (α = 0.05).

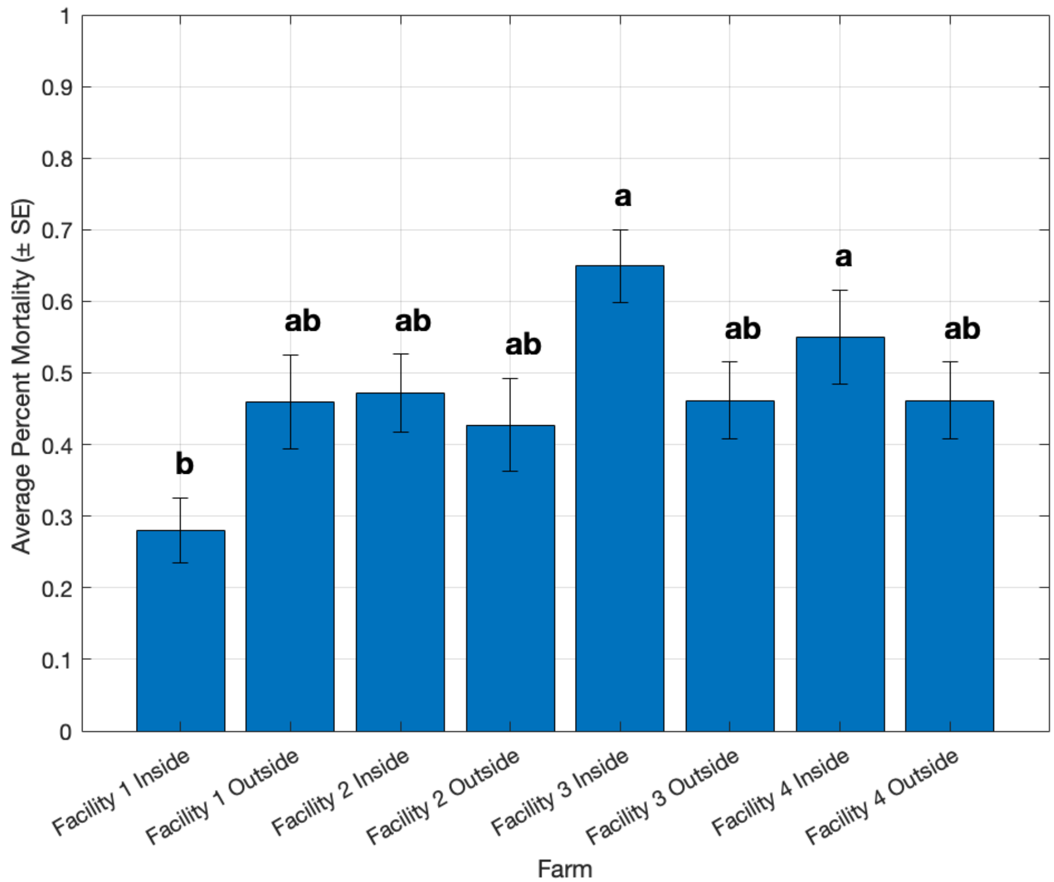

Figure 1.4. Susceptibility differences among sampled facilities for populations collected inside conventionally managed sweetpotato storage rooms and outside in open spaces. Means separation conducted with Tukey HSD (α = 0.05).

CHAPTER 2

Characterizing *Drosophila* spp. Bait Preference to Optimize Population Monitoring Tools in Sweetpotato Storage Facilities

Abstract

Drosophila hydei is a destructive vinegar fly pest within sweetpotato storage houses in North Carolina because of their ability to mechanically transmit *Ceratocystis fimbriata,* a fungal pathogen of sweetpotato roots. Limited information about *D. hydei* population dynamics exists in sweetpotato storage facilities, which undermines the development of effective management practices. To address this knowledge gap, we conducted behavioral choice experiments to assess the most attractive bait for this species, a first step toward improved monitoring. To do this, laboratory-reared flies were placed into a sealed tub with bottle traps containing one of four baits: 1) mashed banana and yeast, 2) fermented sugar water with yeast, 3) apple cider vinegar, and 4) a water control. The number of flies attracted to each bait was counted after 24 hours. The most effective bait from this experiment was a banana yeast mixture. A field experiment was also conducted to compare bait preference between laboratory and field conditions. Field bait assays included the same baits as the laboratory study, distributed across 20 storage rooms in two different replications (N=40 replications total). A sticky card was also placed in each room to passively assess *Drosophila* spp. abundance and total arthropod species diversity. All arthropods were identified and quantified in the various bait traps. The field bait assay showed that apple cider vinegar was the most preferred bait for *D. hydei* and *D. funebris,* which were the two most common *Drosophila* spp. in sweetpotato storage facilities. In contrast to a previous study of *Drosophila* spp. in NC sweetpotato storage, our study found that *D. funebris* was the most common species. Further research will be needed to assess *D. funebris* and its impact on

sweetpotatoes. Determining the optimal bait combination will enable synthesis of chemical baits for population monitoring and management in sweetpotato storage.

2.1 Introduction

Current pest management strategies in sweetpotato storage facilities rely on intensive prophylactic insecticide use to manage *Drosophila* spp. pest populations. *Drosophila* spp. are the most common pest within sweetpotato storage houses (Stahr et al. 2024). Evidence suggests that chemical control effectiveness has eroded due to the intensive use of pyrethrum within storage facilities (Phillips 2025 - Chapter 1). In any integrated pest management (IPM) program, the integration of multiple control methods is beneficial to alleviate reliance on pesticides, especially for *Drosophila* spp. because of their rapid reproductive rate (Tait et al. 2021). Recently, *D. hydei* was documented as a mechanical vector of *Ceratocystis fimbriata* (black rot) within storage houses (Stahr et al. 2024). This prolific pathogen causes immense damage in sweetpotato facilities (Scruggs et al. 2017). Controlling *D. hydei* is vital to minimize the spread of black rot in sweetpotato facilities, but the approaches to fly monitoring to inform application timing remain understudied (Stahr et al. 2024).

