BIOLOGY, ECOLOGY, AND MANAGEMENT OF *PANGAEUS BILINEATUS* (HEMIPTERA: CYDNIDAE) IN SOUTHEAST US COMMERCIAL PEANUT SYSTEMS

by

Benjamin L. Aigner

(Under the Direction of Mark R. Abney)

ABSTRACT

Peanut burrower bug, *Pangaeus bilineatus* (Say), is a piercing-sucking pest of peanut, *Arachis hypogaea* (L.), native to North America. It feeds directly on seed through the hull, reducing the crop's value by approximately $209/MT at ≥3.5% injury by weight. Knowledge of *P. bilineatus* biology and ecology is required to develop an integrated pest management (IPM) strategy. Therefore, studies were conducted to elucidate the pest's biology and ecology, and to optimize the design of light traps. Developmental biology of *P. bilineatus* was studied in a controlled environment chamber in the laboratory on a 14:10 L:D cycle, constant temperature of 29°C ± 1°C, and 55% RH ± 10%. Observations were made daily and date, time, and no. of eggs, nymphs (and life stage), and adults were recorded. Development from oviposition to adult completed in 39.5 ± 3.0 days through 5 nymphal instars. Immature life stages last 3.7 – 8.1 days and head capsule width (0.46 – 1.68 mm) progressively increases as nymphs age. Females produced 129.0 ± 80.8 eggs and lived 88.7 ± 31.2 days. Studies also indicate 1st instar nymphs need an adult female present to molt to the second instar.

Pangaeus bilineatus is sporadic, thus, research was conducted to identify significant contributing landscape and environmental factors to peanut injury. Injury data from Georgia, US was acquired for years 2016 – 2018 and generalized linear models were constructed using compositional, configurational, and environmental covariates to identify significant predictors of injury. Associations of *P. bilineatus* peanut injury were observed with landcover proportions of ‘peanut’, ‘cotton’, ‘forest’, and ‘other agriculture’, richness of crops/non-crops, field size, elevation, edge density, soil texture, precipitation, and irrigation.

Light traps and pitfall traps are the only tools currently available for monitoring *P. bilineatus*. Optimizing light traps with the most attractive light source could provide a more useful tool for IPM. Adults’ response to various wavelengths of light were evaluated in a two-choice bioassay. White fluorescent wavelengths attracted significantly more adults and should be tested in the field. Information from these studies expands our understanding of *P. bilineatus* biology and ecology and should lead to future research and an IPM strategy.

INDEX WORDS: *Arachis hypogaea*, peanut burrower bug, integrated pest management, biology, ecology, landscape complexity, composition, configuration, light trap.

BIOLOGY, ECOLOGY, AND MANAGEMENT OF *PANGAEUS BILINEATUS* (HEMIPTERA: CYDNIDAE) IN SOUTHEAST US COMMERCIAL PEANUT SYSTEMS

by

BENJAMIN L. AIGNER

BA, Virginia Tech, 2013

MSLFS, Virginia Tech, 2016

A Dissertation Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

DOCTOR OF PHILOSOPHY

ATHENS, GEORGIA

2023

BIOLOGY, ECOLOGY, AND MANAGEMENT OF *PANGAEUS BILINEATUS* (HEMIPTERA: CYDNIDAE) IN SOUTHEAST US COMMERCIAL PEANUT SYSTEMS

by

BENJAMIN L. AIGNER

Major Professor:	Mark R. Abney
Committee:	W. Scott Monfort
	Glen C. Rains
	Jason M. Schmidt

Electronic Version Approved:

Ron Walcott
Vice Provost for Graduate Education and Dean of the Graduate School
The University of Georgia
August 2023

DEDICATION

To my parents, John and Shari, for imparting an invaluable work ethic in me from a young age and their boundless love, support, and encouragement throughout the struggles and successes of my personal life, and academic and professional careers. To my brothers and sisters Ashley, Joe, John Jr., Tanyua, Shannon, and Shaun for paving the way and guiding my exploration through a variety of interests as I developed my own. A special thanks to my brother John for providing his mentorship as I sought my own path in entomological science.

ACKNOWLEDGEMENTS

I'd like to extend my gratitude to my department head Dr. Braman, and the entire University of Georgia Department of Entomology faculty. Especially, my advisor Mark Abney and other committee members, Jason Schmidt, Scott Monfort, and Glen Rains. The level of support I've received here at UGA is incomparable and I would not be where I am today without each of you. I am eternally grateful for the opportunity, trust, and mentorship provided to afford me the opportunity to take on the challenges of my project and graduate school.

I also thank my former master's advisors Ames Herbert and Carlyle Brewster for their wisdom and encouragement I needed to pursue my current opportunity. A special thanks to Tom Kuhar for introducing me to the field of agricultural entomology and for guiding me through my early professional career as a laboratory and field assistant in the Vegetable Insect Pest Research Lab at Virginia Tech.

Thank you to my friends and mentors Mike Crossley and Arash Kheirodin for their guidance throughout my program, for pushing me through the learning curve of R, and imparting their wisdom of agroecology.

Additional thanks to my collaborators at USDA, Georgia FSIS, Georgia peanut farmers, and Georgia county extension agents for their assistance and cooperation in completing surveys, sampling, and helping to coordinate certain aspects of my project.

To my friends and classmates in the Entomology Annex in Tifton, thank you for helping me stay sane throughout a uniquely bizarre time during the COVID-19 pandemic.

Last, but certainly not least, I thank the funding sources of my research project, the USDA-NIFA, the National Peanut Board, and The Georgia Peanut Commission.

TABLE OF CONTENTS

Page

APPENDICES

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

Dissertation Overview and Research Objectives

Effective management of agricultural insect pests depends upon human understanding of their biology and ecology. The availability of management tools for monitoring, and biological and chemical treatment agents dictates our ability to efficiently reduce populations to levels that diminish the economic impact of herbivorous insects to cash crops. Besides eradication, there is no “silver bullet” that will provide consistent efficacious results for pest management. Behaviors (e.g., flying, burrowing, etc.) and physiological responses of insects (e.g., resistance development), and environmental challenges prevent the indefinite success of management tactics. Therefore, developing diverse integrated pest management (IPM) programs is essential to managing insect pests over the long-term and prevent resistance development. Furthermore, an ecologically sound IPM approach to managing pests can result in less pesticide use and lower input costs for farmers.

The peanut burrower bug, *Pangaeus bilineatus* Say, is a cydnid species that can cause severe economic loss to peanut farmers in the Southeast US. This pest spends most of its life cycle below ground which makes it difficult to monitor and manage. With very little information of the pest’s biology and ecology, the need to better understand this cryptic subterranean species marks the first hurdle towards development of a comprehensive IPM strategy. The work presented in this dissertation provides a foundation for future research with the ultimate goal of developing an IPM strategy to manage *P. bilineatus* in peanut. Each chapter of this document was written in preparation of separate submission for journal publication and, therefore, are presented in manuscript format.

An ample review of literature relevant to *P. bilineatus* as an agricultural pest is found in Chapter 2. From this review, the reader will develop an understanding of *P. bilineatus* life history, agricultural pest status, past and present management, and the importance of the contributions presented in this document. As much of the groundwork for understanding *P. bilineatus* biology was completed for this project, there is minor overlap between Chapters 2 and 3.

Research to define basic biological information of *P. bilineatus* is found in Chapter 3. The overarching goal of the research objectives presented here is to gain a better understanding of *P. bilineatus* biology. This chapter defines parameters surrounding *P. bilineatus* developmental biology (fecundity, longevity of females, development time, and morphometrics) and behavioral ecology.

Landscape complexity (composition and configuration) and abiotic environmental factors influence insect populations and their ability to thrive in agricultural landscapes. As a sporadic peanut pest, the need to understand why *P. bilineatus* appears in some peanut fields and not others is imperative to IPM strategy development. The research goals presented in Chapter 4 are to elucidate the influence of landscape complexity and abiotic environmental factors on *P. bilineatus* injury to peanut. Metrics of composition (proportions of landcover types and landscape diversity), configuration (e.g., edge density, elevation, etc.), and climate (e.g., rainfall, temperature, etc.) were analyzed to identify a predictive model for peanut injury risk.

A primary component of IPM strategies involves pest monitoring. Use of monitoring tools enables surveying of pest populations (e.g., insect traps) and can help inform management decisions like spray schedules and/or release of biological agents. Monitoring *P. bilineatus* is difficult because it is a soil pest and there are limited tools available. Although light traps have been used in the field, they could potentially be optimized with a light source that elicits a greater response from *P. bilineatus* populations. To evaluate the response of *P. bilineatus* adults to various wavelengths of light, the experiments presented in Chapter 5 were conducted in the laboratory.

Finally, the contributions of the studies presented here are summarized in Chapter 6. Each of the research objectives of this project provide vital contributions to the overall long-term goal of developing a comprehensive IPM program for *P. bilineatus* management in peanut.

CHAPTER 2

LITERATURE REVIEW

Aigner, B.L., M.S. Crossley, and M.R. Abney. 2021. Biology and management of peanut burrower bug (Hemiptera: Cydnidae) in Southeast US Peanut. *Journal of Integrated Pest Management*. 12(1), p.29.
Reprinted here with permission of the publisher

Abstract

Peanut burrower bug, *Pangaeus bilineatus* (Say), is a piercing-sucking pest of peanut, *Arachis hypogaea* (L.), that is native to Central and North America. The insect spends most of its life below the soil surface and is not easily detected in the field. Although injury to peanut is sporadic in the Southern USA, the bug has become a serious economic pest for farmers in the region in recent years. During and after peanut seed formation, adult and immature bugs feed directly on seeds through the hull, reducing the quality and value of the crop. The value of peanut is reduced by approximately $209/MT when feeding injury is present on ≥3.5% of kernels by weight. Deep tillage prior to planting and application of granular chlorpyrifos during the growing season are the only tactics currently available for managing *P. bilineatus* in peanut in the United States. Relatively little research attention has been focused on *P. bilineatus*, and improved knowledge of the insect's biology and ecology is needed to develop an integrated pest management (IPM) strategy that significantly reduces financial losses caused by this insect. The purpose of this article is to provide a review of the taxonomic history, biology, pest status, and management of *P. bilineatus* primarily as it relates to peanut production systems in the Southeast USA.

Key words: Cydnidae, IPM, field and forage crop, host plant resistance, sampling

Taxonomic Treatment

The peanut burrower bug, *Pangaeus bilineatus* (Say), is a subterranean agricultural pest native to Central and North America. It was first described by Say in 1824 as *Cydnus bilineatus* but was moved to the genus *Pangaeus* by Stål in 1862 and placed in the subgenus *Homaloporous* by Uhler in 1877 (Froeschner 1960). The family Cydnidae (burrower or burrowing bugs) is comprised of six subfamilies: Amaurocorinae, Amnestinae, Cephalocteinae, Cydninae, Garsauriinae, and Sehirinae (Schwertner and Nardi 2015). *Pangaeus bilineatus* resides in the Cydninae within the tribe Geotimini. The insect has a confusing taxonomic history as subtle variations observed between species types resulted in the publication of 26 synonyms for *P. bilineatus* (Froeschner 1960). Synonyms include: *Pangaeus uhleri*, *P. fortis*, *P. vicinus*, *P. douglasi*, *P. scotti*, and *P. spangbergi* (Sailer 1954, Froeschner 1960).

Distinguishing Peanut Burrower Bug from Other Burrower Bugs

Proper pest identification is the first step in developing a management strategy. The primary diagnostic feature of peanut burrower bug is the deep, sharply impressed line that parallels the anterior margin of the pronotum from side to side (Fig. 1.1). Burrower bugs found in Southeast USA peanut, *Arachis hypogaea* (L.), fields include *P. bilineatus*, *Dallasiellus lugubris* (Stål), *Cyrtomenus ciliatus* (Palisot), and *Sehirus cinctus* (Palisot); however, *P. bilineatus* is the only species of economic concern (Froeschner 1960, Chapin and Thomas 2003). *Dallasiellus lugubris* is smaller (3.9–5.5 mm length) than *P. bilineatus* (5.3–7.8 mm length) and lacks the deep impressed line parallel to the anterior margin of the pronotum (Froeschner 1960). *Cyrtomenus ciliatus* can be distinguished by its large body size (approx. 8.9 mm length) and bulbous shape (Froeschner 1960). *Sehirus cinctus,* or white-margined burrower bug (4.6–5.2 mm length), is easily distinguished from other groups by the white line that extends around the lateral margins of the pronotum, corium, and abdomen (Froeschner 1960).

Life Cycle and Description

Pangeaus bilineatus is a paurometabolous insect, like other cydnids (Sites and McPherson 1982, Riis et al. 2005, Schwertner and Nardi 2015). According to Cole (1988) in Texas in 1971–72, the majority of adults overwintered in the soil at depths of 6–8 in. (15–20 cm), terminated diapause in late February and early March, and progressively moved toward the soil surface as temperatures increased. Active nymphs have been observed during winter months (December–February) in Georgia, USA, within fields previously planted to peanut, suggesting that at least a portion of the population may not enter diapause in this region (personal observations). Evidence suggests at least three *P. bilineatus* generations/year occur in South Georgia, and a single generation can be completed in approximately one month under favorable conditions in the lab (Abney and Aigner 2018, Aigner and Abney 2020).

Adults

Pangaeus bilineatus adults are oval-shaped, 5.3–7.8 mm in length, and light brown to shiny black in color, depending on their age. Freshly molted adults are white and remain below ground while their exoskeletons harden and gradually darken in color (Fig. 1.2a). The bug’s tibiae are modified with spines that wrap around 360° and project 45° posteriorly (Fig. 1.2b). When disturbed, a pungent odor is emitted from metathoracic glands as a probable defense mechanism. Roth (1961) described the fungistatic and repellent properties of metathoracic gland secretions from another cydnid, *Scaptocoris divergens* (Froeschner). The odor is similar to that released by other species in the pentatomoid superfamily, and is likened by many to the smell of coriander or cilantro. The meso- and meta-pleural evaporatoria on the ventral thoracic plates (Figs. 1.2c-d) facilitate diffusion of these odorous compounds. The terminal segment of the male is a capsule that contains the genital clasper, while the terminal segment of the female consists of two genital plates that control access to the reproductive organs (Figs. 2e-f). Sailer (1954) provides detailed illustrations of *P. bilineatus* males' genital claspers.

Eggs

Eggs are approximately 1 mm in length, oval-shaped, and opaque-white in color (Fig. 1.3a). Oviposition begins less than 8 d after mating and nymphs have been observed within 14 d of mating (Aigner and Abney 2020). Red eyespots appear on the developing embryo after a few days, and the egg retains a pearly white to cream coloration until the nymph emerges (Fig. 1.3b).

Nymphs

Pangaeus bilineatus develops through 5 nymphal instars (Fig. 1.4). Immatures spend most of their development below ground. First instar nymphs (Fig. 1.5a) have an average pronotum width of 0.66 mm (Table 1) and are beige in color. Second instar nymphs (Fig. 1.5b) have an average pronotum width of 0.9 mm and are light brown in color. Third instar nymphs (Fig. 1.5c) have an average pronotum width of 1.27 mm with dark brown (almost black) sclerotized parts (e.g., head, legs, tergal plates) and lighter colored membranous tissue between. Third and fourth instar nymphs are similar in color, but the latter (Fig. 1.5d) is slightly larger with an average pronotum width of 1.8 mm. Fifth instar nymphs (Fig. 1.5e) are slightly larger than fourth instars (2.6 mm average pronotum width) but possess distinctly visible wing pads. In a growth chamber at 29°C, 40% RH, and a 14:10 light–dark cycle, the insect can complete development from egg to adult in approximately 30 d (Aigner and Abney 2020).

Geographic Distribution and Host Range

The distribution of *P. bilineatus* ranges from Guatemala, northward throughout Mexico, and includes much of the southern and eastern U.S. (Fig. 1.6) (Sailer 1954, Froeschner 1960). The species also occurs in eastern Canada, Puerto Rico, Bermuda, and Hawaii (Russell 1934, Sailer 1954, Froeschner 1960, Lis et al. 2000, Paiero et al. 2003, Garcia 2011).

Though its full host range is unknown, *P. bilineatus* is quite polyphagous, and has been reported to feed on a variety of tissue in several crops including peanut seeds, cotton seedlings (*Gossipium hirsutum* L.), newly sprouted wheat (*Triticum aestivum* L.), pepper seeds (*Capsicum spp*. L.), spinach seedlings (*Spinacia oleracea* L.), and strawberry fruit (*Fragaria ananassa* Duchesne) (Swenk 1913, Gould 1931, Russell 1934, Cassidy 1939, Tissot 1939, Watson and Tissot 1942, Sailer 1954, Smith and Pitts 1974, Cole 1988). In a laboratory colony, adults and nymphs have been observed feeding on seeds, stems, and roots of peanut, tubers of yellow nutsedge (*Cyperus esculentus* L.), florets of river oats (*Chasmanthium latifolium* (Michx.) Yates), stems of chickweed (*Stellaria media* (L.) Villars), and stems and roots of white clover (*Trifolium repens* L.) (personal observations). Cydnids are primarily seed and root feeders, and although *P. bilineatus* has been observed feeding on a variety of plant tissue, its host preferences are unknown (Froeschner 1960, Smith and Pitts 1974, Lis et al. 2000, Schwertner and Nardi 2015).

Injury to Peanut and Economic Impact

Pangaeus bilineatus has piercing-sucking mouthparts that inject digestive enzymes into plant tissue and ingest dissolved nutrients in a process known as non-reflux extraoral digestion (Cantón and Bonning 2020). Adults and nymphs feed directly on peanut seed through the hull which can cause commercial grade reductions and facilitate aflatoxin contamination leading to a loss in crop value of more than 50% (Smith and Pitts 1974, Chapin et al. 2006, Mbata and Shapiro-Ilan 2013). Feeding injury can be seen as yellow to dark brown spots or pits on mature seed once the testa is removed. (Fig. 1.7) (Smith and Pitts 1974).

The potential for *P. bilineatus* to become a serious economic pest of peanut in the United States was first mentioned by Smith and Pitts (1974). Injury to peanut was first reported in Georgia in 1959, and significant economic injury was documented in Alabama in 1966 and in the southern peanut growing region of Texas in 1968 (Smith and Pitts 1974). Although *P. bilineatus* injury to peanut is sporadic spatially and temporally, the insect is considered a serious threat to peanut in Georgia, where approximately 50% of United States peanut production occurs annually. (USDA–NASS 2020).

Significant economic losses have been reported throughout Georgia since 2010 (Mbata and Shapiro-Ilan 2013, Abney 2014, Abney 2015, Abney 2016, Abney 2017). Because the insect and the injury occur below ground, injury is usually not detected until peanuts reach a buying point, where grading occurs. Injury greater than 3.49% by weight results in loss of grade from ‘segregation I’ to ‘segregation II’ and a loss in value of approximately $209/MT (USDA 2019). Losses due to *P. bilineatus* injury in Georgia were estimated to be approximately $15 million in 2014, and losses were disproportionately endured by relatively few producers (Abney 2014). Though Sailer (1954) raised concerns that the species might transmit viral diseases through plant roots, no cases of virus transmission have been documented.

Natural Enemies

Very little is known about the natural enemies of *P. bilineatus*, and biological control at a management scale has never been reported in the field. Johnson (1973) described *P. bilineatus* as a host of the strepsipteran parasite, *Triozocera mexicana* (Pierce), in the family Mengeidae.

However, Cook (2015) suggested that *T. mexicana* likely does not occur in the United States, and Johnson (1973) may have misidentified either *Triozocera vernalis* (Kifune and Brailovsky) or *Triozocera texana* (Pierce). Southern fire ant, *Solenopsis xyloni* (McCook) (Hymenoptera: Formicidae), workers were seen foraging on eggs and small nymphs of *P. bilineatus* in Texas, but this behavior was rarely observed (Smith and Pitts 1974). An ascomycotic fungal entomopathogen of the family Ophiocordycipitaceae and (newly revived) genus *Paraisaria* was identified from field-collected specimens in Emanuel County, Georgia, but it has not yet been identified to species (L. Castrillo, USDA, personal communication).

Management

Sampling and Monitoring

There are no established economic thresholds or sampling protocols for *P. bilineatus* in any crop. The insect spends most of its life cycle below ground where it is difficult to detect, and peanut injury may go entirely unnoticed until pods and seed are inspected after harvest. In the summer, adults actively fly at dusk (Highland and Lummus 1986, Abney and Aigner 2018). Published field sampling techniques for *P. bilineatus* include light trapping and pitfall trapping (Smith and Pitts 1974, Highland and Lummus 1986, Chapin and Thomas 2003, Mbata and Shapiro-Ilan 2013, Abney and Aigner 2018). Light traps have been used to study the insect’s distribution and population dynamics in Texas and Georgia (Highland and Lummus 1986, Abney and Aigner 2018), and pitfall traps have been effectively used to measure the seasonal abundance of *P. bilineatus* in South Carolina and relate trap catch to peanut injury ratings (Chapin and Thomas 2003). While Highland and Lummus (1986) did not find any biotic or abiotic predictors of peak *P. bilineatus* light trap captures in peanut, they did find a close correlation between light trap captures and field infestations. The presence and abundance of *P. bilineatus* in light traps and pitfall traps are dependent on abiotic factors as well as the insect's behavior, but could be useful indicators of field infestation. To date, trap capture data have not been used to inform decision making in peanut pest management programs. In addition, trapping observations could help elucidate farm-scale spatiotemporal distribution patterns of *P. bilineatus* and help identify risk factors (e.g., elevation, soil texture, soil moisture) associated with economic injury (Chapin and Thomas 2003, Holland et al. 2005, Mbata and Shapiro-Ilan 2013). Direct assessment (Hutchins 1994) of *P. bilineatus* population density has been achieved by collecting a known volume of soil and determining the number of bugs present. Although the method of soil extraction was not described, soil samples were used by Smith and Pitts (1974) to measure *P. bilineatus* abundance during insecticide efficacy field trials. Though time consuming and labor-intensive absolute soil sampling of this type avoids the behavioral bias potentially associated with active and passive trapping.

Cultural Management

Host plant resistance is an important element of IPM that can directly counter insect herbivory via physical (e.g., spines, trichomes) and chemical (e.g., herbivore-induced plant volatiles, plant secondary metabolites) plant defenses (Fürstenberg-Hägg et al. 2013, Trapero et al. 2016, Van Emden 2017). Peanut breeding programs have prioritized the development of cultivars with traits conferring resistance to viruses like *Tomato spotted wilt orthotospovirus* (TSWV) and causative agents of leaf spots (*Cercospora arachidicola* Hori and *Cercosporidium personatum* (Berk. and M.A. Curtis) Deighton), and stem rot (*Sclerotium rolfsii* Sacc.) due to the prevalence and economic impact of these pathogens (Chamberlin 2019). For the past 4 decades, three runner-type cultivars have dominated planted acreage in the Southeast USA: 'Florunner' (1980s and 90s), 'Georgia Green' (1990s and 2000s), and 'Georgia-06G' (2000s to present) (Branch and

Culbreath 2015). The increased occurrence of *P. bilineatus* injury following the release and widespread adoption of cultivar 'Georgia-06G' in 2007 (Branch 2007) might indicate a higher relative susceptibility of this cultivar to *P. bilineatus* injury compared to previously grown cultivars. An effort to identify and incorporate host plant resistance traits against *P. bilineatus* into new commercially viable cultivars is needed and would provide benefit for future IPM programs.

Crop rotation and tillage can have significant impact on soil dwelling arthropods (House and Del Rosario Alzugaray 1989, Poggi et al. 2021), but information regarding the effects of specific practices on *P. bilineatus* is lacking. In the early 2000s, adoption of conservation tillage practices (i.e., no-till, strip-till, and mulch-till) steadily increased in cotton, corn, and soybean agroecosystems in the Southeast USA to >60% of planted acres in 2015 (Claassen et al. 2018). Cotton, corn, and bahiagrass are common rotational crops with peanut in the Southeast USA, but actual rotation patterns vary by state and area within a state (Chapin and Thomas 2003, Puppala et al. 2018, Wright et al. 2020, Anco and Thomas 2021, Jordan 2021, Strayer-Scherer 2021). Studies evaluating the effect of tillage practices and rotational crops suggest that conventional moldboard plowing prior to peanut planting can significantly reduce the risk of peanut burrower bug injury compared to strip-till planting into wheat or corn residue (Chapin et al. 2001, Chapin and Thomas 2003). Subsequent studies found significantly less *P. bilineatus* injury to peanut grown in conventional versus conservation tillage (Abney et al. 2017). However, conventional tillage does not guarantee peanut to be free from *P. bilineatus* injury, and the cost of converting from conservation to conventional tillage can be high in economic and environmental terms. Given these facts and the sporadic nature of *P. bilineatus* injury, many growers are reluctant to alter tillage regimes solely for the purpose of managing burrower bug.

