CONFERENCE PROCEEDINGS

Midsouth Entomologist

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Oral Presentations

Ehrlichia chaffeensis co-opts phagocytic hemocytes for systemic dissemination in the Lone Star tick, *Amblyomma americanum*

Abdulsalam Adegoke and Shahid Karim

Hematophagous arthropods can acquire and transmit several pathogens of medical importance. In ticks, the innate immune system is crucial in the outcome between vectorpathogen interaction and overall vector competence. However, the specific immune response(s) elicited by the immune cells known as hemocytes remains largely undefined in *Ehrlichia chaffeensis* and its competent tick vector, *Amblyomma americanum*. Here, we show that granulocytes, professional phagocytic cells, are integral in eliciting immune responses against commensal and pathogen infection. The chemical depletion of granulocytes led to decreased phagocytic efficiency of tissues-associated hemocytes. We demonstrate *E. chaffeensis* can infect circulating hemocytes, and both cell-free plasma and hemocytes from *E. chaffeensis*- infected ticks can establish Ehrlichia infection in recipient ticks. Lastly, we provide evidence to show granulocytes play a dual role in *E. chaffeensis* infection. Depleting granulocytic hemocytes increased Ehrlichia load in the salivary gland and midgut tissues. In contrast, granulocyte depletion led to a reduced systemic load of *E. chaffeensis*. This study has identified multiple roles for granulocytic hemocytes in the control and systemic dissemination of *E. chaffeensis* infection.

Longitudinal Monitoring of Honey Bee Colonies Along an Agricultural Intensification Gradient in Mississippi

Catchot III, Angus, Urita Agana, Audrey Sheridan, Priyadarshini Chakrabarti Basu and Jeffrey Gore

Honey bee (*Apis mellifera* L.) colony declines have been reported extensively nationally and globally over the past decade. Pesticides and poor nutrition are two important factors causing such declines. The main purpose of this study is to evaluate the interactive effects of both pesticide exposure and poor nutrition on honey bee colony health across an agricultural intensification gradient in Mississippi. These field sites also vary in their availability of natural forage. Overall colony health, pesticide exposure and forage availability will be evaluated during an entire year from field sites across the state. Visual inspections, pollen traps and land use mapping will be used to determine the availability of forage in these areas. In addition, pesticide residue analysis will be conducted on pollen and nectar samples to assess the field realistic exposures to such pesticides. Various colony health parameters will be recorded in addition to collecting data on pests, parasites and pathogens from these colonies. The information gleaned from this study will help us determine if optimal nutrition can counteract the detrimental impacts of pesticides, if any, over long term in a field realistic scenario.

Influence of Imidacloprid Seed Treatment Rate on ThryvOn Cotton

Farmer, Brett; Jeff Gore, Angus Catchot, Whitney Crow, Don Cook, and Brian Pieralisi

In the Mid-South, thrips are an important early season pest on cotton. Mississippi planted over 500,000 acres of cotton in 2020, all of which were infested with tobacco thrips *Frankliniella fusca* (Hinds), which resulted in about \$6.5 million of economic losses. Therefore, cotton is typically treated with a preventative thrips control product at planting and generally about 25-30% requires an additional foliar treatment to reduce damage from thrips. A new *Bacillus thuringiensis* trait (ThryvOn[®], Bayer CropScience, St. Louis, MO) has been developed that provides good control of thrips. Currently, there is some debate about the need for an insecticide seed treatment to improve thrips control. We conducted a study to determine if the addition of an imidacloprid seed treatment improves efficacy against tobacco thrips and to determine if reduced rates of seed treatments (100, 75, 50, 25, and 0% of the labeled rate) provide benefits for thrips control in ThryvOn cotton. Preliminary results suggest that a 50% seed treatment rate of imidacloprid provided similar control and yield protection as a 100% imidacloprid seed treatment rate. These preliminary data suggest that ThryvOn cotton could be utilized with a reduced rate of imidacloprid compared to other current commercial varieties.

Evaluation of Selected Insecticides for Control of Rice Stink Bug in Mississippi

Lytle, Mary Jane, Jeff Gore, Whitney Crow, Don Cook, Tyler Towles, Jason Bond, and Angus Catchot

The rice stink bug (*Oebalus pugnax* F.) is a damaging insect pest of late season or heading rice (*Oryza sativa* L.) and inflicts both direct yield loss as well as grain damage that results in poor milling quality. In Mississippi rice production, this pest has historically been controlled using pyrethroids, due to the cost effectiveness of the insecticide class. In recent years, control failures of lambda-cyhalothrin have been reported throughout the Mid-South and Mississippi Delta, likely due to development of resistance in rice stink bug populations. In order to determine efficacy of insecticides for control of rice stink bug, a field study was conducted during 2023 at the Delta Research and Extension Center in Stoneville, Miss. Treatments included Warrior II[®] at 2.56 oz/ac, Tenchu[®] at 8 oz/ac, malathion at 16 oz/ac, bifenthrin at 6.4 oz/ac, Endigo[®] ZCX at 4 and 5 oz/ac, respectively, and a nontreated control. Samples were taken at 3, 5, and 7 days after application. Data analysis is ongoing, and results will be discussed regarding insecticide efficacy for management of rice stink bug in Mississippi and control options for the future.