Effective monitoring is the cornerstone for a successful IPM program because it is important to detect pest populations before economic thresholds are reached and effective control actions can be implemented (Tait et al. 2021). Bait traps are an effective tool for monitoring *Drosophila* spp., such as *Drosophila melanogast*er and *Drosophila suzukii* (Iglesias et. al, 2014). In the sweetpotato storage system, monitoring approaches are not used, which leads to intensive insecticide pyrethrum applications which are costly to the grower and elevate risk of resistance development (Phillips 2025 - Chapter 1). Optimizing baiting techniques is the first step toward more effective population sampling that could be used to develop threshold-based management programs tailored to sweetpotato storage facilities. Specifically, using bait traps to estimate population density could refine pesticide application timing, which lessens the possibility of resistance. It is known that the effectiveness of a trap is dependent on several factors such as color, size, attractant, and ease of handling (Iglesias 2014).

Drosophila suzukii is an invasive pest from eastern Asia threatening the U.S. berry industry (Iglesias et. al, 2014), and it represents a reasonable model framework to design trap research targeting sweetpotato pests within the same genus. Previous studies have focused on developing optimized monitoring tools for *D. suzukii* (Iglesias et. al, 2014). Different techniques for optimizing monitoring tools for *D. suzukii* include varying color attractants or bait attractants. One study used a yellow card in a plastic trap as a color attractant (Iglesias et. al, 2014), while others investigated a wide variety of baits, such as apple cider vinegar and different types of yeasts, to target certain flies (Batista et al. 2017). We know that the esters produced by the yeast during the fermentation of fruits are attractive to many species of insects (Batista et al. 2017). Generally, *Drosophila* spp. use yeasts as a source of food for the larvae, nutrients for egg production, and as nuptial gifts in courtships (Hamby et al. 2012). *Drosophila* are equipped with a sophisticated olfactory sensory system that permits them to recognize and discriminate hundreds of discrete odorants (Vosshall 2000). Fermentation products have been used for many years to attract other *Drosophila* spp., including ethanol, acetic acid, and methanol (Liburd et al. 2013). By optimizing bait preference in *D. suzukii* a robust monitoring system was developed. Iglesias et al. (2014) found that *D. suzukii* was best monitored using a basic cup trap, without visual aids, with a yeast bait, such as yeast + sugar and yeast + flour. This was instead of using vinegar-based traps. This research helps growers estimate *D. suzukii* population density in their fields to inform spray decisions. Growers may use vinegar baits over yeast mixtures because it is less messy to set up and serves as an acceptable bait for monitoring.

Another study used sticky cards as a way of estimating species diversity and density within sweetpotato storage houses (Stahr et al. 2024). Based on sticky card data, *D. hydei* was found to be the most prominent *Drosophila* spp. in four counties in these storage facilities in North Carolina. The findings from this study were used to inform the development of the current study as we sought to improve management strategies for this prolific pest.

Currently, no economic threshold has been developed for *D. hydei* in sweetpotato storage houses. The economic threshold is when the pest density reaches a point at which management action should be taken to prevent an increasing pest population from reaching the economic injury level (Hunt 2024). The economic injury level refers to the smallest number of insects (amount of injury) that will cause yield losses equal to the insect management costs (Hunt 2024). For *D. hydei* little to no information exists on bait preference, population density patterns, or an economic spray threshold. Defining an economic threshold for this species will first require a firm understanding of the relationship between monitoring trap data and to *D. hydei* population dynamics in sweetpotato storage houses. To address this knowledge gap, we conducted a behavioral choice experiment in the laboratory to assess the most attractive bait for this species, which is a first step toward improved field monitoring. Determining the optimal bait combination will enable synthesis of easy-to-use chemical baits to improve population monitoring in sweetpotato storage. To assess bait preference among common *Drosophila* species that are present in sweetpotato storage facilities, a field study was conducted on behavioral choice experiments to assess the most attractive bait for various *Drosophila.* We hypothesized that the banana yeast mixture would be the most preferred bait because it contained fruit that was actively fermenting, making it microbially rich compared to other baits that were already fermented (e.g. apple cider vinegar) (Batista et al. 2017).

2.2 MATERIALS AND METHODS

2.2.1 Trap Design and Deployment

The four different baits used in these study were apple cider vinegar, banana and yeast mixture, fermented sugar water, and water as a control. The apple cider was sourced from a local grocery store (1 Gallon (3.79 L.) Food Lion Apple Cider Vinegar, Non-GMO, Jug, Food Lion , Salisbury NC). The banana mixture was composed of 35 blended bananas, roughly 5 kilograms, that are approximately a seven on the ripeness scale (USDA 2004). Each banana bait cup was sprinkled with yeast on top, roughly 5 grams yeast per batch of 35 blended bananas. The fermented sugar water mixture was composed of fermented sugar, yeast and water; 3.7 liters of water was mixed with 185 grams of sugar and 61.6 grams of yeast. This mixture is fermented and aged for 2 months before implementation in a dim room at 23°C. Bait traps were constructed using either 1 L or 2 L plastic bottles [1 L (33.81 oz.) Clear PET Smooth Water Bottle with 28 mm PCO Neck, US Plastics Corp, Lima Ohio] [2 L (67.62 oz.) Food Lion 2L Soda, Food Lion , Salisbury NC]. To construct the trap, the top of the bottle was cut off, inverted, and inserted back into the bottom section of the bottle. This created a funnel for the flies to enter but to prevent them from escaping. Each trap contained a bait cup filled with approximately 50 mL of the selected bait. The bait cup is assembled with an 89 mL plastic cup and two lids: one a porous lid to contain the bait and a lid used as the base of the bait cup, hot glued to the bottom of the cup for stability. The bait cup was inserted into the base of the bottle before the top was inverted and taped to secure the connection.