Irrigation represents another potential cultural management tool for *P. bilineatus*. Although the cause is unknown, *P. bilineatus* injury tends to occur at a higher rate in non-irrigated than irrigated peanut fields (Highland and Lummus 1986, Riis et al. 2005, Aigner et al. 2019). Current availability of irrigated land and the need to maintain crop rotations for agronomic reasons limit the amount of peanut that can be irrigated in Georgia to approximately 50% of the total crop each year.

The effects of cover crop (Chapin and Thomas 2001, Chapin et al. 2003), planting and harvest date, and weed seed bank on peanut burrower bug populations and peanut injury are largely unknown, but the insect's broad host range suggests that investigation in these areas is necessary. Adjustment of planting and/or harvest dates could have an impact on incidence of injury. In warm climates, later harvest dates expose the crop to risk of feeding injury through October and into November, enabling further damage to the crop. According to Hart (2020), early burn down of cover crops and winter weeds that may be suitable hosts for insect pests can reduce the 'green bridge' – where polyphagous pests in warm climates move from cover crop to cash crop (Alyokhin et al. 2020) – between successive plantings. Additionally, weed seed bank could provide food resources to facilitate the survival of populations within fields during fallow periods.

Chemical Management

The only insecticide labeled for use against *P. bilineatus* in peanuts in the United States is the granular formulation of the organophosphate chlorpyrifos (Chapin et al. 2001, Chapin and Thomas 2003, Abney 2021). Granular chlorpyrifos applied at pegging (R2 growth stage) was shown by Chapin et al. (2001) to significantly reduce peanut burrower bug feeding injury in peanut even in high-risk fields planted following a rotation of wheat or corn. The recommended application rate is 2 lbs. AI per acre, which can be delivered by a 10–18″ banded application (Abney 2021). Specialized equipment is required to apply granular chlorpyrifos, and rainfall or irrigation is needed within 10–14 d to activate the insecticide and to optimize residual efficacy (Pike and Getzin 1981). Broad-spectrum insecticides like chlorpyrifos can have negative impacts on beneficial arthropod communities leading to secondary pest outbreaks (Ruberson et al. 1998, Lu et al. 2012, Hill et al. 2017), but there is currently no chemical alternative for *P. bilineatus* management. Lambda cyhalothrin, bifenthrin, and imidacloprid have been examined for efficacy but none have consistently reduced injury (Chapin and Thomas 2003, Abney et al. 2017). Mbata and Shapiro-Ilan (2013) concluded that a combination of the entomopathogenic nematode, *Heterorhabditis bacteriophora* (Poinar), and chlorpyrifos results in a synergistic effect under laboratory conditions. The nematode apparently did not cause *P. bilineatus* mortality in the absence of insecticide, and field efficacy of the nematode and insecticide combination has not been tested. The regulatory fate of chlorpyrifos is uncertain, and there is a need for continued research to find safe and effective chemical alternatives (Eaton et al. 2008, Grandjean and Landrigan 2014, Mie et al. 2018, Foong et al. 2020).

Closing Remarks

Though not fully described, the host range of *P. bilineatus* is certainly large and contains a wide variety of wild and cultivated plants (Sailer 1954, Highland and Lummus 1986, Lis et al. 2000). The insect is a severe economic threat, especially to peanut (Smith and Pitts 1974, Lis et al. 2000). The mechanism(s) responsible for the upward trend in peanut burrower bug injury witnessed since 2010 in the Southeast USA is a mystery. Several factors may be at play, including increasing adoption of reduced-till farming techniques throughout the early 2000s, a transition to greater use of the peanut cultivar ‘Georgia-06G’, and an increasingly warm climate.

Effective management of *P. bilineatus* in peanuts will require improved knowledge of the insect's biology, more effective monitoring and management tools, and a greater understanding of the factors that place peanut at risk of economic injury. For instance, treating light traps with sex pheromone could increase trap efficacy and improve the correlation between trap counts and pest densities in peanut fields. There are currently no economic thresholds or economic injury levels for *P. bilineatus* in any crop. These are the foundations of IPM programs and would provide growers with more precise information regarding the appropriate use of management tools, including insecticides, to prevent economic loss. Furthermore, a better understanding of *P. bilineatus*' interactions with crop and non-crop habitats could reveal critical places where targeted sampling and management efforts could enhance pest control. Given the limitations of current monitoring and management tools, continuation of efforts to elucidate the biology and ecology of the cryptic, native, peanut burrower bug will be critical to develop safe, effective, and sustainable pest management practices in peanut agroecosystems.

Acknowledgments

We thank Dr. Elizabeth McCarty (UGA) for volunteering her laboratory space and microphotography equipment for our use and Dr. John Shields (UGA) of the Georgia Electron Microscopy laboratory for his time in producing high-quality SEM images of *Pangaeus bilineatus*.

References Cited

Abney, M. R. 2014. Georgia Entomological Society Arthropod Survey. Georgia Entomological Society. https://site.caes.uga.edu/ges/arthropod-surveys/.

Abney, M. R. 2015. Georgia Entomological Society Arthropod Survey. Georgia Entomological Society. https://site.caes.uga.edu/ges/arthropod-surveys/.

Abney, M. R. 2016. Georgia Entomological Society Arthropod Survey. Georgia Entomological Society. https://site.caes.uga.edu/ges/arthropod-surveys/.

Abney, M. R. 2017. Georgia Entomological Society Arthropod Survey. Georgia Entomological Society. https://site.caes.uga.edu/ges/arthropod-surveys/.

Abney, M. R. 2021. Georgia Pest Management Handbook, 2021 Commercial Edition, Vol. 1. Peanut: peanut insect control. https://extension.uga.edu/content/dam/extension/programs-and-services/integrated-pest-management/documents/handbooks/2021-pmh-comm-chapters/Peanut.pdf

Abney, M. R. and B. L. Aigner. 2018. Spatial Abundance and Temporal Flight Activity of Peanut Burrower Bug, *Pangaeus bilineatus*, in Georgia. Abstract *In*: 50th Proceedings of the American Peanut Research and Education Society, Inc. Annual Meeting. July 10—12, 2018. Williamsburg, VA.

Abney, M.R., B.L. Aigner, P.M. Crosby, and S. Hollifield. 2017. Peanut burrower bug (Hemiptera: Cydnidae) distribution and management in Southeast US runner-type peanut. Abstract *In*: 49th Proceedings of the American Peanut Research and Education Society, Inc. Annual Meeting. July 11—13, 2017. Albuquerque, NM.

Aigner, B. L. and M. R. Abney. 2020. Life Cycle and Fecundity of Peanut Burrower Bug, *Pangaeus bilineatus* Say (Hemiptera: Cydnidae), Under Laboratory Conditions. Abstract

In: 51st Proceedings of the American Peanut Research and Education Society, Inc. Annual Virtual Meeting. Dallas, TX.

Aigner B. L., J. Schmidt, and M. R. Abney. 2019. Developing a predictive risk model for peanut burrower bug, *Pangaeus bilineatus,* in Southeast US peanut systems. Abstract *In*: Proceedings of the Entomological Society of America, National Annual Meeting 2019, November 17—20. St. Louis, MO.

Alyokhin, A., B. Nault, and B. Brown. 2020. Soil conservation practices for insect pest management in highly disturbed agroecosystems–a review. Entomologia Experimentalis et Applicata. 168(1): pp.7-27.

Anco, D. and J. S. Thomas. 2021. Peanut Money-Maker 2021 Production Guide: Crop Rotation https://www.clemson.edu/extension/agronomy/peanuts/docs/moneymaker/peanut_money_maker.pdf.

Branch, W. D. 2007. Registration of 'Georgia-06G' Peanut. J. Plant Regist. 1 (2): 120.

Branch, W. D., and A. K. Culbreath. 2015. Stability of TSWV general field resistance in the 'Georgia Green' peanut cultivar. Plant Health Prog. 16 (2): 95—99.

Cantón, P. E. and B. C. Bonning. 2020. Extraoral digestion: outsourcing the role of the hemipteran midgut. Curr. Opin. Insect. Sci. 41: 86—91.

Cassidy, T. P. 1939. USDA Entomol. and Plant Quar. Bur., Ins. Pes. Surv. Bull. 19: 322.

Chamberlin, K. D. 2019. Not Your Grandma's Goobers: Designing the Future of Peanut Breeding. Peanut Sci. 46: 91–98.

Chapin, J. W. and J. S. Thomas. 2003. Burrower bugs (Heteroptera: Cydnidae) in peanut: Seasonal species abundance, tillage effects, grade reduction effects, insecticide efficacy, and management. J. Econ. Entomol. 96 (4): 1142-1152.

Chapin, J. W., J. S. Thomas, and P. H. Joost. 2001. Tillage and chlorpyrifos treatment effects on peanut arthropods – an incidence of severe burrower bug injury. Peanut Sci. 28: 64-73.

Chapin, J. W., T. H. Sanders, L. O. Dean, K. W. Hendrix, and J. S. Thomas. 2006. Effect of feeding by a burrower bug, *Pangaeus bilineatus* (Say) (Heteroptera: Cydnidae), on peanut flavor and quality. J. Entomol. Sci. 41(1): 33-39.

Claassen, R., M. Bowman, J. McFadden, D. Smith, and S. Wallander. 2018. Tillage Intensity and Conservation Cropping in the United States, EIB-197, U.S. Department of Agriculture, Economic Research Service, September 2018.

Cole, C. L. 1988. Stratification and survival of diapausing burrower bugs. Southwest Entomol. 13(4): 243-246.

Cook, J.L. 2015. Reevaluation of the Genus *Triozocera* Pierce (Strepsipera: Corioxenidae) in the United States. Proceedings of the Entomological Society of Washington. 117(2): pp. 126—134.

Eaton, D. L., R. B. Daroff, H. Autrup, J. Bridges, P. Buffler, L. G. Costa, J. Coyle, G. McKhann, W. C. Mobley, L. Nadel, D. Neubert, R. Schulte-Hermann, and P. S. Spencer. 2008. Review of the Toxicology of Chlorpyrifos with an Emphasis on Human Exposure and Neurodevelopment. Crit. Rev. Toxicol. S2: 1–125. DOI: 10.1080/10408440802272158.

Foong, S. Y., N. L. Ma, S. S. Lam, W. Peng, F. Low, B. H. K. Lee, A. K. O. Alstrup, C. Sonne. 2020. A recent global review of hazardous chlorpyrifos pesticide in fruit and vegetables: Prevalence, remediation and actions needed. J. Hazard. Mater. 400 (123006): 1-10.

Froeschner, R. C. 1960. Cydnidae of the Western Hemisphere. Proc. U. S. Nat. Mus. 111: 337-680.

Fürstenberg-Hägg, J., M. Zagrobelny and S. Bak. 2013. Review: Plant Defense against Insect Herbivores. Int. J. Mol. Sci. 14: 10242-10297.

Garcia, J. N. 2011. *Pangaeus bilineatus* (Say), a burrowing bug new to Hawaii. Proc. Hawaii. Entomol. Soc. 43: 63-64.

Gould, G. E. 1931. *Pangaeus uhleri*, A Pest of Spinach. J. Econ. Entomol. 24: 484—486.

Grandjean, P., and P. J. Landrigan. 2014. Review: Neurobehavioural effects of developmental toxicity. Lancet Neurol. 13: 330–38.

Hart, J. 2020. Cover crop benefits outweigh the pest risk, but manage the ‘green bridge’. https://www.farmprogress.com/cover-crops/cover-crop-benefits-outweigh-pest-risk-manage-green-bridge.

Highland, H. B. and P. F. Lummus. 1986. Use of light traps to monitor activity of the burrowing bug, *Pangaeus bilineatus* (Hemiptera: Cydnidae), and associated field infestations in peanuts. J. Econ. Entomol. 79: 523-526.

Hill, M. P., Macfadyen S., Nash M. A. 2017. Broad spectrum pesticide application alters natural enemy communities and may facilitate secondary pest outbreaks. PeerJ. 5:e4179. DOI 10.7717/peerj.4179.

Holland, J. M., C. F. G. Thomas, T. Birkett, S. Southway, and H. Oaten. 2005. Farm-scale spatiotemporal dynamics of predatory beetles in arable crops. J. Appl. Ecol. 42: 1140—1152.

House, G. J. and M. Del Rosario Alzugaray. 1989. Influence of Cover Cropping and No-Tillage Practices on Community Composition of Soil Arthropods in a North Carolina Agroecosystem. Environ. Entomol. 8(2): 302-307.

Hutchins, S. H. 1994. Techniques for sampling arthropods in integrated pest management, pp. 73—97. *In* L. P. Pedigo and G. D. Buntin (eds.), Handbook of Sampling Methods for Arthropods in Agriculture. CRC Press, Inc., Boca Raton, FL.

Johnson, V. 1973. The female and host of *Triozocera mexicana* (Strepsiptera: Mengeidae). Ann. Ent. Soc. Amer. 66 (3): 671-672.

Jordan, D. L. 2021. NC State Extension 2021 Peanut Information, Chapter 3: Peanut production practices. https://content.ces.ncsu.edu/peanut-information/peanut-production-practices.

Lis, J. A., M. Becker, and C. W. Schaefer. 2000. Burrower bugs (Cydnidae), pp. 405—419. *In* C. W. Schaefer and A. R. Panizzi (eds.), Heteroptera of Economic Importance. CRC Press, Inc., Boca Raton, FL.

Lu, Y., K. Wu, Y. Jiang, Y. Guo, and N. Desneux. 2012. Widespread adoption of Bt cotton and insecticide decrease promotes biocontrol services. Nature. 487: 362—365.

Mbata, G. N. and D. Shapiro-Ilan. 2013. The potential for controlling *Pangaeus bilineatus* (Heteroptera: Cydnidae) using a combination of entomopathogens and an insecticide. J. Econ. Entomol. 106(5): 2072-2076.

Mie, A., C. Rhudén, and P. Grandjean. 2018. Safety of Safety Evaluation of Pesticides: developmental neurotoxicity of chlorpyrifos and chlorpyrifos-methyl. Environ. Health. 17: 77.

Paiero, S. M., S. A. Marshall, and K. G. A. Hamilton. 2003. New records of hemipteran from Canada and Ontario. J. ent. Soc. Ont. 134: 115—129.

Pike, K. S., and L. W. Getzin. 1981. Persistence and movement of chlorpyrifos in sprinkler-irrigated soil. J. Econ. Entomol. 74: 385-388.

Poggi, S., R. Le Cointe, J. Lehmhus, M. Plantegenest, L. Furlan. 2021. Alternative Strategies for Controlling Wireworms in Field Crops: A Review. Agriculture. 11: 436.

Puppala, N., N. P. Goldberg, L. Beck, S. Sanogo, S. Thomas and C. Trostle. 2018. New Mexico Peanut Production. https://aces.nmsu.edu/pubs/_circulars/CR645.pdf.

Riis, L., P. Esbjerg, and A. C. Bellotti. 2005. Influence of temperature and soil moisture on some population growth parameters of *Cyrtomenus bergi* (Hemiptera: Cydnidae). Fla. Entomol. 88(1): 11-22.

Roth, L. M. 1961. A study of the odoriferous glands of Scaptocoris divergens (Hemiptera: Cydnidae). Ann. Entomol. Soc. Am. 54 (6): 900-911.

Ruberson, J., H. Nemoto, and Y. Hirose. 1998. Pesticides and conservation of natural enemies in pest management, pp: 207-220. In Pedro Barbosa (ed.), Conservation Biological Control. Academic Press, Cambridge, MA. ISBN 9780120781478. https://doi.org/10.1016/B978-012078147-8/50057-8.

Russell, T. A. 1934. *Pangaeus bilineatus* ("Negro bug, *Pangaeus bilineatus",* punctured strawberries which subsequently rotted.). Bermuda Rept. Dept. Agriculture for 1933, p. 35.

Sailer, R. I. 1954. Concerning *Pangaeus bilineatus* (Say) (Hemiptera: Pentatomidae, subfamily Cydnidae). J. KS Entomol. Soc. 27 (2): 41-44.

Schwertner, C. F. and C. Nardi. 2015. Burrower Bugs (Cydnidae), pp: 639-680. *In* A.R. Panizzi, J. Grazia (eds.), True Bugs (Heteroptera) of the Neotropics, Entomology in Focus 2. DOI 10.1007/978-94-017-9861-7_21.

Sites, R. W. and J. E. McPherson. 1982. Life History and Laboratory Rearing of *Sehirus cinctus cinctus* (Hemiptera: Cydnidae), with Descriptions of Immature Stages. Ann. Entomol. Soc. Am. 75(2): 210–215.

Smith, J. W. and J. T. Pitts. 1974. Pest Status of *Pangaeus bilineatus* Attacking Peanuts in Texas. J. Econ. Entomol. 67(1): 111-113.

Strayer-Scherer, A. 2021. 2021 Peanut Production Guide [Alabama]: Peanut Disease and Nematode Management. https://www.alpeanuts.com/wp-content/uploads/2021/02/2021-Production-Guide.pdf.

Swenk, M. H. 1913. *Pangaeus bilineatus* (attacking newly sprouted wheat and injuring plants). Bul. State Entomologist. 1: 87.

Tissot, A. N. 1939. USDA Entomol. and Plant Quar. Bur., Ins. Pest Surv. Bull. 19: 455.

Trapero, C., I. W. Wilson, W. N. Stiller and L. J. Wilson. 2016. Enhancing Integrated Pest Management in GM Cotton Systems Using Host Plant Resistance. Front. Plant Sci. 7:500.

USDA. 2019. Farmers' Stock Peanuts: Inspection Instructions. https://www.ams.usda.gov/sites/default/files/media/FarmersStockPeanutsInspectionInstructions.pdf.

USDA National Agricultural Statistics Service. 2020. NASS - Quick Stats. National/State Peanut Production – Lbs. USDA National Agricultural Statistics Service. https://data.nal.usda.gov/dataset/nass-quick-stats

Van Emden, H. F. 2017. Host-plant resistance, pp. 515-532. *In*: Van Emden, H. F. and Harrington, R. (eds.) Aphids as Crop Pests, 2nd edition. CABI, Wallingford, UK.

Watson and Tissot. 1942. *Pangaeus bilineatus* (Discusses injury to strawberries and control). Univ. Fla. Agr. Exp. Station, Bui. No. 370, pp. 97-98.

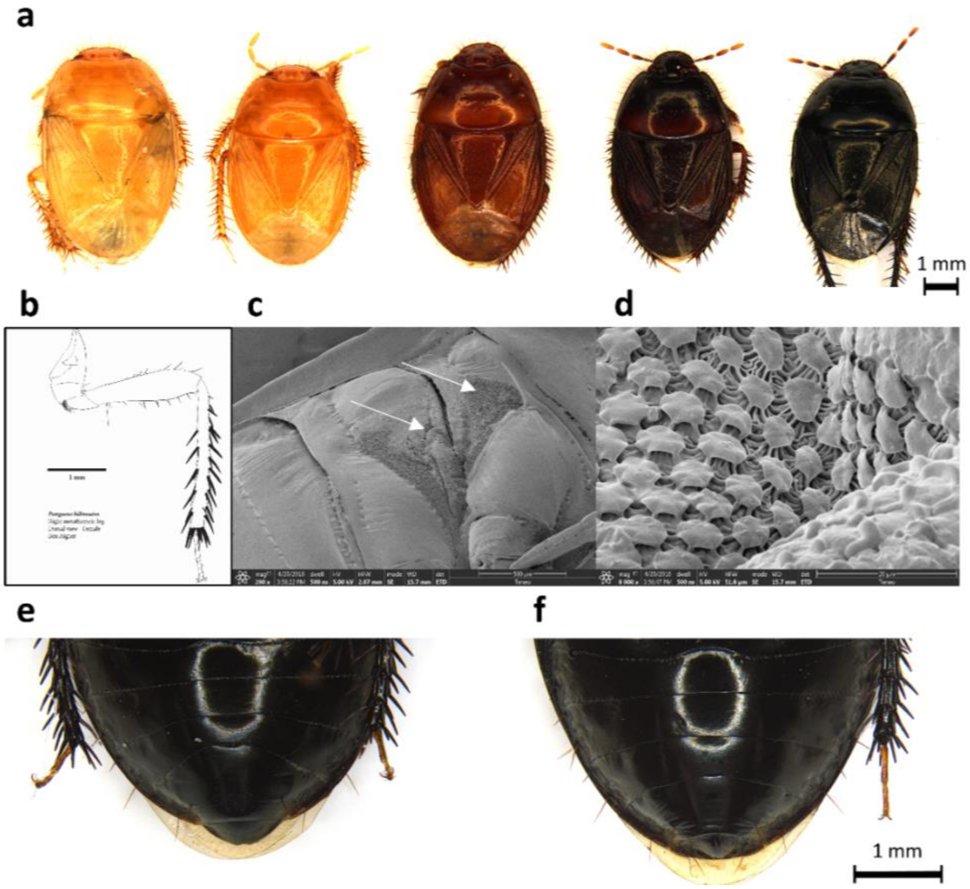
Wright, D. L., B. L. Tillman, I. M. Small, D. Pratap, Z. Grabau, S. Paula-Moraes, E. Carter, J. A. Ferrell, M. J. Mulvaney, C. Barrett, and N. DuFault. 2020. Peanut Production Guide 2020: Management and Cultural Practices for Peanuts. https://nfrec.ifas.ufl.edu/media/nfrecifasufledu/docs/pdf/Peanut-Production-Guide-2020.pdf.

Table 1.1. Average pronotum width of *P. bilineatus* nymphs and adults by life stage and ratio of growth between life stages.

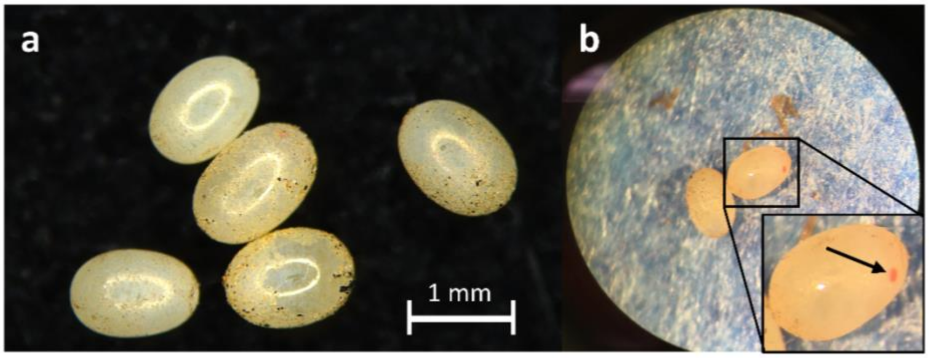
Life Stage	Avg Pronotum Width (μm)	SD	SEM	Growth Ratio
1st Instar	656.87 [a]	24.17	7.64	
2nd Instar	904.82 [a]	47.38	14.98	1.38
3rd Instar	1266.70 [a]	48.21	15.25	1.40
4th Instar	1799.55 [a]	101.10	31.97	1.42
5th Instar	2591.33 [a]	80.99	25.61	1.44
Adult	3582.14[b]	128.40	31.14	1.38

[a]n=10, [b]n=17.

Fig. 1.1. The sharp, deeply impressed line that runs parallel to the anterior and lateral edges of the pronotum serves as the primary diagnostic feature of *Pangaeus bilineatus* (Froeschner 1960).