Poster Presentations

Deciphering the role of Amblyomma maculatum hemocytes during Rickettsia parkeri infection

Abdulsalam Adegoke and Shahid Karim

The increase in tick-borne diseases is a significant threat to public health without preventive measures. The tick immune system is divided into cellular and humoral immune responses. Immune cells, commonly called hemocytes, are a critical component of the immune system and produce immune factors that either facilitate or suppress pathogen development in a tick. Despite their importance in pathogen development, understanding of their basic biology and molecular mechanisms is limited. In this study, a combination of pharmacological and molecular tools was utilized to investigate the functional role of tick hemocytes in rickettsiae infection. In *Amblyomma maculatum*, we morphologically identified five types of hemocytes.

In-vivo bead phagocytosis led to identifying phagocytic and non-phagocytic populations of hemocytes. Clodronate liposome was used to deplete phagocytic hemocytes and elucidate their role in immune response against *Staphylococcus aureus, Escherichia coli*, and *Rickettsia parkeri* infection. Bulk RNA sequencing of hemocytes with and without *R. parkeri* infection revealed a total of 39,249 mRNA transcripts, with 11,301 identified as immune-related. We employed a reverse genetic approach (RNAi) to functionally characterize two differentially expressed marker genes in hemocyte phagocytosis. Together, these results represent a significant advancement in our understanding the biology of tick hemocytes and immune responses.

Uncovering the role of Amblyomma americanum tRNA synthetases in hematophagy and Ehrlichia infection

Anza Ali, L. Downs and S. Karim.

Transfer RNA synthetases are a family of enzymes that catalyze the addition of amino acids to their corresponding transfer RNAs (tRNAs) and play an essential role in protein synthesis. Recent research has uncovered intriguing connections between arthropod protein synthesis, development, and their ability to host and transmit pathogens. Ticks are obligate hematophagous ectoparasites that rely on prolonged feeding periods to complete their life cycle on a vertebrate host. Tick tRNA synthetases help the tick in continuous protein synthesis to evade the host immune system while stealing the host blood. These enzymes present a potential target for developing new tick control strategies. In this study, we investigate the crucial role of transfer RNA (tRNA) synthetases in the lonestar tick (Amblyomma americanum) during blood feeding on the host and propagation of Ehrlichia chaffeensis (EC) infection within the tick vector, the causative agent of human monocytic ehrlichiosis. We hypothesized that tRNA synthetases are indispensable in tick hematophagy, embryogenesis, and Ehrlichia infection. To validate this hypothesis, we used two commercially available tRNA synthetase inhibitors, Halofuginone (targeting prolyl-tRNA synthetase) and Borrelidin (targeting threonyl-tRNA synthetase), to evaluate their effects on the tick and pathogen infection at the cellular and organismal level. Preliminary data obtained from tick cell lines indicate that Halofuginone and Borrelidin affect tick protein synthesis and handicap EC proliferation. Additionally, our results demonstrated that both inhibitors have detrimental effects on the organism, resulting in impairment of tick feeding, leading to the death of ticks attached to the host after 24 hours. These results will provide a deep insight into the molecular mechanisms of these enzymes for designing new tickspecific inhibitors to prevent ticks and tick-borne diseases.

Investigation of Pyrethroid Resistance in Rice Stink Bug

Lytle, Mary Jane, Jeff Gore, Whitney Crow, Don Cook, Tyler Towles, Jason Bond, and Angus Catchot

Throughout the Mississippi Delta and Mid-South, rice (*Oryza sativa* L.) producers have been dependent on pyrethroids to control rice stink bug (*Oebalus pugnax* F.) due to the economic benefit pyrethroids deliver. The rice stink bug is a severe late-season pest of rice, feeding on developing grain and causing blanked heads and damaged kernels. Pyrethroid applications that provided inadequate control of rice stink bug have occurred over the last several years, suggesting potential resistance to the chemistry. Laboratory bioassay experiments were conducted in 2021, 2022, and 2023 at the Delta Research and Extension Center in Stoneville, Miss. to determine the effectiveness of pyrethroids on rice stink bug management. The trial consisted of technical grade lambda-cyhalothrin, a commonly used pyrethroid, applied in liquid scintillation vials at multiple concentrations. Rice stink bugs were collected throughout the rice growing area. One rice stink bug was

infested per vial and rated for mortality after 24 hours. Historical data from Mississippi in 2012 produced an LC₅₀ value of 0.16. Concentrations tested in 2023, produced LC₅₀ values >50. These results indicate a reduction in pyrethroid efficacy and a need for further evaluation of acceptable insecticides for rice stink bug control in the Mid-South.