2.2.2 Bait Assay Peference Laboratory Experiments

The four baits were tested with laboratory reared *D. hydei* in a pairwise choice experimental design over a 24 hour interval. Constructed bottle traps were placed in an 18 1/2" x

14" x 11" storage container (Sterilite Plastic Letter-Size File Box 2025). One hundred adult D. *hydei* were introduced to each container. Fly colonies were collected from sweetpotato storage houses following the methods outlined in Chapter 1 and reared for experimentation using rearing techniques mentioned in section 1.2.1 Insect Collection and Colony Maintenance. The behavioral choice assay consisted of 30 replications of each pair-wise comparison using 2 L bottle traps baited with either apple cider vinegar, banana and yeast mixture, fermented sugar water, or water (four bottle treatments per container). After a 24 hour choice period the containers were placed in a walk-in cooler at approximately 4.4°C to slow fly activity. After 20 minutes of cooling, the container was opened, and each bottle trap was plugged to prevent the flies from escaping. Individual traps were filled with carbon dioxide using a flow pad and gun to anesthetize the flies (Benchtop Flow Buddy Complete System, #59-122 BCU, Genesee Scientific, Morrisville, NC USA). Flies from individual traps were poured on to the flow pad and counted with a paintbrush. The data was then analyzed using a one way ANOVA test in SAS (SAS Institute Inc., Cary, NC). Means separation was conducted using the Tukey-Kramer method, and significance was determined at $P < 0.05$ to compare bait preference (Fig. 2.2).

2.2.3 Bait Assay 1 L and 2 L Bottle Comparison

We found that 2 L bottles were logistically challenging to deploy for field experiments due to their volume when moving them in large numbers. To optimize field deployment, we evaluated the efficacy of smaller 1 L vs 2 L bottles in a controlled pairwise laboratory experiment prior to field deployment. To test if there was a significant difference between the two bottle traps, one, one-liter trap and one two-liter trap were placed in a container with 100 flies for 24 hours. Each bottle was baited with 50ml of banana-yeast bait to standardize attraction among traps. The banana with yeast bait was selected because it was the most preferred bait from the lab bait preference experiments. Thirty replications of paired bottle comparisons were completed then analyzed with a paired t-test to test for differences between bottle types (Fig. 2.3).

2.2.4 Bait Preference in Sweetpotato Storage Facilities

To complement controlled bait preference experiments in the laboratory, field experiments were used to compare preferences of flies at two different storage facilities. One liter (1 L) soda bottle bait traps were used for field experiments; each baited with the standard 50 mL of bait. For each experimental replication, four bait traps, including a water control, were placed in a sweetpotato storage room for 48 hrs. After that period, traps were closed and returned to the laboratory for counting and identification. Placement of the traps was randomized using base R (R Core Team 2000). Traps were placed on the ground at a minimum of 3 m apart to limit confounding attraction. A sticky card was placed in each room as a reference for the non-bait density (Fig. 2.1). Each trap and vial were labeled with repetition number, experimental window, bait, and placement. Sticky cards were placed in clear binder sheets then refrigerated for preservation. Specimens on the sticky cards were identified and quantified. A total of 10 storage rooms were sampled at two different facilities. This experiment was completed twice (creating two experimental windows separated by seven days) for a total of 40 bait preference experimental replications. After the numerous traps were collected and closed, they were put into a walk-in cooler for a minimum of 48 hours to kill and preserve the flies. A rather than a freezer, walk in cooler was used because the traps would not fit in a freezer. The traps were deconstructed, rinsed with ethanol, and poured into a 20 mL vial to preserve the flies for quantification and species identification. The *Drosophila* were identified to species and other arthropods were identified to various levels depending on the group and its difficulty to identify

(Table 2.1). If a sample exceeded 100 flies, a subsample of the arthropods was taken, and total arthropods were noted (data not shown). To ensure a subsample was representative of a sample, the sample was mixed/shaken then arthropods were scooped out of the sample onto a petri dish for identification and quantification. Estimated counts of species from subsamples were determined by the number of species in the subsample multiplied by number of flies total, divided by the number of flies subsampled.

2.2.5 Statistical Analysis

Data analysis was completed using a one way ANOVA in R (version 4.4.1, R Core Team 2025) to test differences in bait preference when *D. hydei* were provided a choice under laboratory conditions. Means separation was conducted using Tukey HSD ($\alpha = 0.05$) with the *emmeans* package (Lenth 2025). Because we wanted to improve the efficiency of trap deployment in the field, we also tested differences between 1 L and 2 L trap designs under the same laboratory conditions using a paired t-test in R. To detect differences in *Drosophila* spp. bait efficiency in the field study, we analyzed the 48-hour bait capture using a two-way ANOVA. The model included as main effect factors bait type, storage house, sampling period, replication, and we also assessed the interaction between bait and storage house. Because *D. funebris* comprised 83% of the total trap capture, we chose to pool all *Drosophila* species into a single predictor variable for analysis in R. Means separation was conducted using Tukey HSD ($\alpha = 0.05$) with the *emmeans* package (Lenth 2025). Summary statistics were generated using the *plyr* package in R. Figures were generated using a combination of MATLAB and *ggplot* in R.

2.3 RESULTS

2.3.1 Laboratory Bait Preference Comparison

Drosophila hydei bait preference was significantly different when provided a choice in the laboratory arenas (F = 24.26, df = 3,140, P < 0.0001; Fig. 2.2). We observed that the banana and yeast mixture was the preferred bait for *D. hydei* when provided a choice (Fig. 2.2). However, banana and yeast was not statistically different from apple cider vinegar, suggesting that either bait could be used for monitoring in the field (Fig. 2.2). An average of 44.61% (± 3.79 SE) of flies tested across all experimental replications did not make a bait choice.

2.3.2 1 L and 2 L Trap Comparison

The number of flies caught between one liter and two-liter bottle were statistically different (t = 3.47, df = 35, P = 0.0014; Fig. 2.3). Similar to choice experiments above (Section 2.4.1), 50.6% (±3.8 SE) of flies in the test made no choice between paired bottles on average. The similarity of these results suggest that baited bottle traps may underestimate local *D. hydei* populations by approximately half; however, additional studies are needed to relate absolute population densities to observed trap capture under realistic field conditions. Comparison of the estimated effect shows that the 1 L is more efficient capturing flies than the 2 L bottle when equivalent baits are used (Fig. 2.3). This result confirms that the smaller bottle is an adequate device for field bait evaluation.