Figs. 1.2.a-f. a) Adult *Pangaeus bilineatus*' gradual sclerotization post imaginal ecdysis at approximately 4 hrs (far left), 7 hrs (near left), 14 hrs (center), 22 hrs (near right), and > 30 hrs (far right). b) Drawing of the dorsal view of the right metathoracic leg of a female *P. bilineatus* (Drawing by B. L. Aigner, 2018). c) Scanning electron microscope (SEM) image of the evaporative structures on the right meso- and metapleura of an adult *P. bilineatus* female. Pictured areas of *P. bilineatus*' ventral thoracic plates are referred to by Froeschner (1960) as the mesopleural evaporatorium and the osteolar peritreme. d) Close-up SEM image of the metapleural evaporatorium near the opening of the metathoracic gland (Images by J. Shields, Georgia Electron Microscopy, UGA, 2018). e) Terminal segment of a male *P. bilineatus* showing the capsule that encloses the male genital clasper. f) Terminal segment of a female *P. bilineatus* showing the genital plates that enclose the female genitalia.



Figs. 1.3.a-b. Eggs of *Pangaeus bilineatus* a) < 3 days old, and b) > 3 days old (arrow pointing to red eyespot).

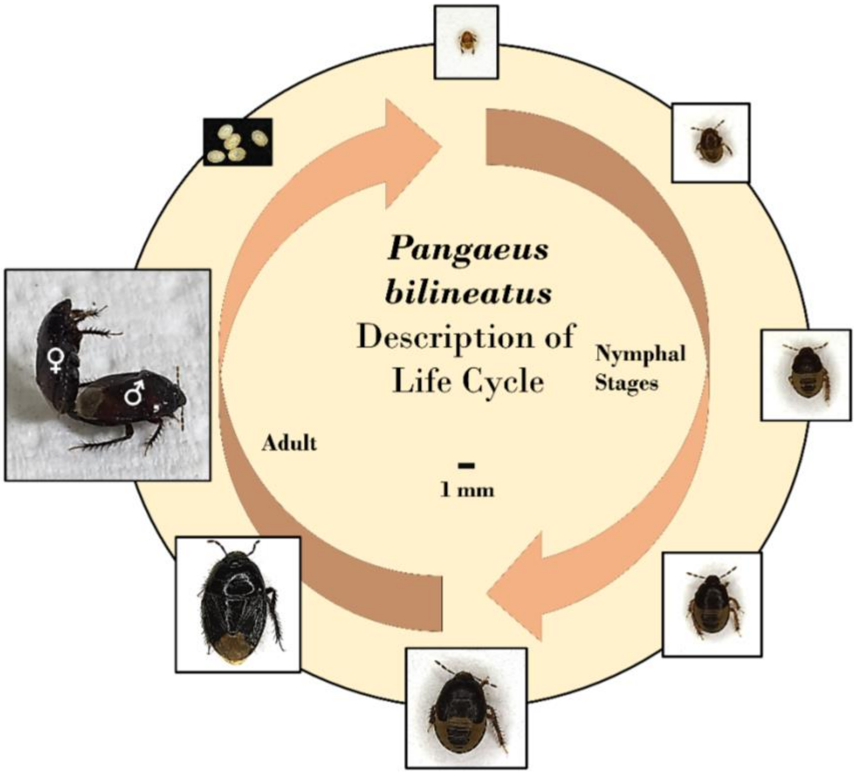
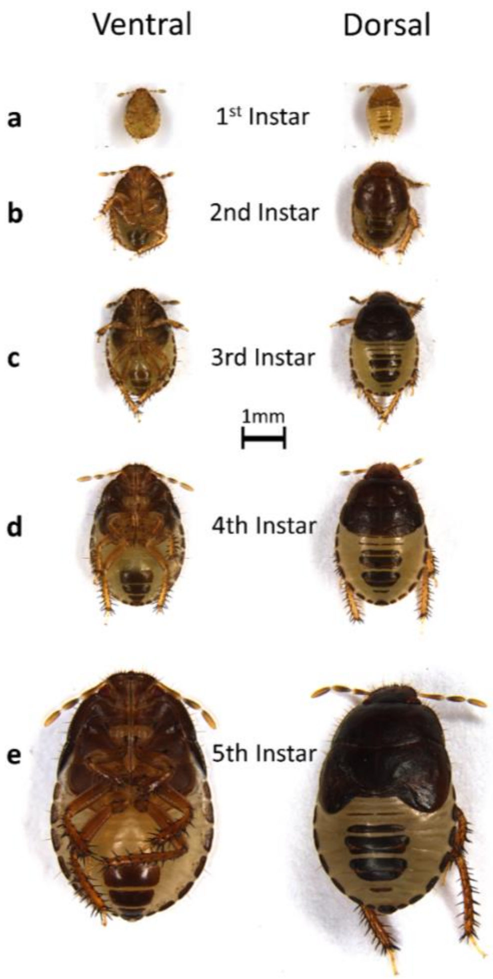


Fig. 1.4. Life cycle of *Pangaeus bilineatus*. After hatching, the bug develops through 5 nymphal instars before eclosion to adulthood. The image at 270° portrays mating adults.



Figs. 1.5.a-e. Ventral and dorsal view of immature *Pangaeus bilineatus* captured in a) first instar, b) second instar, c) third instar, d) fourth instar, and e) fifth instar stadia.

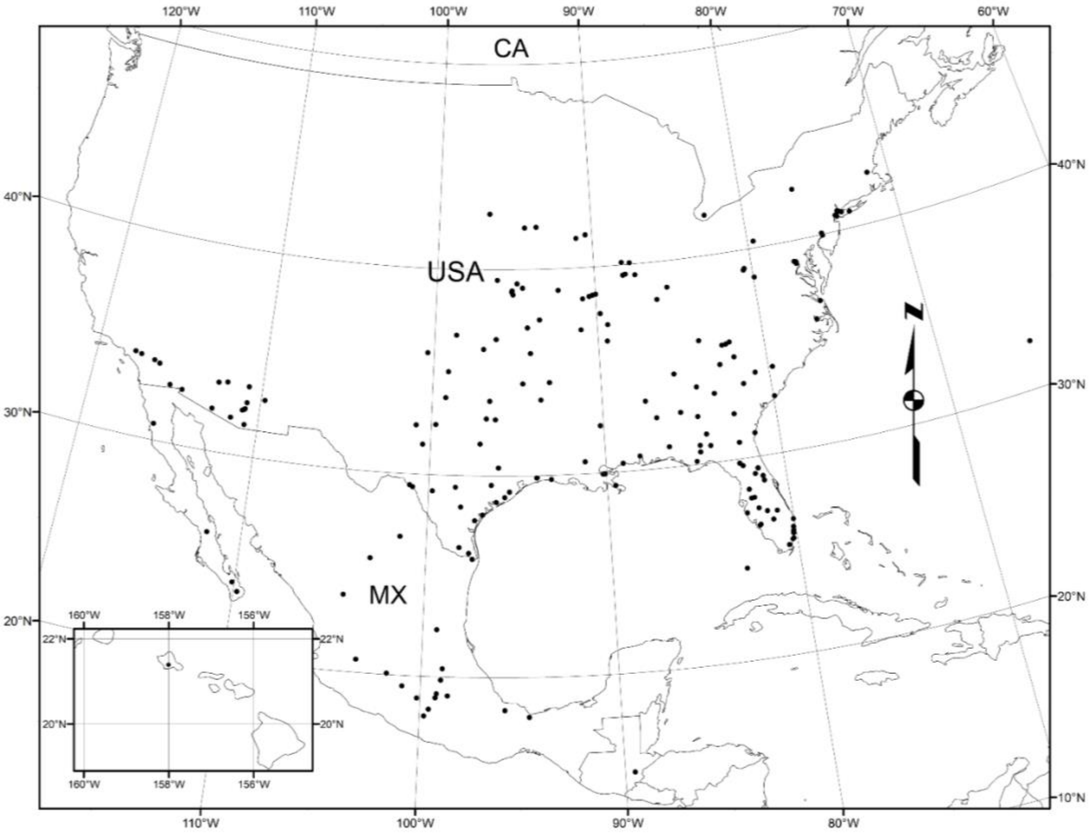


Fig. 1.6. Map of historical occurrence of *Pangaeus bilineatus* as documented in Froeschner's: Cydnids of the Western Hemisphere (1960) (Map drawn by B.L. Aigner and M.S. Crossley). Additional locations not listed by Froeschner (1960) include Ontario, CA (Paiero et al. 2003) and Oahu, Hawaii (Garcia 2011).

Fig. 1.7. Peanut seed with testa removed showing severe *P. bilineatus* feeding injury.

CHAPTER 3

LIFE CYCLE, MORPHOMETRICS, AND BEHAVIORAL ECOLOGY OF *PANGAEUS BILINEATUS* (HEMIPTERA: CYDNIDAE) UNDER LABORATORY CONDITIONS

Aigner, B.L. and M.R. Abney. To be submitted to the *Journal of Entomological Science*.

Abstract

Effective integrated pest management (IPM) strategies often exploit behaviors and biology of a target pest. Little is known of the behavior or biology of *Pangaeus bilineatus* Say, a severe peanut, *Arachis hypogaea* L., pest in the Southeast, US. Laboratory studies were conducted to elucidate *P. bilineatus* developmental biology, morphometrics, fecundity, longevity, and behavioral ecology. To define *P. bilineatus* biological parameters multiple experiments were conducted in a controlled environment chamber in the laboratory and observations were made daily for each study. Environment chambers were checked daily for eggs, nymphs, and adults; date, time, number of eggs, number of nymphs and their life stage, and/or number of adults were recorded. Time of development from oviposition to adult completed in 39.5 ± 3.0 days through 5 nymphal instars. Immature life stages last 3.7 – 8.1 days and head capsule width (.46 – 1.68 mm) progressively increases as nymphs age. Females produced a mean total of 78.5 ± 25.2 offspring after pairing 14 days and lived an average of 88.7 ± 31.2 days. Behavioral ecology studies indicate 1st instar nymphs need an adult female present to molt to the second instar. All studies were conducted in a controlled environment chamber on a 14:10 L:D cycle, constant temperature of 29°C, and 55% RH ± 10%.

Key Words: development, fecundity, longevity, life cycle, behavioral ecology.

Pangaeus bilineatus Say, is a polyphagous hemipteran soil pest in the family Cydnidae, subfamily Cydninae, and tribe Geotimini. It is native to the US and has been reported in 27 southern and eastern US states (Aigner 2021, Froeschner 1960). *Pangaeus bilineatus* was initially reported as a potential agricultural pest in 1934 (Gould 1931) after it was observed feeding on spinach, *Spinacia oleracea* L., seedlings in Virginia, and it caused significant peanut, *Arachis hypogaea* L., injury in Texas and Alabama in the late 1960's (Smith and Pitts 1974). Despite its reported pest status, the insect remained poorly studied, at least in part, due to cryptic life stages that are primarily subterranean. Since 2010, *P. bilineatus* has been a significant annual economic threat to peanut throughout Georgia's coastal plain (Abney 2014, Abney 2015, Abney 2016, Abney 2017, Aigner et al. 2021, Mbata and Shapiro-Ilan 2013). The revocation of food use tolerances for the insecticide chlorpyrifos in 2022 left no effective, commercially available insecticide(s) for *P. bilineatus* management in peanut (Aigner et al. 2021, Mbata et al. 2022, US EPA 2021). A better understanding of the biology and ecology of the pest is imperative for the development of sustainable integrated management tactics and strategies.

Integrated Pest Management (IPM) involves targeting pests in time and space, based on knowledge of pest biology and behavior within an agricultural production system. Susceptibility of insects to management tactics may vary among life stages (Cuthbertson et al. 2005, Ghidiu and Zehnder 1993, Pedersen et al. 2020), and timing treatment applications according to peak occurrence of susceptible life stages (and stages of host plant vulnerability) can prevent pest populations from reaching economic injury levels (Chi 1990). Prabhaker et al. (2006) assessed toxicity of various classes of insecticides on immature stages of glassy-winged sharpshooter, *Homalodisca coagulata* Say. Results showed, early instar nymphs were most susceptible to all treatments tested, and susceptibility to all treatments decreased as life stage increased.

Determining the life stages of arthropod species relies on a frequency distribution of larval size, usually measured by head capsule width (Floater 1996). Life stage characterization and knowledge of population growth parameters like fecundity and female longevity enable researchers to construct life tables and model population dynamics (Kakde et al. 2014; Nielsen et al. 2008). Moreover, aspects of insect behavioral ecology further our understanding of insect development, health, and fitness in certain environmental conditions (Fukatsu and Hosokawa 2002; Prado and Almeida 2009; Taylor et al. 2014). In turn, prediction of peak occurrence of pest (and/or natural enemy) populations is feasible for field management purposes. For example, in the Southeast US peanut planting date is routinely adjusted to avoid early season peak thrips populations (Srinivasan et al. 2018). A thorough understanding of pest biology and ecology facilitates the modeling of pest phenology and, subsequently, pest distribution throughout agricultural landscapes, as described by Philips et al. (2011) in the case of cereal leaf beetle, *Oulema melanopus* L. Similarly, understanding the biology of *Diabrotic virgifera* Leconte led to the use of crop rotation as an effective management tool (Gillette 1912). Research within the scope of insect biology and ecology may lead to further study of the influence of planting date, tillage practices, irrigation schedules, biocontrol, insecticide use patterns, etc. for improved efficacy of interventions and optimization of sustainable management tactics (Barzman et al. 2015, Kogan 1998). These examples highlight how knowledge of pest biology and ecology can be used to develop and hone sustainable IPM strategies that reduce harm to farmers, crops, and the environment.

Here we report the results of studies designed to elucidate aspects of *P. bilineatus'* biology and behavioral ecology under controlled conditions including: (1) the number and duration of life stages; (2) the relationship between head capsule width and life stage (adherence to Brooks—Dyar's law); (3) fecundity and female longevity; and (4) the effect of adult presence/absence on nymphal development.

Materials and Methods

Laboratory Rearing.

All experiments were conducted using insects from a laboratory colony of *P. bilineatus* maintained in Tifton, GA. The colony was established with nymphs and adults collected in November 2016 from a field in Terrell County, Georgia, US (31.7929°, -84.3241°). Adults were collected from Emanuel County, GA (32.60534°, -82.26979°) and added to the colony in December 2018. Both fields had been planted to peanut the year collections were made.

Plastic food storage containers (27.5 × 25.5 × 19.5 cm; MPN: FG631200CLR; Rubbermaid Commercial Products Inc., Winchester, VA, USA) were filled with approximately 10 L of sandy loam soil collected at the UGA Lang-Rigdon Farm (31.511239°, -83.549084°) in Tifton, GA. All soil used for the studies reported in this manuscript was collected from this location and heat treated for at least 2 hours at 107°C (Wilen et al. *Revised continuously*). Containers were covered with a plastic lid. A single 16 × 8.5 cm hole was cut into the center of each lid to provide ventilation, and 1.5 mm cloth mesh screen was hot-glued over the hole. Populations were allowed to grow in each container for 4 to 6 months, then 20 healthy adult bugs were transferred into a clean container with new soil so they could reproduce.

Bugs in the laboratory colony were fed raw, untreated peanut seed (3 – 7% moisture content), and soil in rearing containers was wetted with approximately 100 mL filtered tap water (Model Number: 56151-03; 3M Purification Inc., Meriden, CT, USA) twice per week (every 4th day or as needed). Soil moisture was maintained at 10-15% volumetric water content (VWC) as averaged over four measurements per container with a Field Scout TDR (time domain reflectometry) 300 Soil Moisture Probe (12.2 cm probe length; Model Number: 6430FS; Spectrum Technologies, Inc., Aurora, IL, USA). Roughly 1 peanut seed/6.5 cm^2 was placed on the soil surface in each container. Containers were kept in an insect rearing room maintained at 28°C ± 1.1°C, 14: 10 L: D cycle, and 55% ± 10% RH. WatchDog Data Loggers (1000/2000 Series Spectrum Technologies, Inc., Aurora, IL.) were used to monitor temperature and relative humidity in the rearing room. Bugs for all studies were reared using the procedures and conditions described here.

Developmental Biology – Fecundity and Longevity.

Roughly 50 fifth instar nymphs were placed in each of five 437 mL plastic cups with vented lids (MRP Number: P160100002; Placon Inc., W. Springfield, MA, US) and approximately 300 g of Tifton sandy loam soil, 30 mL filtered tap water, and raw peanut seed. The cups were placed in an environment chamber (Model Number: I36LLVLC8; Percival Scientific Inc., Perry, IA, USA) at 29°C ± 1.1°C, 14: 10 L: D cycle, and 55% ± 10% RH. The bugs were provided new peanut seed daily, and water was added to cups as needed to maintain soil moisture at 5 – 15 % VWC. Containers were examined each day for newly eclosed adults, which were removed before they had the opportunity to mate. Virgin adults were isolated by sex and stored in vented 437 mL plastic cups as described previously; insects were fed raw peanut seed and watered daily until ready for use.

Seventy grams of Tifton sandy loam soil was added to each of sixteen 266 mL resealable plastic containers (each container = 1 rep) to a depth of 1 cm (11 × 8 × 5 cm.; Mfr Number: 459224; Glad Products Co., Oakland, CA, US). One virgin male and female were placed in each container within 1 day of adult eclosion. Then, raw, untreated peanut seed (1 seed/6.5 cm^2) and approximately 6 mL water were added, and the container was covered with a vented lid. Containers were stored in a controlled environment chamber at 29˚C ± 1.1°C, 55% ± 10% RH, and 14:10 L:D cycle. Eggs were collected from the soil by placing the contents of each container separately in a solution of 2 mol $MgSO_4$ and filtered tap water (Shaw et al. 1976) in a 7.5 L plastic food storage container (27.5 × 25.5 × 19.5 cm; MPN: FG631200CLR; Rubbermaid Commercial Products Inc., Winchester, VA, USA), and gently mixed by hand for approximately 1 min. After allowing sediment to settle for approximately 5 mins, contents were poured through a 1 mm (number 18 mesh) sieve (Model Number: EISC-BI0139; Hubbard Scientific Co., Northbrook, IL, USA) into another 7.5 L food storage container. Eggs caught in the sieve were rinsed with filtered water from a spray bottle (P.F. Harris Manufacturing Co., LLC, Cartersville, GA, USA) to remove the $MgSO_4$ solution and debris, and the eggs were counted. Adult pairs were transferred to clean, vented 266 mL plastic containers as described previously. The number of days from pairing to first appearance of eggs (post-adult eclosion + preoviposition period), number of eggs/female, number of eggs/day (oviposition rate), total number of days oviposition occurred (oviposition period), and longevity of females were recorded. Effect of location within the environment chamber on the number of eggs/female, oviposition rate, oviposition period, and female longevity was analyzed using ANOVA in R (R Core Team 2020).

Developmental Biology – Development Time.

Adults (3:3, male: female) of unknown age were randomly selected from the laboratory colony and placed in each of 72 vented plastic containers (266 mL) previously filled with 70 g of moist soil and 12 raw peanut seed. Approximately 6 mL filtered tap water was added to each container daily. The adults were transferred daily into new containers prepared as described previously. The soil in containers from which adults were taken was used for the study and contained eggs laid within the previous 24 hours. Though the mechanism responsible for the phenomenon is unknown, *P. bilineatus* nymphs will not molt to subsequent life stages if a female adult is not present. Therefore, a single virgin female was added to each container at the first sighting of 1st instar nymphs to ensure normal nymph development without the possibility of a mated female laying additional eggs. The 72 individual containers (each container = 1 rep) were placed in an environment chamber at 29˚C ± 1.1°C, 55% ± 10% RH, and 14:10 L:D cycle. Eight containers were randomly chosen each day for 45 days to determine the number of individuals present in each developmental stage. After sampling, containers were returned to the same location in the environment chamber and removed from the pool of random sample selections for the following 3 days. Each container was sampled 3 to 7 times over the course of the experiment. The number of bugs in each life stage was determined by pouring the contents of a container onto a white tray, searching through the soil with a number 2 camel hair paintbrush, and counting the number of bugs in each life stage. The number of days to detection of each life stage was recorded. The effect of location within the environment chamber on life stage duration was analyzed using ANOVA in R.

Morphometrics – Predicting Life Stage by Head Capsule Width.

Twenty bugs of each life stage were individually photographed, and their head capsules were measured from the outer edge of each eye with a Leica M165-C compound microscope (Model/PN: MDG34/10 450 124; Leica Microsystems (SEA) Pte. Ltd., Radin Mas, Singapore), Leica digital microscope camera (Model Number: DFC450 (12730411); Leica Microsystems Ltd., Wetzlar, Hesse, DE) and Las X Software (Leica Microsystems Inc., Buffalo Grove, IL, USA). Life stage was plotted by head capsule width, and the relationship between head capsule with and life stage was examined by fitting linear and exponential regression models to determine best fit to the data and determine whether head capsule width is an accurate predictor of life stage in *P. bilineatus*. Additionally, the natural log of the mean head capsule width was plotted by life stage to determine if the increase in *P. bilineatus* head capsule width adheres to Dyar's rule (1890).

Egg Collection for Behavioral Ecology Studies.

Eggs for behavioral ecology studies were collected from the laboratory colony. Approximately 300 g of soil were taken from rearing containers. Eggs were extracted from the soil using the flotation method described previously (Shaw et al. 1976). Approximately 200 eggs were transferred from the sieve with a number 2 camel hair paintbrush into a 9 cm Petri dish containing germination paper (Model Number: WHA-2181-090; Capitol Scientific Inc., Austin, TX) moistened with filtered water. The dish was sealed with Parafilm and stored in an environment chamber (28°C ± 1.1°C and 55% ± 10% RH) until ready for use.

Behavioral Ecology – Male vs. Female Interaction with Nymphs.

Twenty grams of Tifton sandy loam soil were placed in 30 mL plastic cups (Model Number: P125; SOLO Cup Co., Urbana, IL, USA). A 3 mm depression was made in the soil in the middle of each cup using the handle of a number 2 camel hair paintbrush, and 1, 2, or 4 eggs were placed in the depression (Table 2.1). Cups were covered with perforated lids, labeled by treatment, and arranged in a 9-treatment × 5-rep RCBD in 44 × 34.5 cm trays inside a controlled environment chamber at 29°C ± 1.1°C, 60% ± 10% RH, and 14:10 L:D. Treatments consisted of the following egg/adult combinations: 1 egg/0 adult, 2 eggs/0 adult, 4 eggs/0 adult, 1 egg/1 virgin female, 2 eggs/1 virgin female, 4 eggs/1 virgin female, 1 egg/1 virgin male, 2 eggs/1 virgin male, and 4 eggs/1 virgin male (Table 2.1).

Approximately 1 mL filtered tap water and a single, raw peanut seed were added to each cup daily. After 21 days the soil in each cup was poured onto a white tray and searched using a number 2 camel hair paintbrush. The number and life stages of live and dead bugs were recorded. The data were subjected to ANOVA to identify significant differences in nymph mortality and life cycle progression between treatments. Means separations were performed via two sample t-tests using a .05 significance level and Bonferroni's p-value adjustment for comparisons.

Behavioral Ecology – Nymph Development.

Arenas were constructed by gluing 50 × 9 mm Petri dishes (Fig. 2.1A; Model Number: 351006; Corning Inc., Corning, NY, USA) into the center of 90 × 25 mm Petri dishes (Fig. 2.1B; Model Number: D210-8R; Electron Microscopy Sciences, Hatfield, PA, USA). A hot number 3 insect pin was used to make 12 ventilation holes in the sides of the interior dish. A 2.2 cm diameter hole was cut in the center of the lid of the interior dish and covered with 15 µm polyolefin mesh attached with hot glue to allow air movement. An even layer (3 mm deep; 15 g total for each arena) of Tifton sandy loam soil was applied to the inner and outer dish (Fig. 2.1) of each arena. Ten eggs were placed in the exterior dish of each arena and one live female or one recently killed female – frozen within 10 mins of setup – was placed in either the interior dish or the exterior dish according to treatment (Table 2.2). Treatments consisted of: (1) no adult; (2) a live female in the interior dish (no contact with eggs/nymphs); (3) a dead female in the interior dish; (4) a live female in the exterior dish (contact with eggs/nymphs allowed); and (5) a dead female in the exterior dish. Dead females used in the experiment were replaced after 10 days with freshly killed specimens. Arenas were arranged in a 5 treatment × 5 rep RCBD (blocked by shelf) inside a controlled environment chamber at 29°C ± 1.1°C, 14:10 L:D, and 60% ± 10% RH. After 21 days the soil in each arena was searched using a number 2 camel hair paintbrush, and the number and life stage of live and dead bugs were recorded. Data were subjected to ANOVA to identify significant treatment effects on nymph mortality and development. Means separations were performed via two sample t-Tests using a .05 significance level and Bonferroni's p-value adjustment for comparisons.