Updates on exotic ants (Hymenoptera: Formicidae) in Mississippi

MacGown, Joe A. and JoVonn G. Hill

Largely due to extensive global trade in the modern era, exotic organisms, including ants, have spread throughout much of the world. If conditions are favorable, some species become established in new regions and may become invasive causing negative effects upon humans and other organisms. The southeastern United States, which has numerous ports of entry and a warm climate suitable for many tropical and subtropical species, continues to be a hotspot with at least 69 species of exotic ants now established in the region. Historic collections of ants in Mississippi prior to 2001 by Marion Smith and others resulted in eight exotic ant species being reported from the state. In 2001, the Mississippi Entomological Museum (MEM) began surveying the state for ants and since then have added an additional 22 species of exotic ants to the known fauna of the state. During this time, many of these species have significantly expanded their range in the state. The exotic ants reported from Mississippi are native to many regions including Central and South America, Europe, Africa, Australia, and Asia. Here, we report the exotic ant species currently known to occur in the state and provide updated distribution maps showing county distributions for each species based on records from the MEM collection, SCAN (https:// scan-bugs.org), and antmaps.org.

Characterization of phagocytic hemocytes of Honey bee Apis mellifera

Michael Oeth, Abdulsalam Adegoke, and Shahid Karim

Honey bees are ecologically vital in maintaining a balanced ecosystem. Honey bee colonies face many biotic and abiotic stressors, including parasites, pathogens, and chemical pesticides, which all have led to significant losses during the last two decades. The innate immune system, including hemocytes (immune cells), represents the first line of defense against invading pathogens. The innate immune system of bees is divided into cellular and humoral components, which protect against a wide range of invading microorganisms. Hemocytes, professional immune cells equivalent to vertebrate leukocytes, represent the cellular arm. Following microbial infection, hemocytes execute cell-mediated responses, including phagocytosis, encapsulation, and nodulation, clearing pathogens. This study aims to investigate the morphological and functional role of hemocyte populations in bee biology. Hemolymph collection techniques examined included antennae removal, abdominal puncture, leg amputation, and decapitation. The antennae method yielded the most number of total hemocytes compared to other collection methods. The antennae collection method also generated the highest number of granulocytes and Plasmatocytes from the nursing bees. Upon collecting hemolymph from the antennae and staining the hemocytes with lectin stain (wheat germ agglutinin, WGA) and a nuclear stain (DAPI), the results revealed the presence of two predominant hemocyte types: granulocytes and plasmatocytes. Next, we injected fluorescent beads into the bee hemolymph before hemocyte labeling. The hemocytes were incubated with nuclear stain DAPI and the cell membrane stain Vybrant CM-DiL. Phagocytosis was demonstrated by the presence or association of yellow-green fluospehere (beads) in the cytoplasm of the hemocytes. In conclusion, we established a functional approach to hemocyte collection in worker bees. In addition, we developed clodronate liposome as a method to reduce hemocyte populations within circulating hemolymph. In future work, we will introduce immunosuppressed worker bees with a bacterial challenge to quantify mortality when hemocyte populations are low.

Assessments of in-furrow fungicide applications to manage southern blight in Mississippi peanut fields

Tripathi, Subina, Tessie Wilkerson, and Tom Allen

Southern blight (SB) is a soil-borne disease caused by *Athelia rolfsii* (AR). Symptoms include water-soaked lesions, wilting, and plant death. Production of sclerotia serves as the primary inoculum. Under severe conditions, losses can be up to 80%. In-furrow application of fungicides may provide effective management for SB by creating a zone of protection for seed and seedlings. The study was conducted on a (soil type) with seven fungicides including Quadris, Solatenol, Omega 500, Velum, Proline, Elatus, and Revytek. Each plot was planted to 7 seed per foot and consists of 4 rows, 20 ft long on, 40-inch row spacing using a RCBD design. Rows 1 and 2 were inoculated with AR infested millet and rows 3 and 4 were left non-inoculated. Stand count, vigor, and phytotoxicity were evaluated 15 and 30 DAP. The severity of SB was taken 90 and 110 DAP using a scale of 0 to 9. No significant differences were observed. Among all the fungicides tested, Elatus was found to be effective in managing the SB. Omega treated plots yielded 3200 pounds per acre, followed by Revytek, Solatenol, and Velum, which exhibited a similar nine percent increment compared with Omega.