2.3.3 Bait Assay Preference Field Experiments

The field study showed that the apple cider vinegar bait was most preferred by the *Drosophila* spp. in the storage houses (Fig. 2.5). The diversity of species per bait is shown in Table 2.2. *Drosophila funebris* was the dominant *Drosophila* spp. at the sampled facilities (Table 2.2; Fig. 2.4). Dark-winged fungus gnats (Diptera: Sciaridae) were the dominant arthropod taxa caught on sticky cards (Fig. 2.6). Interestingly, we observed clear differences between capture frequency on sticky cards when compared to average bait capture (Figs. 2.4 & 2.6). Moreover, the composition of the species caught differed markedly. The dominant species in bait traps, *D. funebris,* comprised only 7% of total arthropod abundance on the sticky card traps ($N = 2{,}614$ total arthropods sampled).

A simple regression approach was used for testing the effect of bait, storage room, replication, and the interaction between bait and house. The main effects for bait type were significant ($F = 37.91$, df = 3,78, $P < 0.01$) and storage room were significant ($F = 7.82$, df = 19,78, $P < 0.01$). However, the interaction between both factors was not significant ($F = 1.35$, df = 3,78, $P = 0.11$). We also did not observe a significant effect of the sample window ($F = 1.23$, df = 1,78, $P = 0.27$). These results suggest that the response to baits does not differ among storage rooms despite considerable variation in population densities observed across rooms (Table 2.4). We observed clear differences between baits that show apple cider vinegar, and the banana and yeast mixture baits were superior to fermented sugar baits (Figs. 2.2 & 2.5).

2.4 DISCUSSION

Choice assays showed that the banana and yeast mixture was the preferred bait for attracting *D. hydei* under controlled laboratory conditions (Fig. 2.2). However, there was considerable variation in treatment choice which resulted in statistically similar results between banana and yeast mixture and apple cider vinegar (Fig. 2.2). There was a statistically significant reduction in trap catch when comparing fermented sugar or water only controls to the banana and yeast mixture. These results suggest that under controlled situations, either banana and yeast mixture or apple cider vinegar could be a reasonable baits for *D. hydei* monitoring. We also observed significant differences in trap efficiency depending on bottle size used (Fig. 2.3). In the laboratory choice assay we used 2 L bottle traps with 50 mL of bait while the field bait assay used 1 L bottles with an equivalent bait amount. In the pairwise comparison of bottle size in the laboratory, the one liter bottles caught more flies than the 2 L bottles (Fig. 2.3). This result suggests that a 1 L bottle for field monitoring may more effectively estimate population densities due to more efficient capture while alleviating logistical challenges related to transporting larger bottles. It is unclear why flies preferred the smaller bottle. However, it is possible that a greater concentration of fermentation odors may occur in the smaller diameter 1 L bottle trap opening leading to increased attraction. In the field bait assay, apple cider vinegar bait was most preferred among *Drosophila* (Fig. 2.5). *Drosophila hydei* preferred apple cider vinegar the most (Table 2.2). Each bait was not significantly different except for water in the field and laboratory assay based on Tukey *post hoc* pairwise treatment comparisons (Figs. 2.2 & 2.5).

Apple cider vinegar or other vinegar products have been previously reported as effective at attracting *Drosophila* spp. baits in previous bait studies (Iglesias et al. 2014). *Drosophila* are often attracted to fermentation products for their microbial content as a food source and oviposition site (Hamby et al. 2012). Since all the baits are significantly different, it can be concluded that utilizing any fermentation or vinegar bait will effectively attract *Drosophila* spp. present in sweetpotato storage rooms. Apple cider vinegar was the most preferred bait in the field study, making it the most effective monitoring tool in sweetpotato storage facilities.

A wide variety of arthropods were caught in bait traps or sticky cards, providing an estimate of species diversity and abundance within sweetpotato storage rooms. We documented 13 groups of arthropods across 20 different storage rooms in two different facilities (Table 2.1). A previous study also reported a wide variety of species within storage facilities (Stahr et al. 2024). Cleanliness of the facility (or the lack thereof) may contribute to the presence of storage pests as well as a variety of nuisance species that seek out pooling water or moist environments. Facilities often have a wash station that expels grey water to a pooling area along with organic debris. The area around the facility is often muddy or wet due to washing and transport of sweetpotato bins which may provide additional habitat for these species. Some species diversity could be attributed to urban pests such as Blattodea or Psocodea feeding on residues in the storage bins or other organic debris. The species diversity may also relate to the number of crops the facility stores. One of the sampled storage facilities processes sweetpotatoes, a variety of greens, and watermelon, all of which could attract a greater variety of species. Facility location and landscape may also play a factor as sweetpotato storage bins are taken inside and outside of the storage rooms, giving local insects an opportunity to move into the rooms via bins.

Sampling across two North Carolina counties, *D. funebris* was the most prominent *Drosophila* spp. detected in the field bait traps where it accounted for 64% of arthropods captured (Table 2.2; Fig. 2.4). *Drosophila funebris* accounted for 64% of arthropods captured. *Drosophila hydei* composed 10% of total diversity while Sciaridae and "other" composed 12% (N=64,128 of total arthropods sampled in bottle traps). Sticky cards reported different numbers of the different species. Sciaridae were the most common arthropods caught on sticky cards at 66% percent of captured specimens; in bait traps they were much less common (Fig. 2.4 & 2.6). "Other" athropods accounted for 19% of sticky card specimens, while D. *hydei* accounted for 8% and *D. funebris* accounted for 7% (N = 2,614). A previous study that was conducted within sweetpotato storage rooms reported that *D. hydei* was the most prominent *Drosophila* spp. across four North Carolina sweetpotato facilities. In those experiments, *Drosophila hydei* accounted for 67.55% of the samples, where *D. funebris* accounted for 9.75% of samples (Stahr et al. 2024)