Results

Developmental Biology – Fecundity and Longevity.

All 16 pairs of adults successfully mated and produced eggs. The mean number of days from pairing to the first egg (post-eclosion + preoviposition period) was 7.81 ± 0.91 (SEM = 0.23; *n* = 16). The mean total number of eggs produced per female was 129.0 ± 80.78 (SEM = 20.86; *n* = 15). The mean number of eggs laid per day per female was 2.48 ± 1.43 (SEM = 0.37; *n* = 15), and the average duration of the oviposition period was 62.25 ± 30.72 (SEM = 7.68; *n* = 16). Females lived an average of 88.67 ± 31.22 days (SEM = 8.06; *n* = 15). No location effects were noted for any of the parameters measured. Peak oviposition occurred in the first 11 days of the oviposition period and gradually decreased until the end of the experiment (Fig. 2.2). The mean number of days from the end of *P. bilineatus'* oviposition period until death was 14.5 ± 12.2 (SEM = 3.16; Table 2.3) and ranged from 1 to 39 days. Descriptive statistics are reported in Table 2.3.

Developmental Biology – Development Time.

Sixty two out of 72 adult pairings produced viable offspring. The proportion of bugs in each life stage on each sample day is displayed in Fig. 2.3. *Pangaeus bilineautus* is paurometabolous and develops through five nymphal instars before adult eclosion. The mean days from oviposition to egg hatch (egg incubation period) was 11.25 ± 1.87 (SEM = 0.33; Fig 2.4). Mean days from hatch until molt to the second instar was 4.00 ± 1.88 days (SEM = 0.33; Fig 2.4). Mean days from the second instar until molt to the third instar was 3.74 ± 2.05 days (SEM = 0.35; Fig 2.4). Mean days from the third instar until molt to the fourth instar was 4.77 ± 3.80 days (SEM = 0.64; Fig 2.4). Mean days from the fourth instar until molt to the fifth instar was 6.55 ± 3.85 days (SEM = 0.58; Fig 2.4). And, mean days from the fifth instar until molt to adult was 8.65 ± 3.21 days (SEM = 0.36; Fig 2.4). Overall time elapsed from oviposition to adult eclosion was 39.51 ± 2.98 days (SEM = 0.34, n = 330 bugs; Fig 2.4). No location effects were detected within the environment chamber.

Morphometrics – Predicting Life Stage by Head Capsule Width.

The exponential model provided the best fit to the data ($y = 360.55e^{0.2626x}$; $R^2 = .99$; Fig. 2.5). A significant linear relationship between log transformed head capsule width and growth stage was observed (p = 1.98 e -.06; $R^2 = .99$; Fig. 2.6), validating the correct identification of life stage (Daly 1985). The mean ratio of head capsule width between life stages was 1.3: 1 (successive life stage: previous life stage; Table 2.4).

Behavioral Ecology – Male vs. Female Interaction with Nymphs.

The number of eggs in each treatment had no significant effect on nymph mortality or the development of nymphs to 4th/5th instars. Therefore, treatments with the same adult pairing condition were combined for analysis (3 groups: no adult, adult female, and adult male). Mortality of nymphs paired with a female was significantly lower than of nymphs paired with a male or no adult (F = 7.36; p = 2.281e-3; df = 35; Fig. 2.7). Significantly more nymphs matured to 4th/5th instar when paired with a female than when paired with a male or no adult (F = 15.43; p = 1.855e-6; df = 35; Fig. 2.8).

Behavioral Ecology – Nymph Development.

Nymph mortality was significantly lower when nymphs had direct contact with a live female compared to all other treatments ($F = 19.73$; $p = 1.02\text{e-}6$; $df = 24$; Fig. 2.9). Likewise, significantly more nymphs with direct contact to females developed to successive instars compared to nymphs in all other treatments. Ninety-six percent of nymphs matured to at least the 2nd instar, and 88% of nymphs matured to 4th/5th instars ($F = 26.95$; $p = 8.32\text{e-}8$; $df = 24$; Fig. 2.10) when in direct contact with a female. Twenty-three percent of nymphs matured to the 3rd instar in the treatment with a live female but no direct contact. No other treatments had any nymphs mature beyond the 2nd instar, and there were no significant differences between remaining treatments.

Discussion

Pangaeus bilineatus has relatively low fecundity compared to some major lepidopteran (Luginbill 1928, Quaintance and Brues 1905) and coleopteran pest species (Brown et al. 1980), but similar fecundity to other heteropteran pests such as *Halyomorpha halys* Stål (Nielsen et al. 2008), *Euschistus servus* Say (Rolston and Kendrick 1961), and the cydnid *Cyrtomenus bergi* Froeschner (Riis et al. 2005a, b). Aigner et al. 2021 reported that multiple *Pangaeus bilineatus* generations likely occur each year in South Georgia. Multiple generations of a pest with relatively low fecundity occurring in the same crop field increase the risk that the population will reach an economically damaging level, and management tactics that target early generations of the pest could prevent injury later in the season. While little is known of *Pangaeus bilineatus* movement patterns, the insect is capable of flight and is readily collected in light traps (Highland and Lummus 1986, Aigner et al. 2021). The mean *Pangaeus bilineatus* female life span of 88 days provides ample opportunity for the insect to locate suitable habitat for oviposition.

Pangaeus bilineatus is largely subterranean, and mating duration and frequency are not observable without disturbing the insects and, in the case of research, potentially affecting the outcome of experiments. For this reason, mating duration, frequency, and polyandry were not measured in this study.

Pangaeus bilineatus develops through five nymphal instars (Aigner et al. 2021), as do other cydnid species and most other terrestrial heteroptera (Riis et al. 2005a, b, Schwertner and Nardi 2015, Sites and McPherson 1982). Nevertheless, the duration of these life stages was unknown. The results of the work presented here show that *P. bilineatus* can complete development from egg hatch to adult in about 30 days; this is considerably shorter than the 60 days reported for the larger Cydnidae *C. bergi* and the 50 days reported for *Sehirus cinctus* Palisot (Riis et al. 2005a, b, Sites and McPherson 1982), albeit under different conditions. The pest potential of an insect species is often linked to development time and number of generations per field season (Porter et al. 1991); the relatively rapid development of *P. bilineatus* may contribute to its status as an economic pest.

Pangaeus bilineatus is largely subterranean and mating duration and frequency are not observable without disturbing the insects and, in the case of research, potentially affecting the outcome of experiments. For this reason, the studies described here were not controlled for mating duration, frequency, or polyandry.

William K. Brooks (1886), and later Harrison G. Dyar (1890), proposed that arthropods (namely crustaceans and lepidopterans, respectively) express consistent patterns of geometric progression of growth between molts that can be measured via head capsule width (or length of other highly sclerotized parts). This biological phenomenon is known as Dyar’s Law/Rule, or Brooks – Dyar’s Law/Rule, and can enable accurate prediction of life stage when molts are unobservable. To determine if Dyar's Law is applicable to *P. bilineatus*, the natural log of the mean head capsule width of each life stage should fall on a straight line when plotted against the presumed life stage. Any deviation of points from a straight line indicates an incorrect identification of the life stage in question, or a missing life stage (Daly 1985). When the natural log transformation of mean *P. bilineatus* HCW data were plotted against the presumed life stage, all of the points fell on the linear regression line (Fig. 2.6). This indicates that *P. bilineatus* life stage can be predicted by its HCW. The mean growth ratio of 1.297 ± 0.046 µm for *P. bilineatus* falls within the range 1.2 – 1.4 described by Dyar, which further supports the conclusion that *P. bilineatus* life stage can be predicted by its HCW (Dyar 1890). The ability to accurately identify *P. bilineatus* life stage via head capsule width could provide utility in an IPM program by facilitating optimum timing of management tactics to target the most vulnerable life stages.

Patterns of subsociality and maternal investment have been observed in several Cydnidae species in the subfamily Sehirinae, including *Adomerus rotundus* Hsiao (Inadomi et al. 2014), *Adomerus triguttulus* Motschulsky (Kudo et al. 2006, Kudo et al. 2021, Nakahira and Kudo 2008), *Adomerus variegatus* Signoret (Mukai et al. 2010), *S. cinctus* (Agrawal et al. 2001, Agrawal et al. 2004, Agrawal et al. 2005, Sites and McPherson 1982), and *Canthophorus niveimarginatus* Scott (Filippi et al. 2009). Each of these species lay eggs in clusters in soil and displays maternal investment in the form of maternal defensive behaviors (guarding of eggs and early instar nymphs) and food provisioning behaviors. All but *S. cinctus* lay trophic eggs to enhance survivorship of offspring (Inadomi et al. 2014). *Pangaeus bilineatus* does not lay their eggs in clusters, but females in a laboratory colony have been observed burying seed and ovipositing near the food source (personal observation). In the experiments described here, *P. bilineatus* nymphs that did not have direct physical contact with an adult female did not successfully develop, alluding to a further degree of maternal investment, although the mechanism is unclear.

This work shows that *P. bilineatus* 1st instar nymphs rely on postembryonic physical interaction with female adults to develop and molt properly. Interestingly, interaction with a virgin or mated adult female can enable development of nymphs to the next life stage. It is unknown whether the absence of an adult female inhibits the development of 2nd, 3rd or 4th instars. Fifth instar nymphs that were exposed to adult females early in development matured to adults, demonstrating that *P. bilineatus* can mature through the 5th instar without direct physical contact with an adult female. This was observed as bugs were collected for mating studies.

The role of female adults in nymph development is a mystery, but there are a few potential explanations that require detailed exploration in future studies. Endosymbionts are well known contributors to the overall fitness, development, and survivability of several insect species (Dillon and Dillon 2004, Elston et al. 2022). Postembryonic acquisition of endosymbionts by *H. halys* occurs via feeding on the chorion of hatched eggs (Taylor et al. 2014), and *Megacopta punctatissima* nymphs similarly acquire endosymbionts from ‘symbiont capsules’ deposited by females on the underside of egg masses (Hosokawa 2005). Mode of transmission can occur vertically via egg smearing, germline, gland secretions, and/or transovarial transmission or horizontally via mating, food source, and/or coprophagy (fecal – oral ingestion) (Beard et al. 2001, Elston et al. 2022). Although these behaviors have not been observed in *P. bilineatus,* they should be considered in future research.

DNA-methylation is a form of epigenetic information that influences phenotypic expression in animals by altering chromosome structure through attachment of methyl groups at cytosines in CG dinucleotides facilitated by enzymes in the DNA methyltransferase (DNMT) family (Cardoso-Júnior et al. 2021, Glastad et al. 2017, Vogt 2022). Transcription factor binding (Wang et al. 2012), alternative splicing (Li-Byarlay et al. 2013, Shukla et al. 2011), and transcriptional elongation (Zilberman et al. 2007) are known mechanisms by which methylation regulates gene function (Glastad et al. 2017). De novo methyltransferases are enzymes responsible for new methylation patterns and necessary for a functional methylation system (Glastad et al. 2011) and cytosine isoforms of DNMT1 and DNMT3 were revealed in the genome of pea aphids, *Acyrthosiphon pisum* Harris, by Walsh et al. (2010). Methylation has been reported as an important factor in the mediated repression of specific genes that regulate ecdysone – a critical hormone secreted during the molting process – biosynthesis in some insects (Yang et al. 2021). In vertebrates, parental care of offspring has a strong influence on brain development, and Weaver et al.'s (2004) studies of rats revealed variation in parental care results in variation in DNA – methylation and stress responses. In some eusocial insects, methylation is shown to influence developmental plasticity (Champagne 2013), and recent findings by Arsenault et al. (2018) describe maternal care as role player in the developmental and behavioral response of young honey bees, *Apis mellifera* L. The role of DNA methylation has not been explored in *P. bilineatus,* but it could explain how female contact enables nymphal development.

The studies presented here illuminate previously unknown aspects of *P. bilineatus* biology and ecology. There is still much to learn about *P. bilineatus*, but knowledge of fecundity, longevity, development time, morphometrics, and behavioral ecology furthers our understanding of the bug and provides a foundation for future research and development of comprehensive integrated pest management.

Acknowledgements

We thank the Georgia Peanut Commission, National Peanut Board, and USDA for funding this project. We also thank the UGA Peanut Entomology Lab for assistance in completing these experiments, and an additional thanks to Dr. Elizabeth McCarty for allowing the use of her laboratory equipment.

References cited

Abney, M.R. 2014. Georgia Entomological Society Arthropod Survey. Georgia Entomological Society. https://site.caes.uga.edu/ges/arthropod-surveys/.

Abney, M.R. 2015. Georgia Entomological Society Arthropod Survey. Georgia Entomological Society. https://site.caes.uga.edu/ges/arthropod-surveys/.

Abney, M.R. 2016. Georgia Entomological Society Arthropod Survey. Georgia Entomological Society. https://site.caes.uga.edu/ges/arthropod-surveys/.

Abney, M.R. 2017. Georgia Entomological Society Arthropod Survey. Georgia Entomological Society. https://site.caes.uga.edu/ges/arthropod-surveys/.

Abney, M.R., B.L. Aigner, P.M. Crosby, and S. Hollifield. 2017. Peanut burrower bug (Hemiptera: Cydnidae) distribution and management in Southeast US runner-type peanut. Abstract. In *49th Proceedings of the American Peanut Research and Education Society, Inc*. Annual Meeting. July 11–13, 2017. Albuquerque, NM.

Agrawal, A., E.D. Brodie, and J. Brown. 2001. Parent–offspring coadaptation and the dual genetic control of maternal care. *Science.* 292: 1710–1712.

Agrawal, A.F., J.M. Brown, and E.D. Brodie. 2004. On the social structure of offspring rearing in the burrower bug, *Sehirus cinctus* (Hemiptera: Cydnidae). *Behav. Ecol. Sociobiol.* 57(2): 139-148.

Agrawal, A.F., N. Combs, and E.D. Brodie. 2005. Insights into the costs of complex maternal care behavior in the burrower bug (*Sehirus cinctus*). *Behav. Ecol. Sociobiol*. 57(6): 566-574.

Aigner, B.L., M.S. Crossley, and M.R. Abney. 2021. Biology and Management of Peanut Burrower Bug (Hemiptera: Cydnidae) in Southeast US Peanut. *J. Integr. Pest Manag.* 12(1): 29. https://doi.org/10.1093/jipm/pmab024

Arsenault, S.V., B.G. Hunt, and S.M. Rehan. 2018. The effect of maternal care on gene expression and DNA methylation in a subsocial bee. *Nature communications*. 9(1): 1-9.

Azmy, N.M. 1976. Sexual activity, fecundity and longevity of *Nezara viridula* (L.). *Bulletin de la Société d' Égypte*. 60: 323-330.

Barzman, M., P. Bàrberi, A.N.E. Birch *et al.* 2015. Eight principles of integrated pest management. *Agron. Sustain. Dev.* 35: 1199–1215. https://doi.org/10.1007/s13593-015-0327-9.

Beard, C.B., E.M. Dotson, P.M. Pennington, S. Eichler, C. Cordon-Rosales, and R.V. Durvasula. 2001. Bacterial symbiosis and paratransgenic control of vector-borne Chagas disease. *Int. J. Parasitol.* 31(5-6): 621-627.

Bellotti, A.C., O. Vargas, B. Arias, O. Castaño, and C. Garcia. 1985. July. *Cyrtomenus bergi* Froeschner, a new pest of cassava: biology, ecology and control. In *7th Symposium of the International Society for Tropical Root Crops.* Gosier (Guadeloupe). pp: 1-6.

Brooks, W.K. 1886. Report on the stomatopoda. Report on the scientific results of the voyage of H.M.S. challenger during the years 1873–76. *J. Zool.* 16(45): 1–116. doi:10.5962/bhl.title.9891

Brown, J.J., T. Jermy, and B.A. Butt. 1980. The influence of an alternate host plant on the fecundity of the Colorado potato beetle, *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae). *Ann. Entomol. Soc. Am*. 73:197-99

Champagne, F.A. 2013. Epigenetics and developmental plasticity across species. *Dev. Psychobiol. 55*(1): 33-41.

Chapin, J.W. and J.S. Thomas. 2003. Burrower bugs (Heteroptera: Cydnidae) in peanut: seasonal species abundance, tillage effects, grade reduction effects, insecticide efficacy, and management. *J. Econ. Entomol.* 96(4): 142-1152.

Chi, H. 1990. Timing of control based on the stage structure of pest populations: a simulation approach. *J. Econ. Entomol.* 83(4): 1143-1150.

Cuthbertson, A.G., K.F. Walters, and P. Northing. 2005. The susceptibility of immature stages of *Bemisia tabaci* to the entomopathogenic fungus *Lecanicillium muscarium* on tomato and verbena foliage. *Mycopathologia.* 159: 23-29.

Daly, H.V. 1985. Insect morphometrics. *Annu. Rev. Entomol.* 30(1): 415-438.

Dillon, R.J. and V.M. Dillon. 2004. The gut bacteria of insects: nonpathogenic interactions. *Annu. Rev. Entomol.* 49(1): 71-92.

Dyar, H.G. 1890. The number of molts of lepidopterous larvae. *Psyche (N. Y.)*. *5*(175-176): 420-422.

Elston, K.M., S.P. Leonard, P. Geng, S.B. Bialik, E. Robinson, and J.E. Barrick. 2022. Engineering insects from the endosymbiont out. *Trends Microbiol.* 30(1): 79-96.

Filippi L., N. Baba, K. Inadomi, T. Yanagi, M. Hironaka, S. Nomakuchi. 2009. Pre- and post-hatch trophic egg production in the subsocial burrower bug, *Canthophorus niveimarginatus* (Heteroptera: Cydnidae). *Sci. Nat.* 96(2): 201-11. doi: 10.1007/s00114-008-0463-z.

Filippi, L., M. Hironaka, S. Tojo, and S. Nomakuchi. 2000. Insemination success discrepancy between long-term and short-term copulations in the provisioning shield bug, Parastrachia japonensis (Hemiptera: Cydnidae). *J. Ethol.* 18(1): 29-36.

Froeschner, R. C. 1960. Cydnidae of the Western Hemisphere. *Proc. U. S. Nat. Mus.* 111: 337-680.

Fukatsu, T., and T. Hosokawa. 2002. Capsule-transmitted gut symbiotic bacterium of the Japanese common plataspid stinkbug, *Megacopta punctatissima*. *Appl. Environ. Microbiol.* 68(1): 389–396. https://doi.org/10.1128/AEM.68.1.389-396.2002

Gillette, C.P. 1912. *Diabrotica virgifera* Lec. as a corn rootworm. *J. Econ. Entomol.* 5: 364-366.

Glastad, K.M., B.G. Hunt, S.V. Yi, and M.A.D. Goodisman. 2011. DNA methylation in insects: on the brink of the epigenomic era. *Insect Mol. Biol.* 20(5): 553-565.

Glastad, K.M., S.V. Arsenault, K.L. Vertacnik, S.M. Geib, S. Kay, B.N. Danforth, S.M. Rehan, C.R. Linnen, S.D. Kocher, and B.G. Hunt. 2017. Variation in DNA methylation is not consistently reflected by sociality in Hymenoptera. *Genome Biol. Evol.* 9(6): 1687-1698.

Gould, G. E. 1931. *Pangaeus uhleri*, A Pest of Spinach. *J. Econ. Entomol*. 24: 484 – 486.

Highland, B.H. and P.F. Lummus. 1986. Use of light traps to monitor flight activity of the burrowing bug, *Pangaeus bilineatus* (Hemiptera: Cydnidae), and associated field infestations in peanuts. *J. Econ. Entomol.* 79(2): 523-526.

Honěk, A. 1993. Intraspecific variation in body size and fecundity in insects: a general relationship. *Oikos*. 483-492.

Hosokawa, T., Y. Kikuchi, X.Y. Meng, and T. Fukatsu. 2005. The making of symbiont capsule in the plataspid stinkbug *Megacopta punctatissima*. *FEMS Microbiol. Ecol.* 54(3): 471-477.

Inadomi, K., M. Wakiyama, M. Hironaka, H. Mukai, L. Filippi, and S. Nomakuchi. 2014. Postovipositional maternal care in the burrower bug, *Adomerus rotundus* (Hemiptera: Cydnidae). *Can. Entomol.* 146(2): 211-218.

Kakde, A.M., K.G. Patel, and S. Tayade. 2014. Role of life table in insect pest management – A review. *J. Agric. Vet. Sci*. 7(1): 40-43.

Kogan, M., 1998. Integrated pest management: historical perspectives and contemporary developments. *Annu. Rev. Entomol.* 43: 243.

Kudo, S.I., T. Nakahira, and Y. Saito. 2006. Morphology of trophic eggs and ovarian dynamics in the subsocial bug *Adomerus triguttulus* (Heteroptera: Cydnidae). *Can. J. Zool.* 84(5): 723-728.

Kudo, S.I., N. Baba, H. Mukai, M. Hironaka, and K.D. Tanaka. 2021. Interaction between prenatal and postnatal provisioning in a subsocial bug. *Biol. J. Linn. Soc*. 132(4): 925-930.

Leather, S.R. 2018. Factors affecting fecundity, fertility, oviposition, and larviposition in insects. In *Insect reproduction* . CRC Press. pp: 143-174.

Li-Byarlay, H., Y. Li, H. Stroud, S. Feng, T.C. Newman, M. Kaneda, K.K. Hou, K.C. Worley, C.G. Elsik, S.A. Wickline, and S.E. Jacobsen. 2013. RNA interference knockdown of DNA methyl-transferase 3 affects gene alternative splicing in the honey bee. *Proc. Natl. Acad. Sci. U.S.A.* 110(31): 12750-12755.

Luginbill, P. 1928. The fall army worm. *US Dep. Agric*. No. 34.

Mbata, G. N. and D. Shapiro-Ilan. 2013. The potential for controlling *Pangaeus bilineatus* (Heteroptera: Cydnidae) using a combination of entomopathogens and an insecticide. *J. Econ. Entomol.* 106(5): 2072-2076.

Mbata, G.N., Y. Li, and D. Shapiro-Ilan. 2022. Evaluation of chemical and microbial control options for *Pangaeus bilineatus* (Say) (Hemiptera: Cydnidae) infesting peanut crop. *Pest Manag. Sci.* 78(11): 4719-4727.

Michelotto, M.D., D. Bolonhezi, R.S.D. Freitas, J.F.D. Santos, I.J. Godoy, and C.F. Schwertner. 2022. Population dynamics, vertical distribution and damage characterization of burrower bug in peanut. *Sci. Agric.* 80.

Mukai, H., M. Hironaka, N. Baba, T. Yanagi, K. Inadomi, L. Filippi, and S. Nomakuchi. 2010. Maternal-care behaviour in *Adomerus variegatus* (Hemiptera: Cydnidae). *Can. Entomol.* 142(1): 52–56. doi: 10.4039/n09-045.

Nakahira, T. and S.I. Kudo. 2008. Maternal care in the burrower bug *Adomerus triguttulus*: defensive behavior. *J. Insect Behav.* 21(4): 306-316.

Nielsen, A.L., G.C. Hamilton, and D. Matadha. 2008. Developmental rate estimation and life table analysis for *Halyomorpha halys* (Hemiptera: Pentatomidae). *Environ. Entomol*. 37(2): 348-355.

Patterson, D.T., J.K. Westbrook, P.D. Lingren, and J. Rogasik. 1999. Weeds, insects, and diseases. *Clim. Change*. 43(4): 711-727.

Pedersen, K.E., N.N. Pedersen, N.V. Meyling, B.L. Fredensborg, and N. Cedergreen. 2020. Differences in life stage sensitivity of the beetle *Tenebrio molitor* towards a pyrethroid insecticide explained by stage-specific variations in uptake, elimination and activity of detoxifying enzymes. *Pestic. Biochem. Phys.* 162: 113-121.