This study used sticky cards to determine species diversity and density and sampled a total of 1,017 individual arthropods, fitting into 16 broad taxonomic groups (Stahr et al. 2024). Counts for groups other than *Drosophila* were low, ranging from 0.1 to 11.11% of total arthropods sampled (Stahr et al. 2024). The genus *Drosophila* comprised approximately 79% of all specimens recovered ($N = 809$) (Stahr et al. 2024). Because *D. hydei* was the dominant species recovered during the survey it was selected for investigation as the main vector of C. *fimbriata* (black rot) in the current study; however, the sampling strategies used in Stahr et al. (2024) may be biased for *D. hydei*. *D. funebris* was the dominant drosophila species in the storage houses in the current studies. Future research needs to be conducted to determine whether *D. funebris* or Sciaridae can vector black rot in a similar way. The previous study is evidence that different trapping techniques catch different species. It could be that *D. funebris* have different flight behaviors than Sciaridae, making *D. funebris* less likely to be trapped via sticky cards. Sciaridae are also known for being attracted to the color yellow, thus sticky cards are often used to monitor Sciaridae populations (Stukenberg et al., 2018).

The species diversity in each bait provides insight into storage house diversity and bait preference among species (Table 2.2). The water control, caught mostly Sciaridae and a variety of insects that are categorized as "Other". *Drosophila* spp. were not strongly attracted to water in this study. Sciaridae may have been attracted to water because of moisture since they prefer a moist environment for oviposition (Cloyd and Zaborski 2008). The category "Other", consisted of a variety of Diptera, such as Scatopsidae and Psychodidae, that prefer moist environments for their habitats which may explain the larger amount of "Other" in the water bait. Fermented sugar water, banana yeast mixture, and apple cider vinegar baits attracted *Drosophila* spp. with similar proportions for each bait. This result suggests that any of these three baits provide similar relative results across baits. Here, *D. hydei* (7%) was less abundant than *D. funebris* (64%), which differs from results of Stahr et al. (2024). Moreover, we observed small numbers of *Drosophila repleta* Wollaston, 1858 (Diptera: Drosophilidae) and *D. melanogaster* in the three baits.

In conclusion, this study found that apple cider vinegar bait was the most preferred by *D. hydei* in the field bait assay. *Drosophila* spp. in general preferred it the most, including *D. funebris,* which was found in large numbers in the sweetpotato storage facilities. The full impact of the *Drosophila* species complex within sweetpotato facilities is unknown. This begs the question: what is the role of multiple *Drosophila* in disease transmission in storage facilities. Specifically, further research is necessary to determine if *D. funebris* is a competent vector of *C. fimbriata*. *Drosophila hydei* was first investigated as a carrier for *C. fimbriata* because it was the most abundant based on sticky card traps at a single time point (Stahr et al. 2024). Here, we clearly show that other similar species may need to be considered as potential vectors as well. An optimal bait such as apple cider vinegar may enable preparation of easy to use chemical baits that can be used to improve population monitoring in sweetpotato storage houses. Farmers could then use this information to inform management practices to effectively manage fly populations and suppress *C. fimbriata* disease movement.

REFERENCES

Batista, M. R. D., Uno, F., Chaves, R. D., Tidon, R., Rosa, C. A., and Klaczko, L. B. (2017). Differential attraction of drosophilids to banana baits inoculated with *Saccharomyces cerevisiae* and *Hanseniaspora uvarum* within a Neotropical forest remnant. *PeerJ*. vol. 5, no. 9: pp. e3063–e3063, https://doi.org/10.7717/peerj.3063 Accessed 29 Mar. 2025.

eFly SWD Working Group (2012). Spotted wing drosophila impacts in the eastern United States. Available at: http://www.sripmc.org/WorkingGroups/eFly/Impacts%20of%20SWD%20in%20the%20Eastern%20US%202012.pdf. Accessed on: March, 17th, 2024

Cloyd, R. A., and Zaborski, E. R. (2004). Fungus Gnats, *Bradysia* spp. (Diptera: Sciaridae), and Other Arthropods in Commercial Bagged Soilless Growing Media and Rooted Plant Plugs. J. of Econ. Entomol. vol. 97, no. 2 : pp. 503–10, https://doi.org/10.1603/0022-0493-97.2.503. Accessed 16 Apr. 2025.

Hamby KA, Hernández A, Boundy-Mills K, Zalom FG (2012) Associations of Yeasts with Spotted-Wing Drosophila (*Drosophila suzukii*; Diptera: Drosophilidae) in Cherries and Raspberries. Appl Environ Microbiol, vol. 78, no. 14 : pp. 4869–4873 https://doi.org/10.1128/aem.00841-12.

Hawkins, Nichola J., Chris Bass, Andrea Dixon, Paul Neve. 2018. The Evolutionary Origins of Pesticide Resistance. *Biological Reviews*, vol. 94, no. 1: pp. 135–155, https://doi.org/10.1111/brv.12440.

Hunt, Tom (2024). The Economic Injury Level and the Economic Threshold in IPM | CropWatch | Nebraska. *Unl.edu*, 2024, cropwatch.unl.edu/economic-injury-level-and-economic-threshold-ipm/. Accessed on: April, 13th, 2024

Iglesias, L. E., Nyoike, T. W., and Liburd, O. E. (2014). Effect of Trap Design, Bait Type, and Age on Captures of *Drosophila suzukii* (Diptera: Drosophilidae) in Berry Crops. *J. of Econ. Entomol.* vol. 107, no. 4: pp. 1508–1518, https://doi.org/10.1603/ec13538.

Lenth R. (2025). emmeans: Estimated marginal means, aka least-squares means. https://rvlenth.github.io/emmeans/

Liburd, O. E., and Iglesias, L. E. (2013). Spotted Wing Drosophila: Pest Management Recommendations for Southeastern Blueberries. EDIS. vol. 2013, no. 6, https://doi.org/10.32473/edis-in998-2013. Accessed 25 Sept. 2021.