Philips, C.R., D.A. Herbert, T.P. Kuhar, D.D. Reisig, W.E. Thomason, S. and Malone. 2011. Fifty years of cereal leaf beetle in the US: an update on its biology, management, and current research. *J. Integr. Pest Manag.* 2(2): C1-C5.

Porter, J.H., M.L. Parry, and T.R. Carter. 1991. The potential effects of climatic change on agricultural insect pests. *Agric. For. Meteorol.* 57(1-3): 221-240.

Prabhaker, N., S.J. Castle, and N.C. Toscano. 2006. Susceptibility of immature stages of *Homalodisca coagulata* (Hemiptera: Cicadellidae) to selected insecticides. *J. Econ. Entomol.* 99(5): 1805-1812.

Prado, S.S. and R.P. Almeida. 2009. Role of symbiotic gut bacteria in the development of *Acrosternum hilare* and *Murgantia histrionica*. *Entomol. Exp. Appl.* 132(1): 21-29.

Quaintance, A.L. and C.T. Brues. 1905. The cotton bollworm. *US Government Printing Office.* No. 50.

R Core Team. 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Ridley, M. 1988. Mating frequency and fecundity in insects. *Biol. Rev.* 63(4): 509-549.

Riis, L., A.C. Bellotti, and B. Arias. 2005a. Bionomics and population growth statistics of *Cyrtomenus bergi* (Hemiptera: Cydnidae) on different host plants. *Fla. Entomol.* 88(1): 1-10.

Riis, L., P. Esbjerg, and A.C. Bellotti. 2005b. Influence of temperature and soil moisture on some population growth parameters of *Cyrtomenus bergi* (Hemiptera: Cydnidae). *Fla. Entomol.* 88(1): 11-22.

Rolston, L.H. and R.L. Kendrick. 1961. Biology of the brown stink bug, *Euschistus servus* Say. *J. Kans. Entomol. Soc*. 34(3): 151-157.

Shaw, J.T., R.O. Ellis, and W.H. Luckmann. 1976. Apparatus and procedure for extracting corn rootworm eggs from soil. *Biol. Notes Ill. Nat. Hist. Surv.* No. 096.

Shukla, S., E. Kavak, M. Gregory, *et al.* 2011. CTCF-promoted RNA polymerase II pausing links DNA methylation to splicing. *Nature.* 479: 74–79. https://doi.org/10.1038/nature10442.

Sites, R.W. and J.E. McPherson. 1982. Life History and Laboratory Rearing of *Sehirus cinctus cinctus* (Hemiptera: Cydnidae), with Descriptions of Immature Stages. *Ann. Entomol. Soc. Am.* 75(2): 210–215.

Smith, J.W. and J.T. Pitts. 1974. Pest Status of *Pangaeus bilineatus* Attacking Peanuts in Texas. *J. Econ. Entomol*. 67(1): 111-113.

Srinivasan, R., M.R. Abney, P.C. Lai, A.K. Culbreath, S. Tallury, and S.C. Leal-Bertioli. 2018. Resistance to thrips in peanut and implications for management of thrips and thrips-transmitted orthotospoviruses in peanut. *Front. Plant Sci.* 9:1604.

Taylor C.M., P.L. Coffey, B.D. DeLay, and G.P. Dively. 2014. The Importance of Gut Symbionts in the Development of the Brown Marmorated Stink Bug, *Halyomorpha halys* (Stål). *PLoS ONE*. 9(3): e90312. https://doi.org/10.1371/journal.pone.0090312

US EPA. 2021. EPA takes action to address risk from chlorpyrifos and protect children. https://www.epa.gov/newsreleases/epa-takes-actionaddress-risk-chlorpyrifos-and-protect-childrens-health.

Vogt, G. 2022. Paradigm shifts in animal epigenetics: Research on non-model species leads to new insights into dependencies, functions and inheritance of DNA methylation. *BioEssays*. 44(8): 2200040.

Walsh, T.K., J.A. Brisson, H.M. Robertson, K. Gordon, S. Jaubert-Possamai, D. Tagu, and O.R. Edwards. 2010. A functional DNA methylation system in the pea aphid, *Acyrthosiphon pisum*. *Insect Mol. Biol.* 19: 215-228.

Wang, H., M.T. Maurano, H. Qu, K.E. Varley, J. Gertz, F. Pauli, K. Lee, T. Canfield, M. Weaver, R. Sandstrom, and R.E. Thurman. 2012. Widespread plasticity in CTCF occupancy linked to DNA methylation. *Genome Res.* 22(9): 1680-1688.

Weaver, I.C., N. Cervoni, F.A. Champagne, A.C. D'Alessio, S. Sharma, J.R. Seckl, S. Dymov, M. Szyf, and M.J. Meaney. 2004. Epigenetic programming by maternal behavior. *Nat. Neurosci.* 7(8): 847-854.

Wilen C.A., S.T. Koike, A. Ploeg, S.A. Tjosvold, J.A. Bethke, D.M. Mathews, J.J. Stapleton. *Revised continuously*. UC IPM Pest Management Guidelines: Floriculture and Ornamental Nurseries. *UC ANR Publication 3392*. Oakland, CA.

Yang, Y., T. Zhao, Z. Li, W. Qian, J. Peng, L. Wei, D. Yuan, Y. Li, Q. Xia, and D. Cheng. 2021. Histone H3K27 methylation–mediated repression of Hairy regulates insect developmental transition by modulating ecdysone biosynthesis. *Proc. Natl. Acad. Sci. U.S.A.* 118(35). p.e2101442118.

Zilberman, D., M. Gehring, R.K. Tran, T. Ballinger, and S. Henikoff. 2007. Genome-wide analysis of *Arabidopsis thaliana* DNA methylation uncovers an interdependence between methylation and transcription. *Nat. Genet.* 39(1): 61-69.

Table 2.1. Description of treatments for *P. bilineatus* behavioral ecology – male vs. female interaction with nymphs – experiment. Treatments contained 1, 2, or 4 eggs and either no adult, a virgin male, or a virgin female.

Eggs count	Paired adult
1	No
2	No
4	No
1	Virgin female
2	Virgin female
4	Virgin female
1	Virgin male
2	Virgin male
4	Virgin male

Table 2.2. Description of treatments for *P. bilineatus* behavioral ecology – nymph development – experiment. Arenas contained either no adult, a live female, or a dead female, and the nymphs hatched in each arena were either prevented from contacting the adult or allowed contact.

Adult Paired	Contact allowed
None	No adult
Live female	No
Dead female	No
Live female	Yes
Dead female	Yes

Table 2.3. Mean, standard deviation, and standard error of the mean of the number of days from *P. bilineatus* adult pairing to first appearance of eggs (post-eclosion + preoviposition period), total number of eggs/female, number of eggs/day (from first egg to last egg), oviposition period (days), longevity of females (days), and the number of days from the end of oviposition period until death.

	Mean	**SD**	**SEM**
No. days from pairing to first egg (post-eclosion + preoviposition period) *	7.81	0.91	0.23
Total no. eggs/female**	129.00^{+}	80.78^{+}	20.86^{+}
No. eggs/day (first to last egg) **	2.38^{+}	1.43^{+}	0.37^{+}
Oviposition period (days) *	62.25	30.72	7.68
Longevity of females (days)**	88.67	31.22	8.06
Days from end of oviposition period to death**	15.80	12.82	3.31

*n = 16, **n = 15

$^{+}$ Outliers removed

Table 2.4. Mean, standard deviation, standard error of the mean, and growth ratios of *P. bilineatus* head capsule width by life stage (*n* = 20).

Life Stage	Mean HC Width (μm)	SD	SEM	Ratio	Mean Ratio
1st Instar	459.327	16.574	3.71		**1.297**
2nd Instar	610.508	20.258	4.53	1.329	
3rd Instar	807.152	33.199	7.42	1.322	
4th Instar	1038.152	44.453	9.94	1.286	
5th Instar	1378.791	49.714	11.41	1.328	
Adult	1683.936	56.737	12.69	1.221	

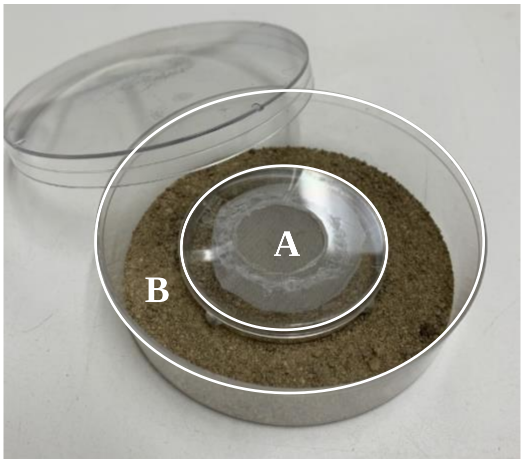


Fig. 2.1. Arena for *P. bilineatus* behavioral ecology – nymph development – experiment. A) inner arena; B) outer arena. Ten eggs were place in the outer arena, and a dead or live female was placed in either the inner arena or outer arena to prevent or allow nymphs to contact the female.

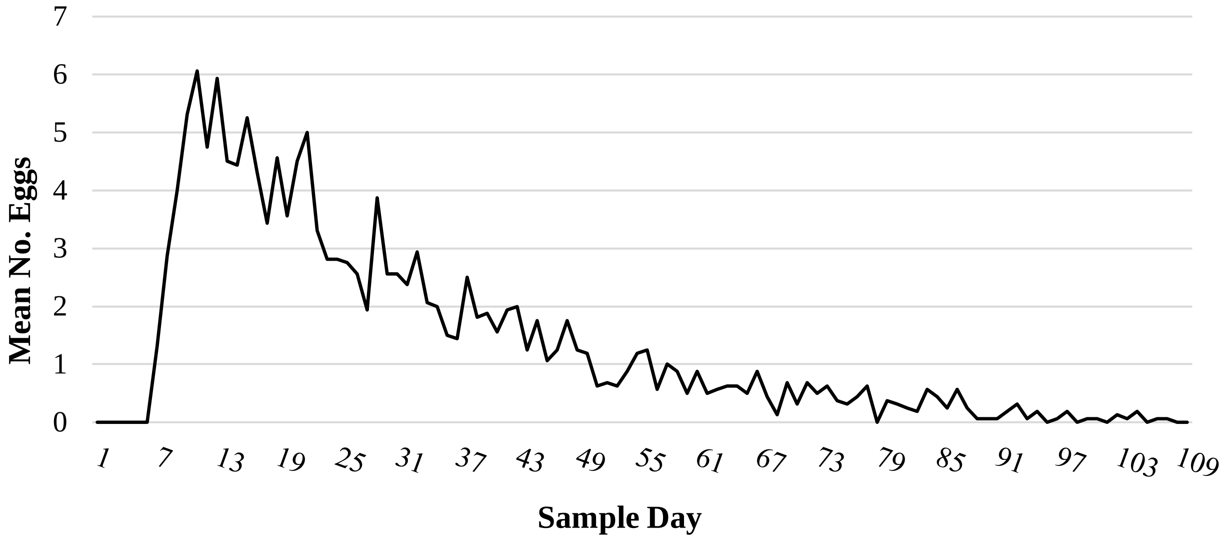


Fig. 2.2. Mean number of *P. bilineatus* eggs laid (*x*) per sample day (*y*) over the course of the 110-day fecundity/longevity experiment. Peak oviposition occurred in beginning of the oviposition period around day 8 and gradually decreased until death.

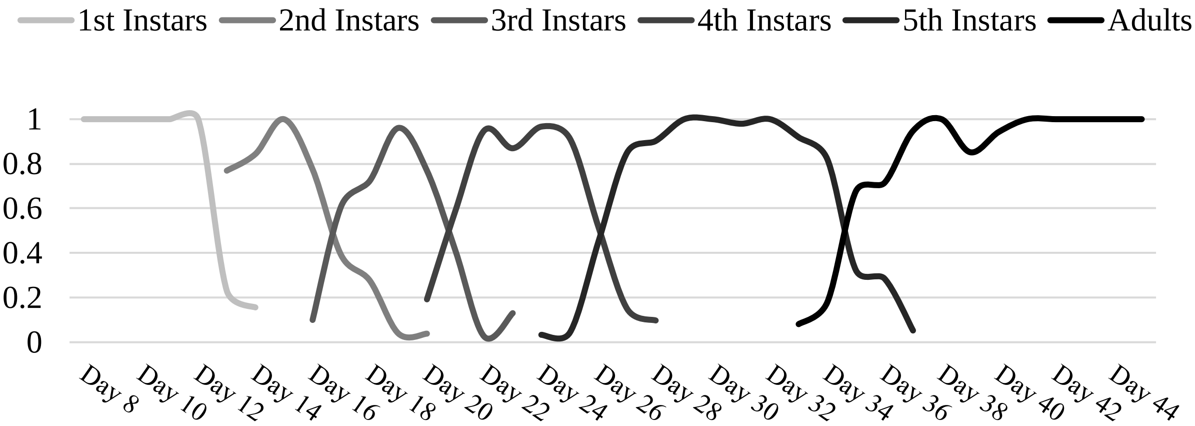


Fig. 2.3. Proportion of *P. bilineatus* (*y*) in each life stage by sample day (*x*) from study of development time.

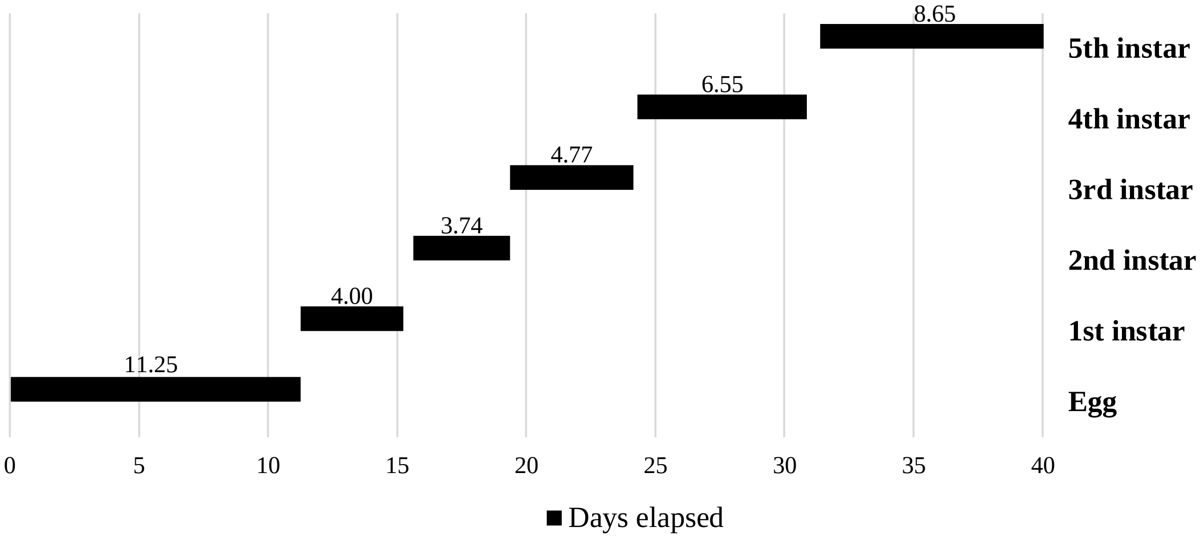


Fig. 2.4. Mean number of days elapsed (*x*) during each *P. bilineatus* life stage (*y*) from the study of development time.

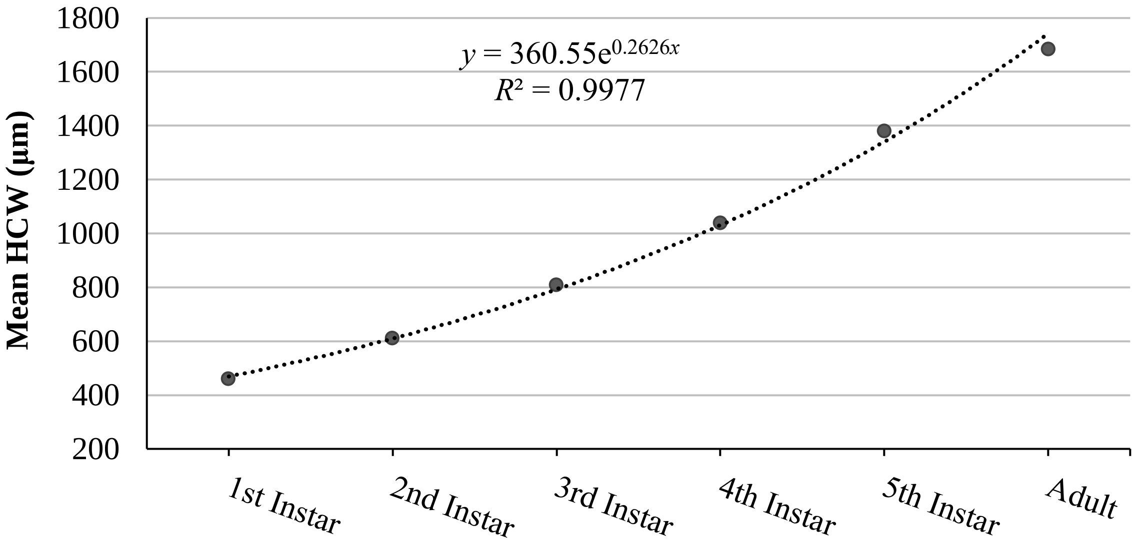


Fig. 2.5. Exponential regression of *P. bilineatus* life stage (*x*) plotted by mean head capsule width (*y*) (*n* = 20 bugs/life stage) from the study of *P. bilineatus* morphometrics.

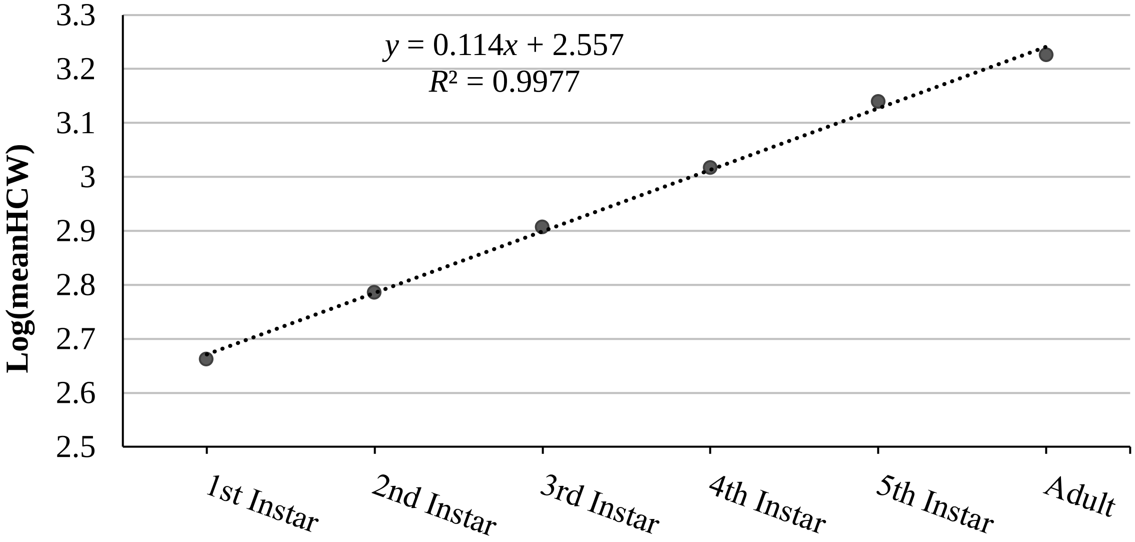


Fig. 2.6. Linear regression of *P. bilineatus* life stage (*x*) plotted by log transformed mean head capsule width (*y*) (*n* = 20 bugs/life stage) from the study of *P. bilineatus* morphometrics.

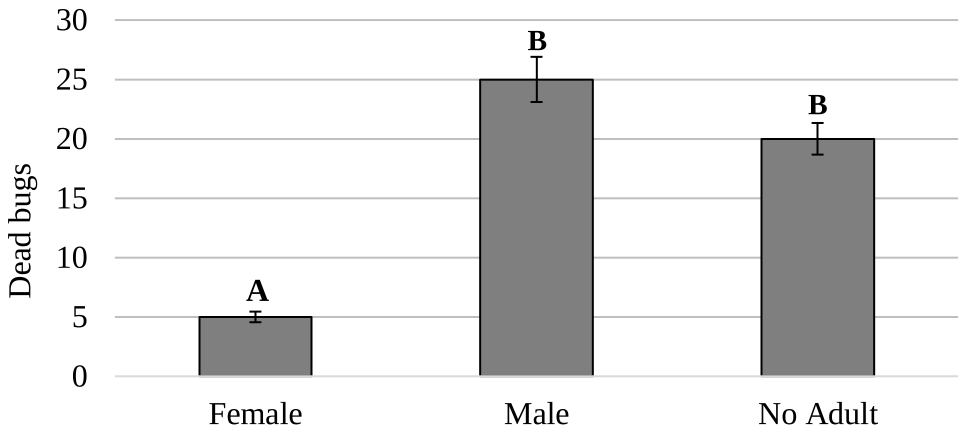


Fig. 2.7. Number of dead or unaccounted for *P. bilineatus* nymphs after 21 days (*y*) by group (*x*; *n* = 28 bugs/group) from the study of *P. bilineatus* behavioral ecology – male vs. female interaction with nymphs.

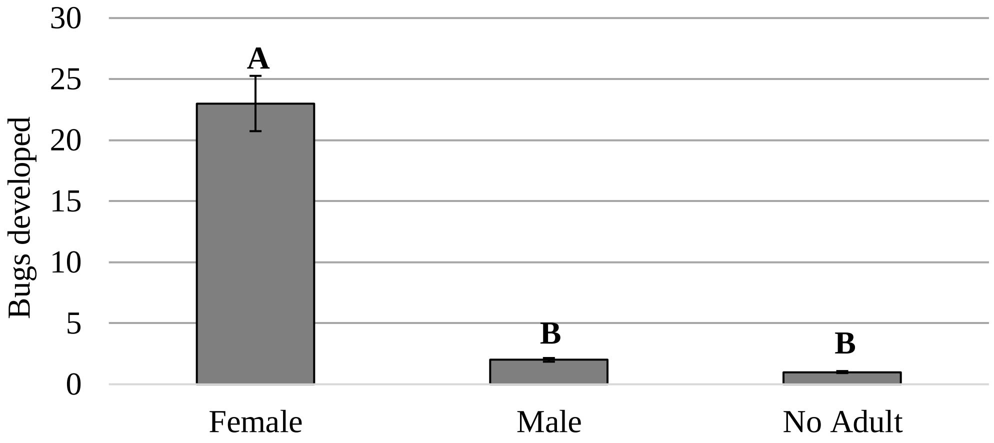


Fig. 2.8. Number of *P. bilineatus* nymphs that developed to 4th/5th instars (matured as expected) after 21 days (*y*) by group (*x*; *n* = 28 bugs/group) from study of *P. bilineatus* behavioral ecology – male vs. female interaction with nymphs experiment.

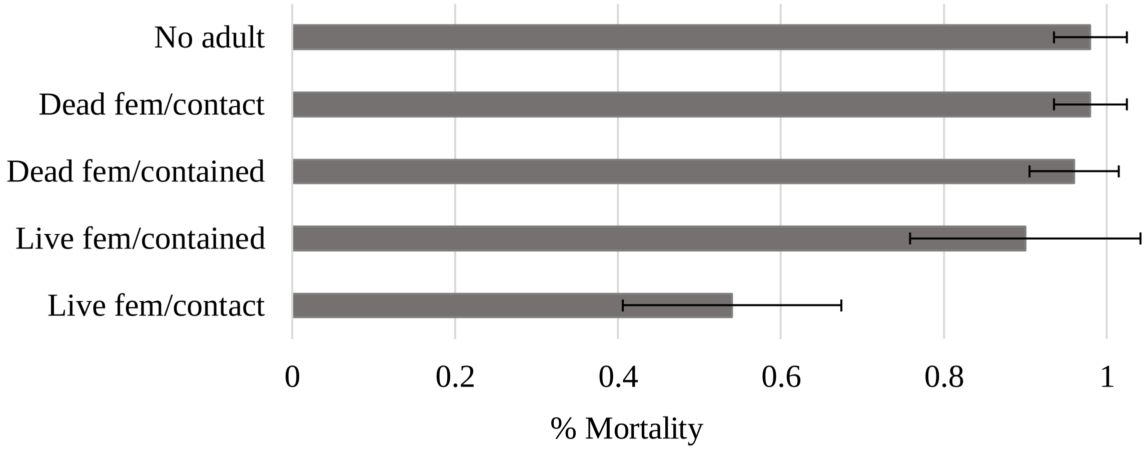


Fig. 2.9. ***Pangaeus bilineatus*** **nymph mortality rate (*x*) of behavioral ecology – male vs. female interaction with nymphs – study treatments (*y*). The live female/contact treatment resulted in significantly lower mortality than all other treatments (*p* = 1.02e-06).**

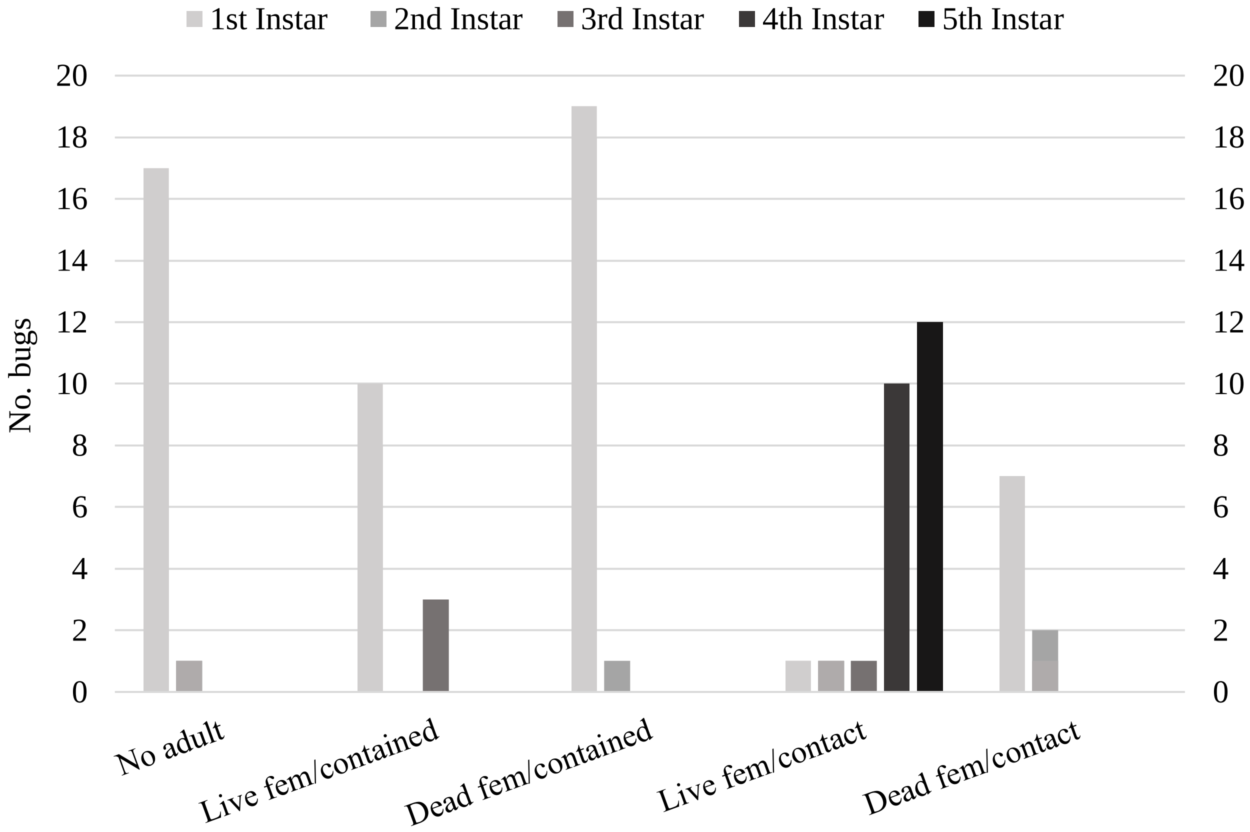


Fig. 2.10. Progression of *P. bilineatus* nymph development (*y*) by treatment (*x*). The live female/contact treatment had significantly more bugs develop through successive life stages than all other treatments (*p* = 8.32e-08).

CHAPTER 4

LANDSCAPE COMPLEXITY AND ABIOTIC ENVIRONMENTAL FACTORS INFLUENCE *PANGAEUS BILINEATUS* (HEMIPTERA: CYDNIDAE) INJURY TO PEANUT IN THE SOUTHEAST US

Aigner, B.L., A. Kheirodin, M.S. Crossley, J.M. Schmidt, and M.R. Abney. To be submitted to the *Journal of Applied Ecology*.

ABSTRACT

Pangaeus bilineatus Say is a severe economic pest of peanut in the Southeast US that feeds directly on seed and reduces its quality, and value by $209/MT. Therefore, research was conducted to identify significant contributing landscape and environmental factors to peanut injury caused by this US native hemipteran soil pest. Peanut injury data from Georgia, US was acquired from the Georgia Federal State Inspection Service for years 2016 – 2018 and curated. Sample sites for all years totaled 442. Areas of landcover types were extracted from the USDA Cropland Data Layer in ArcGIS 10.7 within 1 and 1.5 km buffers and compositional and configurational covariates were quantified in R. Rainfall and temperature data were acquired from the Parameter-elevation Regressions on Independent Slopes Model (PRISM) and curated in Excel. Generalized linear models were constructed separately among three categories (compositional, configurational, and environmental) to determine the best combination of covariates for predicting injury. The final model of significant compositional covariates included 'peanut', 'cotton', 'other agriculture', 'crop richness', 'non-crop richness', and 'forest'. Significant covariates from the final model of configurational covariates included 'field area', 'average roughness', 'edge density of contrasting crop families', and 'edge density of peanut to seminatural habitat'. Finally, the model of environmental covariates resulted in significant associations of peanut injury with winter and summer precipitation, soil organic matter, and irrigation. The model of configurational covariates at the 1.5 km scale resulted in the lowest AIC_c of all model results, and predicted the greatest variation in peanut injury.

Introduction

The development of satellite technologies and high-powered computing and mapping systems (e.g., GPS, satellite/aerial imagery, GIS, etc.) has enabled broad-scale analyses to unravel relationships of landscape complexity (i.e., landscape compositional and configurational heterogeneity; landscape diversity) with insect biodiversity, migratory behaviors of herbivorous insects, and consequent injury to cash crops (Chaplin-Kramer et al. 2011; Rusch et al. 2013; Haan et al. 2020). The availability of large-scale landscape and climate data from open access online sources – the United States Department of Agriculture (USDA), United States Geological Survey (USGS), etc. – enables scientists to conduct these studies by harvesting large amounts of data from a single or few sources. This capability for mining big data greatly reduces the cost to undertake large scale studies and has led to ground-breaking insight in the field of agroecology (Rosenheim and Gratton 2017).

The insect pest management approaches implemented by farmers are linked to the potential risk of insect injury to cash crops driven by the ecological mechanisms that underlie spatial heterogeneity of arthropod populations. These threats and their impact on crops (e.g., quality/yield reduction, introduction of pathogens, etc.) can sometimes be predicted or assessed in real-time during growing seasons by scouting or trapping to monitor pests and natural enemies (Hein and Tollefson 1985), post-harvest during a grading process (Adedeji et al. 2020), or in storage (Throne et al. 2003). The ability to predict pest outbreaks and subsequent crop injury relies heavily on predictive models that utilize ecoinformatics to quantify the effects of field practices, landscape complexity, and environmental factors (e.g., soil texture, precipitation, etc.)

on arthropod diversity and prevalence within agricultural contexts (Hermann et al. 2013; Rusch et al. 2013; Poggi et al. 2018).

Agricultural landscape complexity can influence species abundance and richness (Veres et al. 2013), dispersal tendencies (i.e., aggregation, in-field distribution, etc.)(Wan et al. 2018), top down effects of natural enemies (i.e., rate of parasitism/predation) (Karp et al. 2018), propensity to feed on crops (i.e., crop injury)(Hermann et al. 2013; Rusch et al. 2013; Poggi et al. 2018), and provision of ecosystem services (i.e., pollination/pollinator visitation rate)(Bommarco et al. 2012; Bukovinszky et al. 2017). Moreover, herbivorous pests vs. natural enemies, generalist vs. specialist arthropods, above vs. below-ground arthropods (i.e., mobile vs. *less* mobile arthropods, respectively), and different functional groups (e.g., detritivores, scavengers, etc.) may respond differently to landscape complexity gradients and other environmental factors (Scherber et al. 2010; Chaplin-Kramer et al. 2011; Tscharntke et al. 2012; Karp et al. 2018).

In a bottom-up model of plant species richness effects on arthropod and microbial biodiversity, Scherber et al. (2010) found dissimilar positive responses between functional groups and above vs. below-ground organisms to increasing plant species richness (i.e., increasing landscape complexity); responses of below-ground and lower trophic level organisms were weaker in comparison to above-ground and higher trophic level organisms. This trend is supported by a Chaplin-Kramer et al. (2011) meta-analysis of arthropod responses to landscape complexity which found generalist herbivores and generalist natural enemies exhibited consistent positive responses to landscape complexity across all scales observed, while specialist natural enemies exhibited the strongest positive responses to landscape complexity at lesser scales.

Several studies also explain insect herbivores' relationships with abiotic environmental factors (e.g. precipitation, temperature, soil texture, etc.) (Milosavljević et al. 2016; Riis et al. 1998); however, the concurrent effects of landscape complexity and abiotic environmental factors are rarely assessed to explain crop injury levels. Wireworm research is an exception, and species' abundance and ensuing crop injury is relatively well studied in response to factors of landscape complexity, field practices, field history, and other environmental factors (Campbell 1937; Lefko 1998; Willis et al. 2010; Jung et al. 2014; Saussure et al. 2015; Milosavljević et al. 2016; Poggi et al. 2018; Poggi et al. 2021). Soil characteristics are especially vital to developmental, migratory, and feeding patterns of ground dwelling insects (Campbell 1937; Poggi et al. 2018 Lefko et al. 1998; Jung et al. 2014; Milosavljević et al. 2016). Soil temperature, pH, organic matter, soil texture, and field history of and proximity to meadows were found to influence wireworm injury to corn (Poggi et al. 2018).

A number of hypotheses have been proposed to explain how landscape complexity affects insect pests and crop injury. The landscape-moderated concentration and dilution hypothesis states that agricultural landscapes subject to frequent spatial and temporal disturbances (e.g., crop rotations, annual harvest, etc.) affect transient concentration and/or dilution of insect populations (Root 1973; Holt and Hochberg 2001; Tscharntke et al. 2012). This effect is commonly associated with specialist arthropods (all trophic levels) that reside in agroecosystems characterized by annually harvested monoculture(s) (Tscharntke et al. 2012). Whether this hypothesis is true of generalists that rely on a specific crop during specific growth stages is unknown. Yachi and Loreau (1999) argue that habitat fragmentation directly reduces specialist herbivore abundance and, sequentially, reduces the ecosystem function of herbivory by specialists. As a result, remaining generalist herbivores less affected by fragmentation of host resources substitute for the lost ecosystem functioning of specialists. This is known as the insurance hypothesis and is defined as long-term effects of biodiversity that insure maintenance or enhancement of ecosystem functioning in response to environmental fluxes (Naeem and Li 1997; Yachi and Loreau 1999).

In some systems, landscape complexity can cause variation in crop injury as an indirect result of natural enemy success (i.e., the enemies hypothesis; Letourneau 1987; Veres et al. 2013; Liere et al. 2015; Tschumi et al. 2015; Balzan et al. 2016; Haan et al. 2020). In Germany, Thies and Tscharntke (1999) showed a positive relationship between non-crop area and parasitism rate of rape pollen beetles, and inversely, a negative relationship between rape bud damage and non-crop area. The strength of these relationships was affected by but remained significant regardless of scale.

Tscharntke et al. (2012) predict that landscape changes that reduce a high-volume resource to smaller fragments should result in concentration of organisms that rely heavily on that resource (especially specialists) for survival and reproduction. Conversely, expanding a concentrated resource should cause dilution of organisms that exploit that resource. This is relevant to high intensity agricultural production areas where crop rotation and disturbance are prevalent. Commercial peanut production in the southeast US generally occurs in rotation with cotton and/or corn, though length of rotation and diversity of crops can vary significantly. *Pangaeus bilineatus* Say is a generalist herbivore commonly found in agricultural landscapes within its geographic range in the USA, but economically significant crop injury occurs only sporadically (Chapin and Thomas 2003; Aigner et al. 2021). Annual habitat disturbance and fragmentation that cause fluctuations between concentration/dilution of resources and insect populations could offer at least partial explanation for the sporadicity of *P. bilineatus* in the routinely disturbed peanut agroecosystems of the Southeast US. Here we focus on the associations between landscape complexity and environmental factors, and *P. bilineatus* injury to peanut. Peanut injury is used as a proxy for *P. bilineatus* abundance, although the relationship of injury to abundance is likely not a perfect correlation.

Economic impact and importance of Pangaeus bilineatus management in peanut

Pangaeus bilineatus is a polyphagous hemipteran soil pest that is native to North America with a range spanning most southern and eastern US states (Froeschner 1960). *Pangaeus bilineatus* was initially reported as a potential agricultural pest by Gould (1931) after it was observed feeding on spinach seedlings in Virginia; it was later reported to cause significant peanut injury in Texas and Alabama in the late 1960's (Smith and Pitts 1974). Nevertheless, its subterranean nature and the sporadic occurrence of economic injury left it largely overlooked until the early 2000's (Chapin and Thomas 2003; Chapin et al. 2006). There are substantial gaps in current knowledge regarding *P. bilineatus* biology and ecology. Since 2010, the pest has become a significant annual economic threat to peanut in Georgia, USA (Aigner et al. 2021). The United States is one of the leading producers and exporters of peanut worldwide with the U.S. state of Georgia accounting for about half of all U.S. peanut production annually (average value of $1.25 bn./yr. since 2015) (USDA – NASS 2021). Peanut farmers in the Southeast USA, especially in Georgia, have been economically impacted by *P. bilineatus*, a subterranean seed feeder that reduces the value of peanuts by approximately $209/MT when the incidence of feeding injury exceeds 3.49% (Aigner et al. 2021).

Given the rise in economic importance and lack of management tools available (Aigner et al. 2021), improving knowledge of the biology and ecology of *P. bilineatus* is imperative for the development of sustainable integrated pest management tactics and strategy. The cryptic and sporadic nature of *P. bilineatus* makes sampling for the pest difficult. But data records of all *P. bilineatus* injured peanuts (for years 2016, 2017, and 2018) in Georgia were made available for this project by the Georgia Federal State Inspection Service (GA – FSIS). These data coupled with ecoinformatics provided a novel opportunity to conduct a landscape-scale study with the objective to identify significant compositional, configurational, and abiotic environmental covariates that contribute to variation in *P. bilineatus* injury to peanut in Georgia.

Methods

Study Sites and Site Selection Criteria

Locations of peanut fields with documented peanut burrower bug injury were georeferenced in ArcMap 10.7.1 (Release 10.7.1, Environmental Systems Research Institute, Inc., Redlands, CA, USA) using geographic information (e.g., county, street names, etc.) from farm maps (USDA) which associate to farm numbers on peanut grade sheets (FV-95 form). Presence of peanut in each georeferenced field was verified using the USDA cropland data layer (CDL) for each year (USDA – NASS 2016). Data acquired for the 3 study years totaled 4,796 FV-95 forms – 2,070 in 2016, 963 in 2017, and 1,763 in 2018, respectively. In total, 2,838 records were georeferenced, including 336 unique peanut field locations for 2016, 301 for 2017, and 456 for 2018. A point was placed at the geometric centroid of the farm parcel which contained peanuts. Farms that contained more than one parcel were excluded unless multiple parcels were composed of continuous landcover of peanut. One and 1.5 km buffers were generated around all georeferenced field sites, and sites with overlapping buffers were randomly eliminated by coin flip. Of the remaining sites, those expressing more than 500 kg/ha (440.1 lbs/ac) of injury were considered outliers and excluded from the final analysis. The data sets from 2016, 2017, and 2018 contained a sample size of 155, 100, and 187 field sites, respectively (Fig. 3.1). All sites were at least 3 km apart to ensure no overlap of 1.5 km buffer zones between any two sites. The minimum distance between peanut field sites was 3.01 km and the farthest distance between sites was 380.40 km. Percentage non-crop (seminatural) habitat within buffers ranged from 7.98% to 95.13%, the majority of which was classified as forest or wetlands (Appendix A).

Pangaeus bilineatus injury data

Peanut burrower bug injury data for the state of Georgia, USA were obtained from the GA – FSIS each year from 2016 – 2018. After harvest, all peanuts are subjected to a standardized grading system which rates them based on specific quality parameters (American Peanut Shellers Assn. 2020). Peanut kernels with any insect injury are considered to be "damaged kernels". Peanuts with <3.5 % damaged kernels from a 500 g sample are graded as "Segregation 1" (Seg. 1), peanuts with ≥3.5 % damaged kernels are graded as "Segregation 2" (Seg. 2), and peanuts contaminated with *Aspergillus flavus* – a fungal pathogen that produces aflatoxin – are graded as "Segregation 3" (Seg. 3). For every peanut load that is graded, an FV-95 grade report form containing a variety of data is generated (e.g., inspection date, USDA farm number, load wt., sample wt., injured peanuts wt., etc.). Because many FV-95 forms will correspond to a single peanut field, injury data were pooled by farm number to calculate the amount of injury from each location. For each georeferenced location, the dependent variable used for generalized linear modeling (kg injured peanuts/ha) was calculated using the formula below:

$$\frac{(kg\ injured\ peanuts/kg\ of\ sample) \times total\ kg\ peanut\ from\ site}{Area\ of\ site\ (ha)} = kg\ injured\ peanuts/ha$$

Landscape Quantification

Compositional

Cropland data layers for years 2016 – 2018 were acquired from the USDA CropScape website (USDA – NASS 2016), and crop and non-crop habitats within 1 – 1.5 km buffers were identified and quantified in ArcGIS. A maximum scale of 1.5 km was used in this study. One kilometer was chosen as the minimum scale for this study due to the large size of peanut fields in Georgia which, in some cases, have radii greater than .5 km from field center to field edge. Pearson's correlation test (Pearson 1931) was used to evaluate multicollinearity among independent variables (habitats) before merging them in to broader categories for statistical analysis (Appendix A).

Peanut is a primary crop host of *P. bilineatus* and experiences economic injury in Georgia (Aigner et al. 2021), therefore was kept as a separate variable for all models. *Pangaeus bilineatus* has anecdotally been observed feeding on cotton, *Gossypium hirsutum*, seed (M.R. Abney, personal communication, 2018). Because cotton is a primary rotational crop with peanut, it was also kept as a separate variable for all models. Some woody crops have been identified as probable alternative hosts for *P. bilineatus* (M. Crossley, unpublished data) and were therefore grouped together and analyzed as a separate variable called 'woody crops' (Appendix A) for all models. Exploratory alternative models were conducted initially with 'corn', 'poaceous crops', and 'leguminous crops' as separate variables (Appendix A). These cover types are relevant as potential habitat for *P. bilineatus* during or outside of the peanut growing season (Smith and Pitts 1974; Chapin and Thomas 2003). Due to low mean percent area of ‘corn’, ‘poaceous’, or ‘leguminous crop’ types (<3%) and no significance in exploratory model outcomes, these were combined with all other crop cover types – except ‘cotton’, ‘peanut’, and ‘woody crops’ – in a variable called ‘other agriculture’ for subsequent models. Crop habitat cover type variables used in the final model selection consisted of ‘cotton’, ‘peanut’, ‘other agriculture’, and ‘woody crops’ (Appendix A). Cotton and peanut had the greatest average percentage cover of all crop habitat cover types averaging 18.27% and 7.92%, respectively.

The major non-crop habitat cover types were ‘forest’, ‘wetlands’, and ‘seminatural’ habitats. Forest habitat had the highest percentage cover of all variables averaging 24.87%, the majority of which was evergreen forest (avg. cover = 20.0%). Pine was of special interest due to high coverage across Georgia’s landscape and preliminary evidence suggesting that it may serve as a host of *P. bilineatus* (M. Crossley, unpublished data). Wetland and other seminatural habitat represented the highest percentage covers of all remaining variables averaging 19.50% and 11.73%, respectively. Developed land cover types (avg. cover = 5.21%) were combined and considered in the models due to the occurrence of unmanaged or minimally managed grasses, forbs, and other weeds along road and railroad edges, ditches, and commercial and residential properties which are known as alternate habitat for *P. bilineatus* (Aigner et al. 2021; Appendix A).

Landscape diversity variables were calculated with data from the USDA Cropland Data Layer from years 2016 – 2018. Crop and non-crop richness and evenness were considered separately, and habitat (crop, non-crop, and other) richness and evenness were estimated using

Simpson's diversity index (Simpson 1949). Only *crop* cover types were used for calculation of crop diversity variables and only *non-crop* cover types were used for calculation of non-crop diversity variables. All habitat types were used for calculation of *landscape* diversity variables. The Vegan package in R 4.0.2 (R Core Team 2020) was used to estimate these diversity indices (Oksanen et al. 2015).

Configurational

Field area was measured for each site using the area measurement tool in ArcGIS. Average patch size was calculated using area cover (m^2) of all landcover types at each scale for all sites and years. Edge density (m/ha) variables were calculated in R at each scale (Hesselbarth et al. 2019) with the USDA Cropland Data Layer. Edge density of all edges (interface of contrasting land cover types) was used as a proxy for patch shape complexity in exploratory models. Interfaces of peanut to non-peanut, seminatural habitat, developed, cotton, evergreen forest (pine), and woody crops habitat were of interest due to the polyphagous nature of *P. bilineatus*. Although its full host range is unknown, there is at least anecdotal evidence to suggest potential significance of each of these variables. Edge density of crops to non-crops and interface of contrasting crop families were also assessed in the models.

Average surface roughness – mean of the absolute deviation of each field center height from the mean height – and root mean square of the slope at each scale and site were calculated using the digital elevation model (DEM) of Georgia, US, (US Geological Survey 1996) and were included in the model selection as additional alternatives to patch metrics (McGarigal 2009). These measures of three-dimensional spatial configuration were of interest due to the subterranean nature of *P. bilineatus* and the potential for interference that elevational variation may cause during dispersal and the inherent heterogeneity that elevation change produces in the landscape (e.g., soil moisture, texture, above ground resources, etc.).

Environmental

Soil composition parameters were extracted from the USDA Gridded Soil Survey Geodatabase (gSSURGO) in ArcGIS 10.7.1 (Soil Survey Staff 2019a, b). Average percentage soil composition of sand, clay, and soil organic material (SOM) at each site and scale were calculated in R and included in the final model selection. Precipitation data were acquired from the Parameter-elevation Regressions on Independent Slopes Model (PRISM Climate Group) for each site and year and were unchanged by scale. The summer variables refer to data of the timeframe April 1 – October 31 and winter variables refer to data of the timeframe of November 1 of previous year – March 31. In preliminary models, cumulative winter and summer precipitation (cm) were analyzed. Cumulative days where any amount of precipitation fell during summer and winter were also analyzed and performed better in the model; therefore, cumulative days of precipitation was used in the final model selection. Average daily temperatures during summer and winter at each site were also included as independent variables. In preliminary models, the summer precipitation variables were partitioned into early and late season precipitation to determine whether any differences could be detected in the relationships of early and late season precipitation with *P. bilineatus* injury. Aerial photographs were examined (Google Maps) to determine the presence of center pivot irrigation at each site, and irrigation was treated as a categorical variable (yes/no) in the model selection of environmental variables.