Stahr, M., Lytle, A., Avila, K., Huseth, A. S., Bertone, M., and Quesada-Ocampo, L. M. (2024). *Drosophila hydei* as a potential vector of *Ceratocystis fimbriata*, the causal agent of sweetpotato black rot, in storage facilities. Phytopathology®, 114(7), 1664-1671.https://doi.org/10.1094/phyto-09-23-0328-r

Stukenberg, N., Ahrens, N., & Poehling, H.-M. (2018). Visual orientation of the black fungus gnat, *Bradysia difformis*, explored using LEDs. *Entomologia Experimentalis et Applicata*, *166*(2), 113–123. https://doi.org/10.1111/eea.12652

Tait, G., Mermer, S., Stockton, D., Lee, J., Avosani, S., Abrieux, A., Anfora, G., Beers, E., Biondi, A., Burrack, H., Cha, D., Chiu, J. C., Choi, M.-Y., Cloonan, K., Crava, C. M., Daane, K. M., Dalton, D. T., Diepenbrock, L., Fanning, P., and Ganjisaffar, F. (2021). *Drosophila suzukii* (Diptera: Drosophilidae): A Decade of Research Towards a Sustainable Integrated Pest Management Program. J. of Econ. Entomol., vol. 114, no. 5: pp. 1950–1974, https://doi.org/10.1093/jee/toab158.

[USDA] United States Department of Agriculture – Agricultural Marketing Service. 2004. Bananas: Market Inspection Instructions. Available at: https://www.ams.usda.gov/sites/default/files/media/Bananas_Inspection_Instructions%5B1%5D.pdf Accessed: 24 June, 2025.

Vosshall, Leslie B. (2000) Olfaction in *Drosophila*. Current Opinion in Neurobio., vol. 10, no. 4,:pp. 498–503, https://doi.org/10.1016/S0959-4388(00)00111-2. Accessed on: March, 25th, 2024

TABLES

Table 2.1 Categorization of species. The other category was pooled for subsequent analyses and summary statistics.

Arthropod group	Taxonomic identification
Diptera	*Cecidomyiidae*
	Drosophila hydei
	Drosophila funebris
	Drosophila melanogaster
	Drosophila repleta
	Drosophila sp.
	Psychodidae
	Scatopsidae
	Sciaridae
	Sphaeroceridae
Other	Aleyrodidae
	Aphididae
	Acari
	Araneae
	Blattidae
	Diapriidae
	Latridiidae
	Nitidulidae
	Psocodea
	Tineidae

Table 2.2 Average trap individual taxa per trap (±SE) by treatment across two locations and two experimental replications at each site (N=40 replications). Please note that the sticky card was mounted 1 m above bait traps to passively assess species abundance.

Treatment	***Drosophila hydei***	***Drosophila funebris***	***Drosophila repleta***	***Drosophila melanogaster***	**Sciaridae**
Banana and yeast mixture	7.03 (2.81)	38.85 (6.6)	1.2 (0.55)	1.23 (0.32)	16.05 (10.12)
Apple cider vinegar	8.8 (2.96)	70.6 (18.6)	1.85 (0.49)	0.72 (0.25)	3.6 (1.08)
Fermented sugar solution	8.22 (4.12)	68.62 (34.07)	1.45 (0.79)	0.17 (0.1)	8.5 (2.79)
Water control	3.54 (1.64)	3.44 (2)	0.95 (0.51)	0.18 (0.12)	5.79 (1.67)
Sticky card control	5.3 (2.3)	4.32 (1.36)	0.07 (0.04)	0.25 (0.08)	43.1 (17.24)

FIGURES

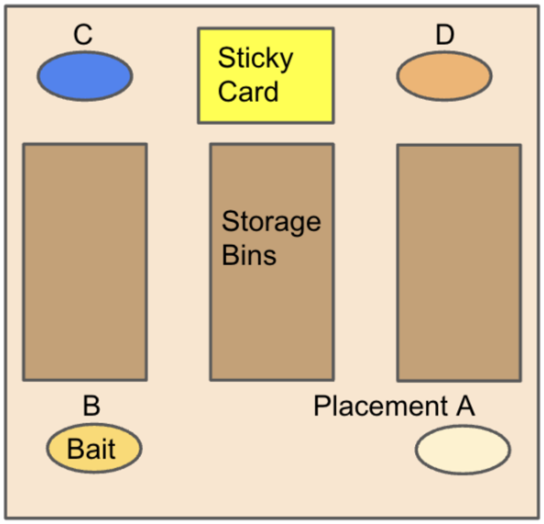

Figure 2.1 Experimental setup in sweetpotato storage room. Four baits were placed randomly in a transect within 20 storage houses across two packing facilities.