Statistical Analysis

All statistical analyses were conducted in R 4.0.2 (R Core Team 2020). Generalized linear models (GLMs) were used to analyze the effect of landscape complexity and environmental variation on injury to peanuts caused by *P. bilineatus*. Due to the high number of covariates in this study, each covariate was categorized as either compositional, configurational, or environmental (described above), and models were constructed separately for each category to ensure model convergence. The Poisson distribution was tested initially, but these models resulted in overdispersion of the data; therefore, a negative binomial distribution was used for subsequent models. The dependent variable for all models was the weight per unit area of injured peanuts from each site (kg injured peanuts/ha). Independent compositional variables were crop and non-crop diversity indices (i.e., richness, evenness, and diversity), landscape diversity indices, peanut, cotton, woody crops, other agriculture, forest, wetlands, seminatural, and developed land cover types. Forest and wetland cover types were kept separate from other seminatural habitats due to the high percent cover of those classes. Woody crops, cotton, and peanut were kept separate from other agriculture due to the high percent cover of each and potential for standalone significance as alternative habitat for *P. bilineatus*.

Independent configurational variables included average roughness, root mean square (rms) of the slope, edge density of peanut to non-peanut, edge density of peanut to seminatural, edge density of peanut to pine forest, edge density of peanut to woody crops, edge density of peanut to cotton, edge density of peanut to developed land cover (roads), edge density of crop to non-crop, edge density of all edges (patch shape complexity), and edge density of contrasting crop families.

Environmental variables used in the final model selection were irrigation (yes or no), temperature (average daily) and precipitation (cumulative days) during respective winters prior (Nov 1 – Mar 31) and summers (Apr 1 – Oct 31), percent clay, percent sand, and percent soil organic matter (SOM). Prior to fitting models, all variables were analyzed with Pearson's correlation tests to ensure the exclusion of correlated independent variables in the same models. All models were constructed to avoid multicollinearity, and this was further tested after model convergence using the car package in R to compare variance inflation factors (VIF). Any variable combinations that caused VIF to exceed 3 were reconstructed without the correlated variable(s) (Fox and Weisberg 2018).

The model selection was completed with the multi-model inference approach in R using the MuMIn package (Anderson and Burnham 2004; Barton 2009). Models were averaged based on Akaike's information criterion (AIC), then the best fitted model among each set of competing models with the lowest $\mathrm{AIC_c}$ at each scale was selected. All models within <2 $\Delta\mathrm{AIC_c}$ were reported as these had similar goodness of fit. This allowed comparison of models with alternate variables that similarly explained variation of peanut injury but could not be included in the same model because of multicollinearity (Harrison et al. 2018). The overall variation in peanut injury explained by models was estimated by comparing the full model log-likelihood with the baseline model containing only intercepts with the rcompanion package, which output the Cox and Snell pseudo R^2 (Mangiafico 2017). The model X^2 was estimated with the nagelkerke command of the rcompanion package (Mangiafico 2017). Partial correlation of each variable was calculated with the rsq package (Crawley 2013). This shows the partial effect of each independent variable on peanut injury within the model. Partial residuals of independent variables within the best fitted models were visualized using the visreg package (Breheny and Burchett 2017). Partial residual plots allow display of the independent effect of an explanatory variable on the outcome while parsing out the effect of other independent variables in the model (Meehan et al. 2011; Moya-Laraño and Corcobado, 2008).

Extrapolating risk of P. bilineatus peanut injury

All injury risk prediction maps were produced in R 4.0 using the tmap (Tennekes 2018) and stars (Pebesma and Bivand 2023) packages with covariate effects of the best performing models at the 1 km scale. Cropland data layers for years 2008 – 2018 (USDA 2008 – 2018) were imported into R, clipped to the study extent, and reclassified to represent the same peanut focused reclassification scheme that was used for the analysis. Raster data sets for covariates that were used to fit the models were curated to a 1x1 km grid to match the spatial scale of the analysis and overlaid to the study extent. Rasters for risk maps of covariates derived from the CDLs (compositional, diversity and edge density variables) were extracted from CDLs that corresponded with those data. The irrigation raster was acquired from Brown et. al (2019). The field size raster was taken from Yan and Roy (2016), and the number of pixels within each field were counted then converted to hectares, and average field size per 1x1 km cell was summarized. Elevation data were available in the DEM in raster format, soil composition rasters were available through the gSSURGO database (Soil Survey Staffa, b), and winter and summer precipitation rasters were generated with the raster package (Hijmans and van Etten 2012) in R using data collected from the PRISM Climate Group.

Raster algebra was used to calculate the level of risk in each pixel within the study extent by multiplying each pixel by the slope (beta) coefficients from the model for each covariate

(Crossley et al. 2021, Chuang et al. 2012). Once risk was calculated, maps were generated for each covariate. Then, predictions from each of the 3 covariate sets (compositional, configurational, and environmental) were averaged and a map was generated that depicts the combined risk accounting for all covariates found to significantly influence *P. bilineatus* injury.

Results

Pangaeus bilineatus injury response to landscape composition

The best fitting model comprised of compositional variables included: year, peanut, cotton, other agriculture, crop richness, non-crop richness, and forest at the 1 km scale (Table 3.1). Injury was significantly greater in 2018 than in 2016 and 2017 (Fig. 3.6). Crop cover of peanut (Fig. 3.2e), cotton (Fig. 3.2f), and other agriculture (Fig. 3.2d) had significant negative relationships with injury at the 1 km scale, and while peanut remained significant at both scales, cotton became nonsignificant at the 1.5 km scale (Table 3.1). Other agriculture was significant at varying levels at both scales, but the trend was consistent. Crop richness (Fig 3.2a) had a significant positive relationship with injury, and non-crop richness (Fig. 3.2b) had a significant negative relationship with injury at the 1 km scale (Table 3.1). Neither had significant relationships with injury at the 1.5 km scale. Forest (Fig. 3.2c) had a significant negative relationship with injury at 1 km but was not significant at 1.5 km. Wetlands had a consistent positive relationship with injury in competing models at both scales (Table 3.1).

Peanut burrower bug injury response to landscape configuration

The best fitting model comprised of configurational variables included field area, average roughness, edge density of contrasting crop families, and edge density of peanut to seminatural habitat at the 1.5 km scale (Table 3.1). Field area (Fig. 3.3a), average roughness (Fig. 3.3b), and edge density of contrasting crop families (Fig. 3.3c) had significant negative associations with *P. bilineatus* injury and these trends were consistent at both scales, and in competing models (Table 3.1). Peanut to pine forest edge density (Fig. 3.3d) had a significant negative relationship with injury at both scales in competing alternative models (Table 3.1).

Peanut burrower bug injury response to abiotic environmental covariates

Percent composition of SOM, cumulative days of summer and winter precipitation, and irrigation consistently affected *P. bilineatus* peanut injury at both scales and in all competing models (Table 3.1). In the best fitting model of environmental variables, SOM had a significant positive relationship with injury (Fig. 3.4a). Cumulative days of winter precipitation also had a significant positive effect on injury (Fig. 3.4b), while cumulative days of summer precipitation (Fig. 3.4c) and irrigation (Fig. 3.5) both had significant negative effects on injury. Percentage sand soil composition had a significant positive relationship at the 1.5 km scale in a competing model.

Extrapolating risk of P. bilineatus *peanut injury*

Predictive risk maps of *P. bilineatus* peanut injury influenced by compositional, configurational, and environmental covariates are shown in figs. 3.7a – f, 3.8a – d, and 3.9a – d, respectively. Maps of combined *P. bilineatus* peanut injury risk influenced by compositional, configurational, and environmental covariates are shown in figs. 3.10a, b, and c, respectively. And, a risk map of *P. bilineatus* peanut injury influenced by all covariates combined is shown in fig. 3.10d. Risk of *P. bilineatus* peanut injury was heterogeneous, but was generally highest in two regions of southwestern GA. (Fig. 3.10d).

Discussion

Pangaeus bilineatus injury response to landscape composition

Model results including landscape composition variables support the characterization of *P. bilineatus* as a polyphagous generalist herbivore that can survive and thrive on both crop and non-crop resources. Identification of *P. bilineatus'* host range outside of the peanut growing season is unresolved, but Aigner et al. (2021) reported readiness to feed on seeds, stems, and roots of a variety of crop and non-crop hosts. Model significance of crop richness, other agriculture, cotton, and peanut in the surrounding areas where injury to peanut was reported suggests an abundance of available crop hosts for *P. bilineatus*.

Other agriculture (excludes peanut, cotton, and woody crops) in the landscape was associated with lower injury. Other crops may provide *P. bilineatus* with temporally diverse alternate, and perhaps preferred, hosts during and outside of the peanut production season. On the other hand, the association of more cotton and other agriculture in the landscape with reduced injury to peanut could be the result of negative impacts on *P. bilineatus* populations; for example, farming practices (e.g., insecticide use and/or tillage) or the unsuitability of some crops as hosts. The association of more peanut in the landscape with less injury could be the result of a resource dilution effect where increasing concentration of host plants results in a negative relationship with herbivore abundance (Root 1973; Tscharntke 2012), and in this case, with peanut injury. In contrast to other agriculture, greater injury to peanut was observed with increasing crop cover richness. It is possible that the *P. bilineatus* population is concentrated in peanut in fragmented landscapes when preferred/alternate hosts are rare. Alternatively, the presence of many suitable hosts in a fragmented landscape could result in an overall increase in pest abundance and subsequently higher injury to peanut. Determining if one or both of these mechanisms is responsible for the observed relationship between crop richness and injury would require a more detailed knowledge of the insect's host range.

Results showed that non-crop land cover also affected the incidence of injury to peanut. Though related, non-crop richness is not the direct inverse of crop richness. Nevertheless, the negative relationship of injury with non-crop richness could be explained by mechanisms similar to those described for crop richness. In both cases, elucidating the cause(s) of the observed effects would require additional knowledge regarding host range and utilization. The negative association of *P. bilineatus* injury with percentage forest cover may reflect the suitability of forest as habitat for *P. bilineatus* and/or its natural enemies. Forest fragments are common in the landscape of Georgia's coastal plain and could serve as a largely undisturbed habitat with potential hosts present all year.

Rossetti et al.'s (2017) meta-analysis of herbivory response to landscape fragmentation found a reduction in specialist herbivore abundance in more fragmented landscapes; however, the ecosystem function of herbivory was maintained by mostly generalist species. Two hypotheses are offered as potential explanations for this effect: (1) previously less abundant species substitute the functional role of abundant species reduced by fragmentation (i.e., the insurance hypothesis) (Yachi and Loreau 1999); or (2) herbivory of the cropping landscape is driven by a few common and dominant species not affected by fragmentation (Rossetti et al. 2017).

The associations of high crop richness with greater peanut injury and high non-crop richness with less peanut injury are difficult to unravel without a closer look into local field conditions and management practices in surrounding crop and non-crop habitats. Further knowledge of the natural enemy pool and the effects of certain crop and non-crop cover types, and management practices on *P. bilineatus* and its predators could elucidate the mechanisms at play. Booij and Noorlander (1992) found that some ground-dwelling insect predators (e.g., carabids, staphylinids, and spiders) responded differently to conventional, organic, and integrated management practices in various crops.

Pangaeus bilineatus injury response to landscape configuration

Fields in South Georgia tend to lack uniformity in soil texture, elevation, and moisture; this is especially true of fields >25 ha (Soil Survey Staff a). The negative association of *P. bilineatus* injury with field size could be a result of a dilution effect. Greater variation in soil texture, elevation, and/or soil moisture in large fields would result in areas that are more or less favorable for population growth. Soil properties affect water holding capacity (Easton 2021), which may drive distribution of *P. bilineatus* by altering the favorability of some hosts. The sporadic nature of *P. bilineatus* injury suggests that specific environmental conditions are required for populations to reach economically damaging levels, and it is likely that only a narrow range of physical and chemical soil conditions is optimal for the insect’s development. The habitat specificity of *P. bilineatus* may result in pockets of a field being heavily infested with bugs while larger portions of the field remain free from injury. The intrinsic rate of increase of *P. bilineatus* may not be high enough to result in infestation across large fields, especially if populations are established as the result of mid to late season immigration. The negative effect of edge density of contrasting crop families with injury supports the general theory that greater crop biodiversity can be beneficial for pest management (i.e., enemies hypothesis; Letourneau 1987; Gurr et al.

2003; Redlich et al. 2018). These pest management benefits are usually attributed to the positive relationships that natural enemy populations have with more diverse cropping arrangements within a fragmented landscape of seminatural habitats (Bianchi et al. 2006; Rusch et al. 2010; Tscharntke et al. 2016). Based on the negative association between *P. bilineatus* peanut injury and the edge density of peanut with seminatural habitat one might hypothesize that increased seminatural edge and fragmentation could: (1) cause spillover of natural enemies into peanut, or (2) reduce spillover of *P. bilineatus* into peanut from seminatural habitat (Rand et al. 2006; Blitzer et al. 2012; Tscharntke et al. 2005).

Pangaeus bilineatus injury response abiotic environmental covariates

Precipitation and SOM are directly related to soil moisture, soil water retention, soil structure, and soil health overall (Karlen 1990; Hudson 1994). Soil moisture has been identified as a critical environmental characteristic in the mating and developmental success of other burrower bug species (e.g., *Cyrtomenus bergi*; Riis and Esbjerg 1998; Riis et al. 2005). The positive relationship of *P. bilineatus* peanut injury with winter precipitation highlights the importance of rainfall during winter months, which was highly variable during the years 2016 – 2018. Though the mechanism(s) by which winter moisture affects *P. bilineatus* is unknown, the observed relationship could be explained by the growth and survival of winter annual host plants or a direct effect of soil moisture requirements for survival of the insect. In contrast to winter precipitation, a negative association of summer precipitation with *P. bilineatus* injury was observed. Similarly, there was a negative relationship of injury with irrigation. This may be due to the relative suitability of peanut compared to alternative host plants under droughty conditions. Alternatively, similar population densities may occur under normal and droughty conditions, but insects may feed more on developing peanut seeds when soil moisture is low. Soil organic matter content at sites was relatively low (0.62 – 3.94%). Nonetheless, the strong positive relationship with injury could be related to soil structure. Soil organic matter of Georgia fields is likely from cover crops or planting directly into residue from previous annual crops (e.g., corn, cotton, etc.). Chapin et al. (2001) found higher *P. bilineatus* injury in peanut planted into wheat and corn stubble compared to conventionally tilled peanut.

Percent soil composition of clay and sand are inversely related, and related to soil tilth and water holding capacity; higher clay content results in greater bulk density and higher water retention (Easton 2021). High bulk density likely reduces the pest's ability to move through soils. Soil moisture and texture have been found to be critical factors for the survival and success of other soil dwelling species of burrower bugs and other soil dwelling insect species, in general (Cherry et al. 1990; Riis et al. 2005; Milosavljević et al. 2016). The results of the study presented here suggest the most important covariates associated with *P. bilineatus* injury are those most related to soil properties and field size.

Final Thoughts

Pangaeus bilineatus is a generalist herbivore that causes significant economic injury to peanut. The fact that it occupies the same environmental niche through all developmental stages and feeds on a host that produces seed below ground makes it unique and difficult to manage. Model results indicate that peanut fields at the highest risk for injury are found in landscapes comprised of high crop diversity. Effects of edge density covariates (edge contrast of crop families and interface of peanut to seminatural habitat) and roughness also suggest that landscapes at high risk for injury lack complexity in shape and elevation. The edge effect could be related to top-down control by natural enemies, although trophic interaction of natural enemies was not tested.

Then, what makes a *particular field* vulnerable? Larger fields dilute injury, and fields with high SOM and low clay make it easier for bugs to burrow through the soil and access peanut pods. Low moisture in the summer (and lack of irrigation) might drive bugs to do more peanut feeding as developing pods are high in water content. The models presented here suggest the risk for economic loss is greatest for non-irrigated fields <25 hectares that are surrounded by a diverse cropping landscape during summer drought conditions. Because management tools for *P. bilineatus* are lacking, the best defense is to avoid it by circumventing high risk fields.

This study focused on the relationships between post-harvest injury level and potential explanatory variables. The addition of pest and natural enemy abundance along with production practice information could further improve the risk assessment models.

The approach to this study did not require extensive field sampling. Therefore, the monetary and intensive physical labor costs relative to other landscape-scale ecological studies was low. Additionally, bulk data collection by GA-FSIS created a large sample size to allow better estimation of the relationships we observed between landscape and environmental factors, and *P. bilineatus* injury to peanut. Crop injury reports paired with ecoinformatics has proven to be an effective approach to identify factors that increase risk of injury caused by this pest that is difficult to observe in real time.

Acknowledgements

We thank the Georgia Peanut Commission, National Peanut Board, and the USDA for funding this project. We also thank the Georgia – Federal State Inspection Service for providing reports of *P. bilineatus* peanut injury.

References

Adedeji, A.A., N. Ekramirad, A. Rady, A. Hamidisepehr, K.D. Donohue, R.T. Villanueva, C.A. Parrish, and M. Li. 2020. Non-destructive technologies for detecting insect infestation in fruits and vegetables under postharvest conditions: a critical review. *Foods*. 9(7): 927.

American Peanut Shellers Association. 2020. Farmers Stock Trading Rules; Shelled, Inshell Trading Rules; Grading Standards. pp: 1-44.

Anderson, D. and K. Burnham. 2004. Model selection and multi-model inference, a practical information-Theoretic approach. *Second edn. Springer-Verlag,* New York.

Aigner, B.L., M.S. Crossley, and M.R. Abney. 2021. Biology and Management of Peanut Burrower Bug (Hemiptera: Cydnidae) in Southeast US Peanut. *J. of Integr. Pest Manag.* 12(1): 29; 1-8.

Balzan, M.V., G. Bocci, and A.C. Moonen. 2016. Landscape complexity and field margin vegetation diversity enhance natural enemies and reduce herbivory by Lepidoptera pests on tomato crop. *BioControl*. *61*: 141-154.

Barton, K. 2009. MuMIn: multi-model inference. R package version 1. 0. 0. Accesses 01/28/2022. http://r-forge. r-project. org/projects/mumin/.

Bianchi, F., C.J.H. Booij, and T. Tscharntke. 2006. Sustainable pest regulation in agricultural landscapes: a review on landscape composition, biodiversity and natural pest control. *Proc. R. Soc. B-Biol. Sci*. 273(1595):1715-27.

Blitzer, E.J., C.F. Dormann, A. Holzschuh, A.M. Klein, T.A. Rand, and T. Tscharntke. 2012. Spillover of functionally important organisms between managed and natural habitats. *Agric. Ecosyst. Environ.* 146(1): 34-43.

Bommarco, R., L. Marini, and B.E. Vaissière. 2012. Insect pollination enhances seed yield, quality, and market value in oilseed rape. *Oecologia*. 169: 1025-1032.

Booij, C.J.H. and J. Noorlander. 1992. Farming systems and insect predators. *Agric. Ecosyst. Environ.* 40:125-135.

Breheny, P. and W. Burchett. 2017. Visualization of regression models using visreg. *R J.* 9(2): 56.

Brown, J.F., D.M. Howard, D. Shrestha, and T.D. Benedict. 2019. Moderate Resolution Imaging Spectroradiometer (MODIS) Irrigated Agriculture Datasets for the Conterminous United States (MIrAD-US): U.S. Geological Survey data release. https://doi.org/10.5066/P9NA3EO8.

Bukovinszky, T., J. Verheijen, S. Zwerver, E. Klop, J.C. Biesmeijer, F.L. Wäckers, and D. Kleijn. 2017. Exploring the relationships between landscape complexity, wild bee species richness and reproduction, and pollination services along a complexity gradient in the Netherlands. *Biol. Conserv*. 214: 312-319. 10.1016/j.biocon.2017.08.027.

Campbell, R.E. 1937. Temperature and moisture preferences of wireworms. *Ecol.* 18(4): 479-489.

Chapin, J.W., J.S. Thomas, and P.H. Joost. 2001. Tillage and chlorpyrifos treatment effects on peanut arthropods – an incidence of severe burrower bug injury. *Peanut Sci.* 28(2): 64-73.

Chapin, J.W., and J.S. Thomas. 2003. Burrower bugs (Heteroptera: Cydnidae) in peanut: seasonal species abundance, tillage effects, grade reduction effects, insecticide efficacy, and management. *J. Econ. Entomol.* 96(4): 1142-1152.

Chapin, J.W., T.H. Sanders, L.O. Dean, K.W. Hendrix, and J.S. Thomas. 2006. Effect of feeding by a burrower bug, *Pangaeus bilineatus* (Say)(Heteroptera: Cydnidae), on peanut flavor and oil quality. *J. of Entomol. Sci.* 41(1): 33-39.

Chaplin-Kramer, R., M.E. O'Rourke, E.J. Blitzer, and C. Kremen. 2011. A meta-analysis of crop pest and natural enemy response to landscape complexity. *Ecol. Lett.* 14(9): 922-932.

Cherry, R.H., F.J. Coale, and P.S. Porter. 1990. Oviposition and survivorship of sugarcane grubs (Coleoptera: Scarabaeidae) at different soil moistures. *J. Econ. Entomol.* 83(4): 1355-1359.

Chuang, T.W., C.W. Hockett, L. Kightlinger, and M.C. Wimberly. 2012. Landscape-level spatial patterns of West Nile virus risk in the northern Great Plains. *Am. J. Trop. Med. Hyg.* 86(4): 724.

Crawley, M.J. 2012. *The R book*. John Wiley & Sons.

Crossley, M.S., O.M. Smith, L.L. Berry, R. Phillips-Cosio, J. Glassberg, K.M. Holman, J.G. Holmquest, A.R. Meier, S.A. Varriano, M.R. McClung, and M.D. Moran. 2021. Recent climate change is creating hotspots of butterfly increase and decline across North America. *Glob. Chang. Biol.* 27(12): 2702-2714.

Easton, Z.M. 2021. Soil and soil water relationships.

Fox, J. and S. Weisberg. 2018. An R companion to applied regression, 3rd edition. Sage Publications, Washington DC. pp: 576.

Froeschner, R.C. 1960. Cydnidae of the Western Hemisphere. *Proc. U. S. Nat. Mus*. 111: 337–680.

Google Maps. Areal search for center pivot irrigation systems in Georgia, US. Accessed October, 2019. https://www.google.com/maps/@31.8373613,-82.4757314,8.54z?entry=ttu.

Gould, G.E. 1931. *Pangaeus uhleri*, a pest of spinach. *J. of Econ. Entomol.* 24(2): 484-486.

Gurr, G.M., S.D. Wratten, and J.M. Luna. 2003. Multi-function agricultural biodiversity: pest management and other benefits. *Basic Appl. Ecol.* 4(2): 107-116.

Haan, N.L., Y. Zhang, and D.A. Landis. 2020. Predicting landscape configuration effects on agricultural pest suppression. *Trends Ecol. Evol.* 35(2): 175-186.

Harrison X.A., L. Donaldson, M.E. Correa-Cano, J. Evans, D.N. Fisher, C.E. Goodwin, B.S. Robinson, D.J. Hodgson, and R. Inger. 2018. A brief introduction to mixed effects modelling and multi-model inference in ecology. *Peerj*. 6: e4794.

Hein, G.L., and J.J. Tollefson. 1985. Use of the Pherocon AM trap as a scouting tool for predicting damage by corn rootworm (Coleoptera: Chrysomelidae) larvae. *J. Econ. Entomol.* 78(1): 200-203.

Hermann, A., N. Brunner, P. Hann, T. Wrbka, and B. Kromp. 2013. Correlations between wireworm damages in potato fields and landscape structure at different scales. *J. Pest Sci.* 86: 41-51.

Hesselbarth, M.H., M. Sciaini, K.A. With, K. Wiegand, and J. Nowosad. 2019. *landscapemetrics*: An open-source R tool to calculate landscape metrics. *Ecography*. 42(10): 1648-1657.

Hijmans, R.J., and J. van Etten. 2012. Raster: Geographic analysis and modeling with raster data. R package version 2.0-12.

Holt, R.A. and M.E. Hochberg. 2001. Indirect interactions, community modules and biological control: a theoretical perspective. In *Evaluating indirect ecological effects of biological control. Key papers from the symposium'Indirect ecological effects in biological control', Montpellier, France, 17-20 October 1999* (pp. 13-37). Wallingford UK: CABI Publishing.

Hudson, B.D. 1994. Soil organic matter and available water capacity. *J. Soil Water Conserv.* 49(2): 189-194.