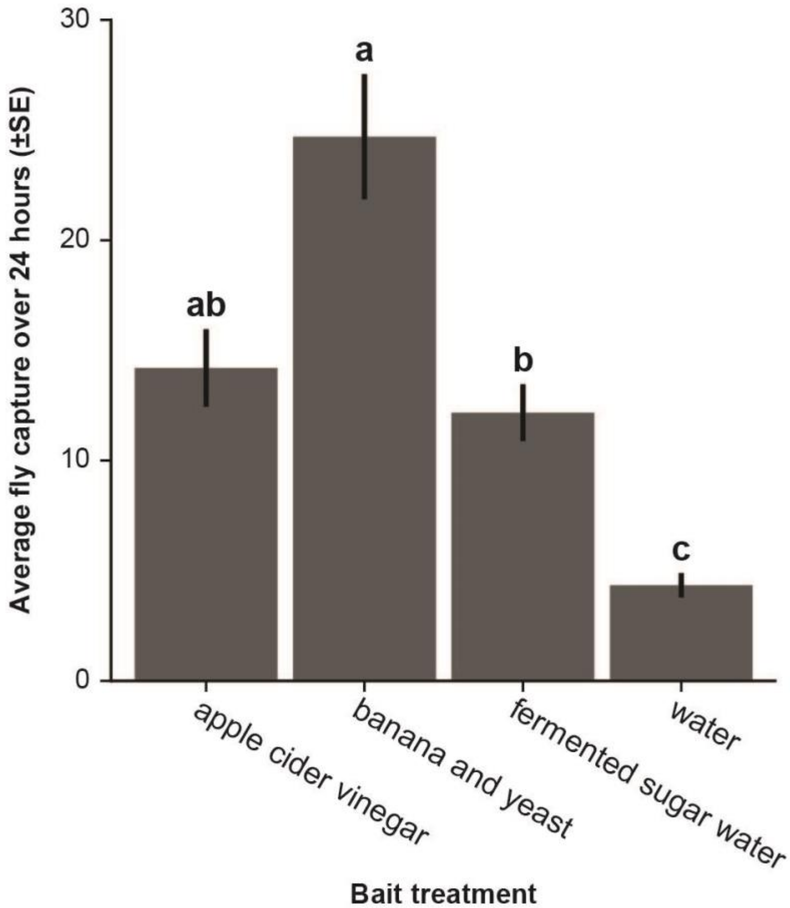

Figure 2.2 *Drosophila hydei* laboratory bait preference choice experiment. Means separation conducted with Tukey HSD (α = 0.05).

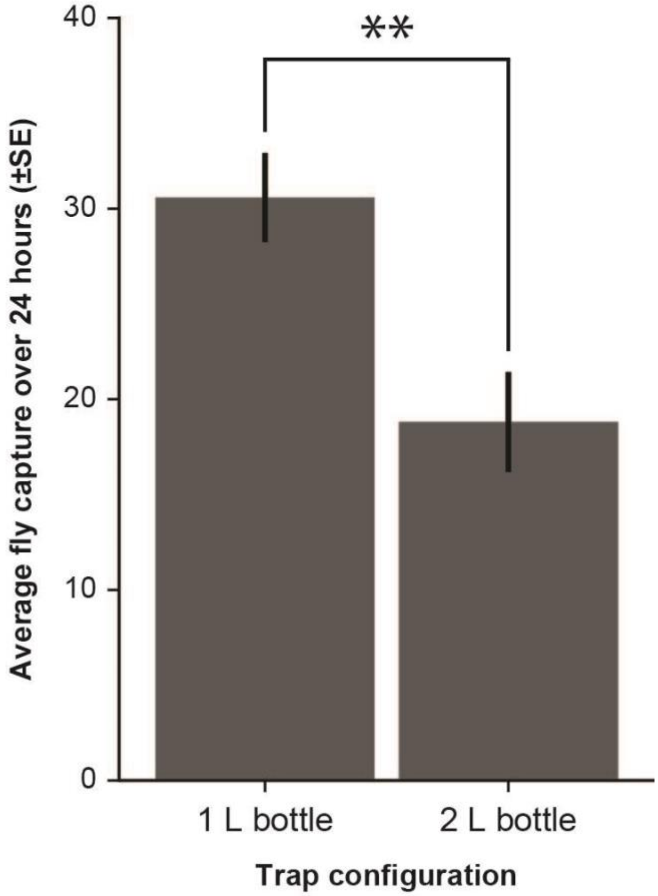

Figure 2.3 Evaluation of laboratory bait trap construction comparing 1 L and 2 L trap designs using the banana yeast mixture. Results of a t-test of trap comparison had a p -value < 0.01 (**).

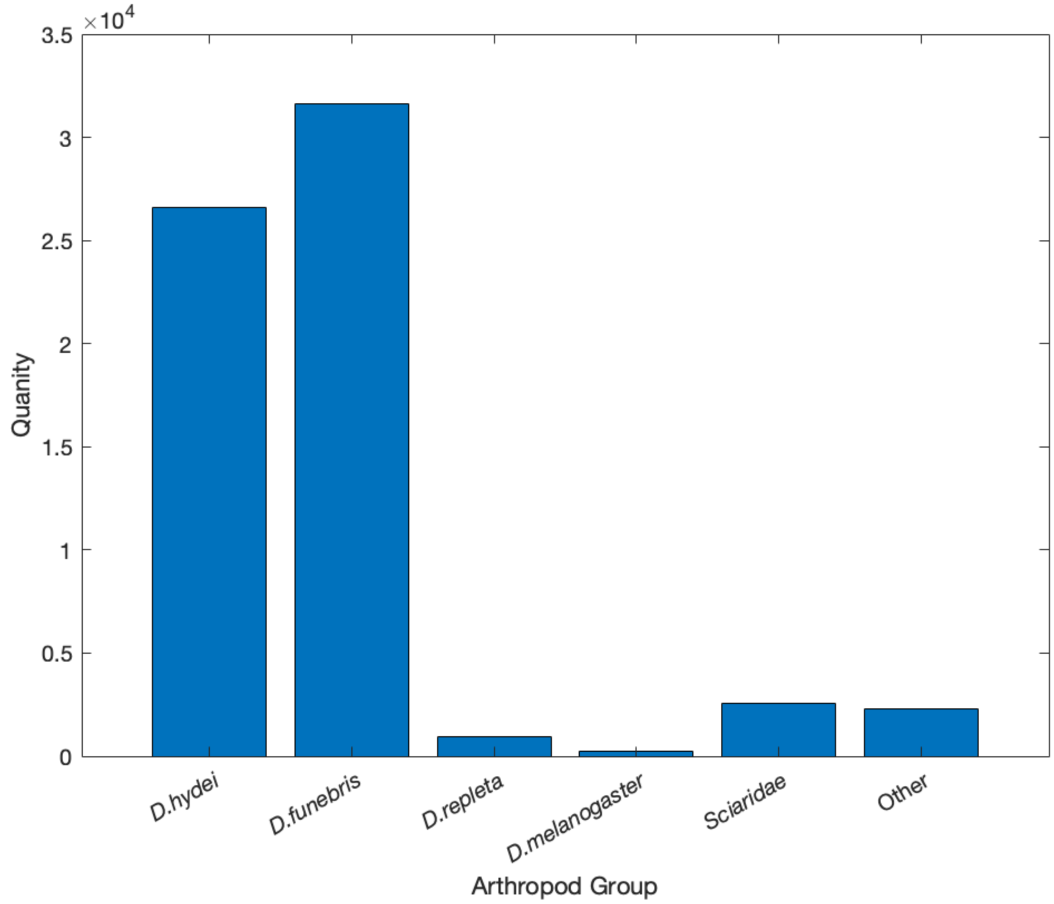

Figure 2.4 Aggregate diversity of arthropod species detected in field collections. Please note, these totals are summed across two locations and two sample dates.

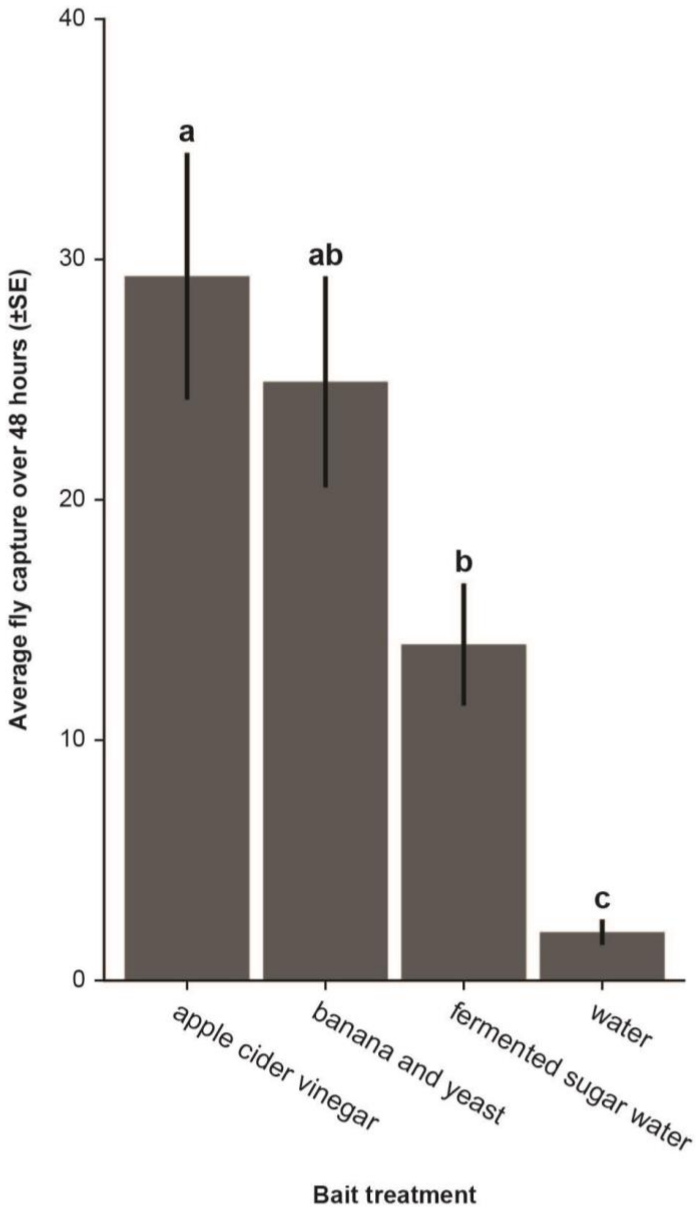

Figure 2.5 Average number of flies caught in traps baited with different lures under field conditions. Means separation conducted with Tukey HSD (α = 0.05).

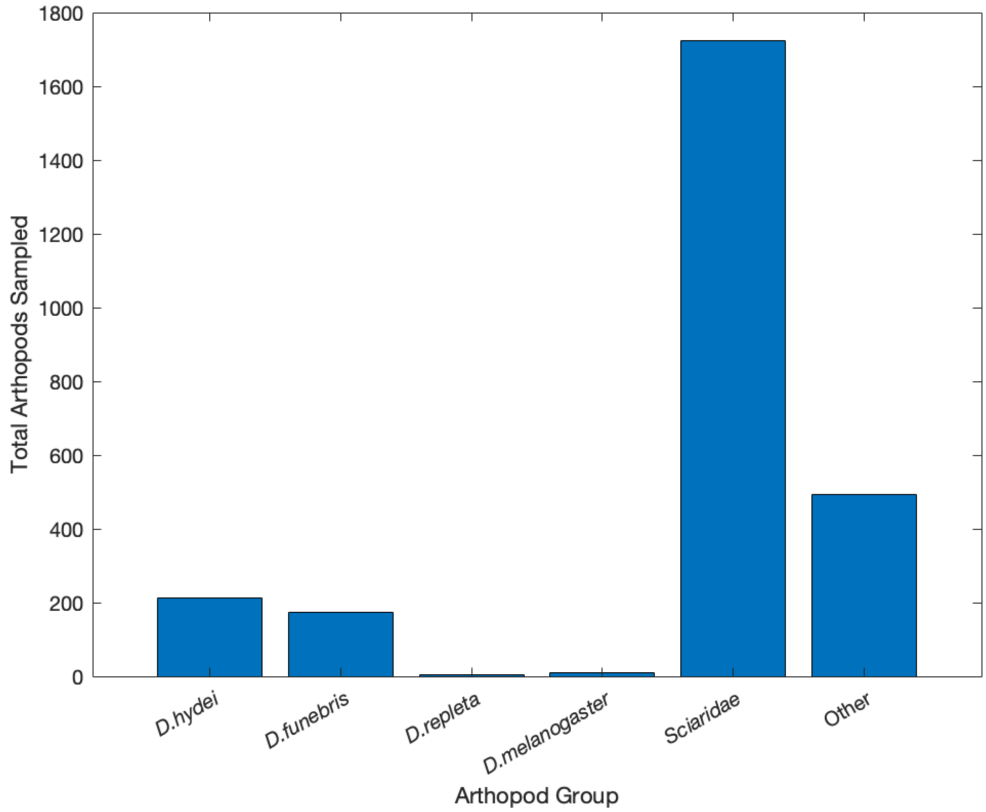

Figure 2.6 Total number of arthropods caught on sticky cards over a 48-hour period within the sampled sweetpotato storage rooms.