Jung, J., P. Racca, J. Schmitt, and B. Kleinhenz. 2014. SIMAGRIO-W: Development of a prediction model for wireworms in relation to soil moisture, temperature and type. *J. Appl. Entomol.* 138(3): 183-194.

Karlen, D.L., D.C. Erbach, T.C. Kaspar, T.S. Colvin, E.C. Berry, and D.R. Timmons. 1990. Soil tilth: A review of past perceptions and future needs. *Soil Sci. Soc. Am. J.* 54(1): 153-161.

Karp D.S., R. Chaplin-Kramer, T.D. Meehan, et al. 2018. Crop pests and predators exhibit inconsistent responses to surrounding landscape composition. *Proc. Natl. Acad. Sci.* 115(33): E7863-E70.

Lefko, S.A., L.P. Pedigo, W.D. Batchelor, and M.E. Rice. 1998. Spatial modeling of preferred wireworm (Coleoptera: Elateridae) habitat. *Environ. Entomol.* 27(2): 184-190.

Letourneau, D.K. 1987. The enemies hypothesis: tritrophic interactions and vegetational diversity in tropical agroecosystems. *Ecol*. 68(6): 1616-1622.

Liere, H., T.N. Kim, B.P. Werling, T.D. Meehan, D.A. Landis, and C. Gratton. 2015. Trophic cascades in agricultural landscapes: indirect effects of landscape composition on crop yield. *Ecol. Appl.* 25(3): 652-661.

Mangiafico, S. 2017. R-companion: functions to support extension education program evaluation. R package version 1.5. 0. The Comprehensive R Archive Network.

McGarigal, K., S. Tagil, and S.A. Cushman. 2009. Surface metrics: an alternative to patch metrics for the quantification of landscape structure. *Landsc. Ecol.* 24(3): 433-450.

Meehan, T.D., B.P. Werling, D.A. Landis, and C. Gratton. 2011. Agricultural landscape simplification and insecticide use in the Midwestern United States. *Proc. Natl. Acad. Sci.* 108(28): 11500-11505.

Milosavljević, I., A.D. Esser, and D.W. Crowder. 2016. Effects of environmental and agronomic factors on soil-dwelling pest communities in cereal crops. *Agric. Ecosyst. Environ.* 225: 192-198.

Moya-Laraño, J. and G. Corcobado. 2008. Plotting partial correlation and regression in ecological studies. *Web Ecol.* 8(1): 35-46.

Naeem, S. and S. Li. 1997. Biodiversity enhances ecosystem reliability. *Nature*. *390*(6659): 507-509.

Oksanen, J., F. Blanchet, M. Friendly, R. Kindt, P. Legendre, D. McGlinn, P.R. Minchin, R.B. O'Hara, G.L. Simpson, P. Solymos, M.H.H. Stevens, E. Szoecs, H. Wagner. 2015. Vegan: community ecology package version 2.3–2. https://cran.r-project.org/web/packages/vegan/index.html.

Pearson, E.S. 1931. The test of significance for the correlation coefficient. *J. Am. Stat. Assoc*. 26(174): 128-134.

Pebesma, E., and R. Bivand. 2023. *Spatial Data Science: With applications in R*. Chapman and Hall/CRC, London. doi:10.1201/9780429459016, https://r-spatial.org/book/.

Poggi, S., R. Le Cointe, J.B. Riou, P. Larroudé, J.B. Thibord, and M. Plantegenest. 2018. Relative influence of climate and agroenvironmental factors on wireworm damage risk in maize crops. *J. Pest Sci.* 91: 585-599.

Poggi, S., R. Le Cointe, J. Lehmhus, M. Plantegenest, and L. Furlan. 2021. Alternative strategies for controlling wireworms in field crops: A review. *Agriculture*. 11(5): 436.

PRISM Climate Group. Oregon State University. https://prism.oregonstate.edu, data created Sep 2019, accessed Feb. 2022.

R Core Team. 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Rand, T.A., J.M. Tylianakis, and T. Tscharntke. 2006. Spillover edge effects: the dispersal of agriculturally subsidized insect natural enemies into adjacent natural habitats. *Ecol. Lett.* 9(5): 603-614.

Redlich, S., E.A. Martin, and I. Steffan-Dewenter. 2018. Landscape-level crop diversity benefits biological pest control. *J. Appl. Ecol.* 55(5): 2419-2428.

Riis, L. and P. Esbjerg. 1998. Season and soil moisture effect on movement, survival, and distribution of Cyrtomenus bergi (Hemiptera: Cydnidae) within the soil profile. *Environ. Entomol.* 27(5): 1182-1189.

Riis, L., P. Esbjerg, and A.C. Bellotti. 2005. Influence of temperature and soil moisture on some population growth parameters of *Cyrtomenus bergi* (Hemiptera: Cydnidae). *Fla. Entomol.* 88(1): 11-22.

Root, R.B. 1973. Organization of a plant–arthropod association in simple and diverse habitats: the fauna of collards (*Brassica oleracea*). *Ecol. Monogr.* 43: 95–124.

Rosenheim, J.A. and C. Gratton. 2017. Ecoinformatics (big data) for agricultural entomology: pitfalls, progress, and promise. *Ann. Rev. Entomol.* 62: 399-417.

Rossetti, M.R., T. Tscharntke, R. Aguilar, and P. Batáry. 2017. Responses of insect herbivores and herbivory to habitat fragmentation: a hierarchical meta-analysis. *Ecol. Lett.* 20(2): 264 – 272.

Rusch, A., M. Valantin-Morison, J.P. Sarthou, and J. Roger-Estrade. 2010. Biological control of insect pests in agroecosystems: effects of crop management, farming systems, and seminatural habitats at the landscape scale: a review. *Adv. Agron.* 109: 219-259.

Rusch, A., M. Valantin-Morison, J.P. Sarthou, and J. Roger-Estrade. 2013. Effect of crop management and landscape context on insect pest populations and crop damage. *Agric. Ecosyst. Environ.* 166: 118-125.

Saussure, S., M. Plantegenest, J.B. Thibord, P. Larroudé, and S. Poggi. 2015. Management of wireworm damage in maize fields using new, landscape-scale strategies. *Agron. Sustain. Dev.* 35: 793-802.

Scherber, C., J. Heimann, G. Köhler, N. Mitschunas, and W.W. Weisser. 2010. Functional identity versus species richness: herbivory resistance in plant communities. *Oecologia*. 163: 707-717.

Simpson, E. H. 1949. Measurement of species diversity. *Nature*. 163: 688.

Smith Jr., J.W., and J.T Pitts. 1974. Pest status of *Pangaeus bilineatus* attacking peanuts in Texas. *J. Econ. Entomol.* 67(1): 111-113.

Soil Survey Staff.a. Gridded Soil Survey Geographic (gSSURGO) Database for Georgia. United States Department of Agriculture, Natural Resources Conservation Service. Available online at http://datagateway.nrcs.usda.gov/. 20180915 (FY2019 official release).

Soil Survey Staff.b. Value Added Look Up (valu1) Table for the Gridded Soil Survey Geographic (gSSURGO) Database. United States Department of Agriculture, Natural Resources Conservation Service. Available online at http://datagateway.nrcs.usda.gov/. 20180915 (FY2019 official release).

Tennekes, M. 2018. "tmap: Thematic Maps in R." *Journal of Statistical Software*, 84(6), 1–39. doi:10.18637/jss.v084.i06

Thies, C., and T. Tscharntke. 1999. Landscape structure and biological control in agroecosystems. *Science*. 285(5429): 893-895.

Throne, J.E., G.J. Hallman, J.A. Johnson, and P.A. Follett. 2003. Post-harvest entomology research in the United States Department of Agriculture–Agricultural Research Service. *Pest Manag. Sci.: formerly Pesticide Science*. 59(6-7): 619-628.

Tscharntke, T., T.A. Rand, and F.J. Bianchi. 2005. The landscape context of trophic interactions: insect spillover across the crop—noncrop interface. In *Annales Zoologici Fennici* (pp: 421-432). Finnish Zoological and Botanical Publishing Board.

Tscharntke, T., J.M. Tylianakis, T.A. Rand, R.K. Didham, L. Fahrig, P. Batáry, J. Bengtsson, Y. Clough, T.O. Crist, C.F. Dormann, and R.M. Ewers. 2012. Landscape moderation of biodiversity patterns and processes-eight hypotheses. *Biol. Rev.* 87(3): 661-685.

Tscharntke, T., D.S. Karp, R. Chaplin-Kramer, P. Batáry, F. DeClerck, C. Gratton, L. Hunt, A. Ives, M. Jonsson, A. Larsen, and E.A. Martin. 2016. When natural habitat fails to enhance biological pest control–Five hypotheses. *Biol. Conserv.* 204: 449-458.

Tschumi, M., M. Albrecht, M.H. Entling, and K. Jacot. 2015. High effectiveness of tailored flower strips in reducing pests and crop plant damage. *Proc. R. Soc. B: Biol. Sci.* 282(1814): 20151369.

USDA – NASS. Accessed Sep. **2021**. https://quickstats.nass.usda.gov/results/E3B3DEC1-5AE5-3A99-9A20-8A742C2A2F67?pivot=short_desc

USDA. 2008. 2008 Georgia Cropland Data Layer | NASS/USDA. United States Department of Agriculture (USDA), National Agricultural Statistics Service (NASS), Research and Development Division (RDD), Geospatial Information Branch (GIB), Spatial Analysis Research Section (SARS). 2008 Edition. *Publication Date:* 12/11/2017. USDA, NASS (Publisher). Marketing and Information Services Office, Washington, D.C. https://nassgeodata.gmu.edu/CropScape/GA

USDA. 2009. 2009 Georgia Cropland Data Layer | NASS/USDA. United States Department of Agriculture (USDA), National Agricultural Statistics Service (NASS), Research and Development Division (RDD), Geospatial Information Branch (GIB), Spatial Analysis Research Section (SARS). 2009 Edition. *Publication Date:* 12/11/2017. USDA, NASS (Publisher). Marketing and Information Services Office, Washington, D.C. https://nassgeodata.gmu.edu/CropScape/GA

USDA. 2010. 2010 Georgia Cropland Data Layer | NASS/USDA. United States Department of Agriculture (USDA), National Agricultural Statistics Service (NASS), Research and Development Division (RDD), Geospatial Information Branch (GIB), Spatial Analysis Research Section (SARS). 2010 Edition. *Publication Date:* 01/10/2011. USDA, NASS (Publisher). Marketing and Information Services Office, Washington, D.C. https://nassgeodata.gmu.edu/CropScape/GA

USDA. 2011. 2011 Georgia Cropland Data Layer | NASS/USDA. United States Department of Agriculture (USDA), National Agricultural Statistics Service (NASS), Research and Development Division (RDD), Geospatial Information Branch (GIB), Spatial Analysis Research Section (SARS). 2011 Edition. *Publication Date:* 01/31/2012. USDA, NASS (Publisher). Marketing and Information Services Office, Washington, D.C. https://nassgeodata.gmu.edu/CropScape/GA

USDA. 2012. 2012 Georgia Cropland Data Layer | NASS/USDA. United States Department of Agriculture (USDA), National Agricultural Statistics Service (NASS), Research and Development Division (RDD), Geospatial Information Branch (GIB), Spatial Analysis Research Section (SARS). 2012 Edition. *Publication Date:* 01/31/2013. USDA, NASS

(Publisher). Marketing and Information Services Office, Washington, D.C. https://nassgeodata.gmu.edu/CropScape/GA

USDA. 2013. 2013 Georgia Cropland Data Layer | NASS/USDA. United States Department of Agriculture (USDA), National Agricultural Statistics Service (NASS), Research and Development Division (RDD), Geospatial Information Branch (GIB), Spatial Analysis Research Section (SARS). 2013 Edition. *Publication Date:* 01/31/2014. USDA, NASS (Publisher). Marketing and Information Services Office, Washington, D.C. https://nassgeodata.gmu.edu/CropScape/GA

USDA. 2014. 2014 Georgia Cropland Data Layer | NASS/USDA. United States Department of Agriculture (USDA), National Agricultural Statistics Service (NASS), Research and Development Division (RDD), Geospatial Information Branch (GIB), Spatial Analysis Research Section (SARS). 2014 Edition. *Publication Date:* 02/02/2015. USDA, NASS (Publisher). Marketing and Information Services Office, Washington, D.C. https://nassgeodata.gmu.edu/CropScape/GA

USDA. 2015. 2008 Georgia Cropland Data Layer | NASS/USDA. United States Department of Agriculture (USDA), National Agricultural Statistics Service (NASS), Research and Development Division (RDD), Geospatial Information Branch (GIB), Spatial Analysis Research Section (SARS). 2008 Edition. *Publication Date:* 02/12/2016. USDA, NASS (Publisher). Marketing and Information Services Office, Washington, D.C. https://nassgeodata.gmu.edu/CropScape/GA

USDA. 2016. 2016 Georgia Cropland Data Layer | NASS/USDA. United States Department of Agriculture (USDA), National Agricultural Statistics Service (NASS), Research and

Development Division (RDD), Geospatial Information Branch (GIB), Spatial Analysis Research Section (SARS). 2016 Edition. *Publication Date:* 01/30/2017. USDA, NASS (Publisher). Marketing and Information Services Office, Washington, D.C. https://nassgeodata.gmu.edu/CropScape/GA

USDA. 2017. 2017 Georgia Cropland Data Layer | NASS/USDA. United States Department of Agriculture (USDA), National Agricultural Statistics Service (NASS), Research and Development Division (RDD), Geospatial Information Branch (GIB), Spatial Analysis Research Section (SARS). 2017 Edition. *Publication Date:* 01/26/2018. USDA, NASS (Publisher). Marketing and Information Services Office, Washington, D.C. https://nassgeodata.gmu.edu/CropScape/GA

USDA. 2018. 2018 Georgia Cropland Data Layer | NASS/USDA. United States Department of Agriculture (USDA), National Agricultural Statistics Service (NASS), Research and Development Division (RDD), Geospatial Information Branch (GIB), Spatial Analysis Research Section (SARS). 2018 Edition. *Publication Date:* 02/15/2019. USDA, NASS (Publisher). Marketing and Information Services Office, Washington, D.C. https://nassgeodata.gmu.edu/CropScape/GA

U.S. Geological Survey. 1996. USGS Digital Elevation Model 1:250,000 (chouse). Digital Data Series. Version 1. Atlanta, GA. Accessed: 2018.

Veres, A., S. Petit, C. Conord, C. Lavigne. **2013**. Does landscape composition affect pest abundance and their control by natural enemies? A review. *Agric. Ecosyst. Environ*. 166: 110-7.

Wan, N.F., X.Y. Ji, L.P. Kiær, S.S. Liu, J.Y. Deng, J.X. Jiang, and B. Li. 2018. Ground cover increases spatial aggregation and association of insect herbivores and their predators in an agricultural landscape. *Landsc. Ecol.* 33: 799-809.

Willis, R.B., M.R. Abney, G.J. Holmes, J.R. Schultheis, and G.G. Kennedy. 2010. Influence of preceding crop on wireworm (Coleoptera: Elateridae) abundance in the coastal plain of North Carolina. *J. Econ. Entomol.* 103(6): 2087-2093.

Yachi, S., and M. Loreau. 1999. Biodiversity and ecosystem productivity in a fluctuating environment: the insurance hypothesis. *Proc. Natl. Acad. Sci.* 96(4): 1463-1468.

Yan, L., and D.P. Roy. 2016. Conterminous United States crop field size quantification from multi-temporal Landsat data. *Remote Sens. Environ*. 172: 67-86. http://www.sciencedirect.com/science/article/pii/S0034425715301851

Table 3.1. Model selection table summarizing the generalized linear models with the best support at two scales ($\Delta AIC_c > 3$ at each scale) which evaluate the associations between the rate of *Pangaeus bilineatus* injury to peanuts, landscape and environmental covariates, and the year of study in Georgia, US. Footnotes include descriptions of column headings [a]; significance symbols [b]; and notations of model covariates: compositional [c], configurational [d], and environmental [e].

Radius (km)	Model (partial correlation coefficient)	*df*	X^2	W*i*	Log-lik	AIC_c	Δi	Pseudo-r^2	*P*
	Landscape composition								
1.0	**I – AG***(0.17) - Y18*** + CR** (0.11) - F** (0.10) - P* (0.10) - NR* (0.09) - CT* (0.08)**	**433**	**40.55**	**0.1039**	**-3861.79**	**3882.30**	**0.00**	**0.09**	**2.54e-06**
1.0	I – Y18*** - AG** (0.13) + CR** (0.12) + W** (0.11) - NR* (0.09) + SN^{ns} (0.08) + WC^{ns} (0.07)	433	40.01	0.2369	-3862.33	3882.84	0.54	0.09	3.19e-06
1.5	I - Y18*** - P** (0.15) + W* (0.09)	436	33.88	0.0350	-3868.46	3882.71	0.00	0.07	2.50e-06
1.5	I – Y18** - P** (0.15)	436	33.72	0.0531	-3868.61	3882.87	0.16	0.07	2.70e-06
1.5	I – P*** (0.17) - Y18** - AG* (0.09)	437	31.45	0.0725	-3870.88	3883.08	0.37	0.07	2.47e-06
	Landscape configuration								
1.0	I - FA*** (0.33) - AR*** (0.20) - ED_{pp}* (0.10)	438	87.31	0.3242	-3815.03	3825.16	0.00	0.18	8.29e-19
1.0	I - FA*** (0.32) - AR*** (0.18) - ED_{cf}* (0.10) - PS^{ns} (0.06)	437	88.53	0.2904	-3813.81	3826.00	0.84	0.18	2.71e-18
1.0	I – FA*** (0.33) - AR*** (0.17) - ED_{cf}* (0.09)	438	86.43	0.5636	-3815.91	3826.04	0.88	0.18	1.28e-18
1.5	**I – FA*** (0.33) - AR*** (0.18) - ED_{cf}* (0.10) – ED_{ps}* (0.09)**	**437**	**90.29**	**0.6061**	**-3812.05**	**3824.24**	**0.00**	**0.18**	**1.14e-18**

Radius (km)	Model (partial correlation coefficient)	*df*	X^2	W*i*	Log-lik	AIC_c	Δi	Pseudo-r^2	*P*
	Landscape configuration								
1.5	I - FA*** (0.33) - AR*** (0.21) - ED_{pp}* (0.10)	438	87.17	0.8364	-3815.17	3825.30	1.06	0.18	8.87e-19
	Environmental								
1.0	**I + SC_o*** (0.19) - SP_d*** (0.15) - IR** (0.12) + WP_d* (0.09) - SC_c^{ns} (0.08)**	**436**	**59.05**	**0.5113**	**-3843.29**	**3857.55**	**0.00**	**0.13**	**1.91e-11**
1.5	I + SC_o*** (0.19) - SP_d*** (0.15) - IR** (0.13) + WP_d* (0.10) + SC_s* (0.09) - WT_d^{ns} (0.07)	435	60.14	0.2929	-3842.20	3858.53	0.00	0.13	4.22e-11
1.5	I + SC_o*** (0.18) - SP_d*** (0.15) - IR** 0.12) + WP_d* (0.09) + SC_s^{ns} (0.07)	436	57.73	0.3074	-3844.61	3858.86	0.33	0.12	3.57e-11

[a] Radius column = the size of the buffer zone (km); Model column = all model covariates and their partial correlation coefficients in parentheses; *df* = model degrees of freedom; X^2 = model chi-square value; W*i* = model weight; Log-lik = log likelihood ratio; AIC_c = Akaike information criterion (corrected); Δi = change in AIC_c between models; Pseudo-r^2 = model pseudo r-squared value; *P* = model p-value/measure of significance.

[b] *** denotes covariate significance at α =.00; ** denotes covariate significance at α = .01; * denotes covariate significance at α = .05; ns = not significant.

[c] Notations of compositional model covariates include AG = other agriculture, CE = crop evenness, CR = crop richness, CT = cotton, F = forest, NR = non-crop richness, P = peanut, SN = seminatural habitat, W = wetlands, WC = woody crops, Y = year.

[d] Notations of configurational model covariates include AR = average roughness, ED_{cf} = edge density of contrasting crop families, ED_{pp} = edge density of peanut to pine forest, ED_{ps} = edge density of peanut to seminatural habitat, FA = field area.

[e] Notations of environmental model covariates include IR = irrigation, SC_c = percent clay soil composition, SC_o = percent soil organic matter soil composition, SC_s = percent sand soil composition, SP_d = cumulative days of summer precipitation, WP_d = cumulative days of winter precipitation, WT_d = average daily winter temperature.

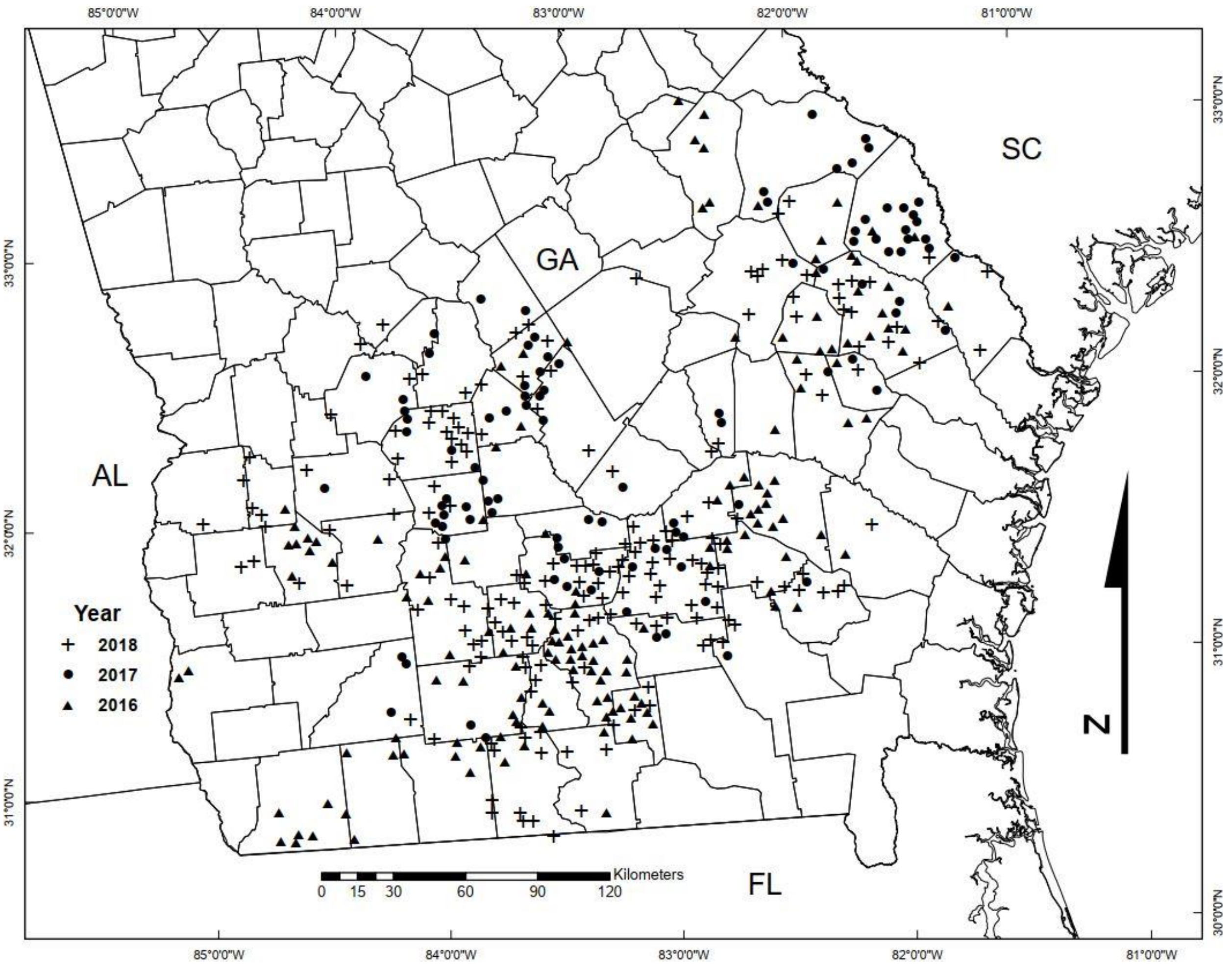
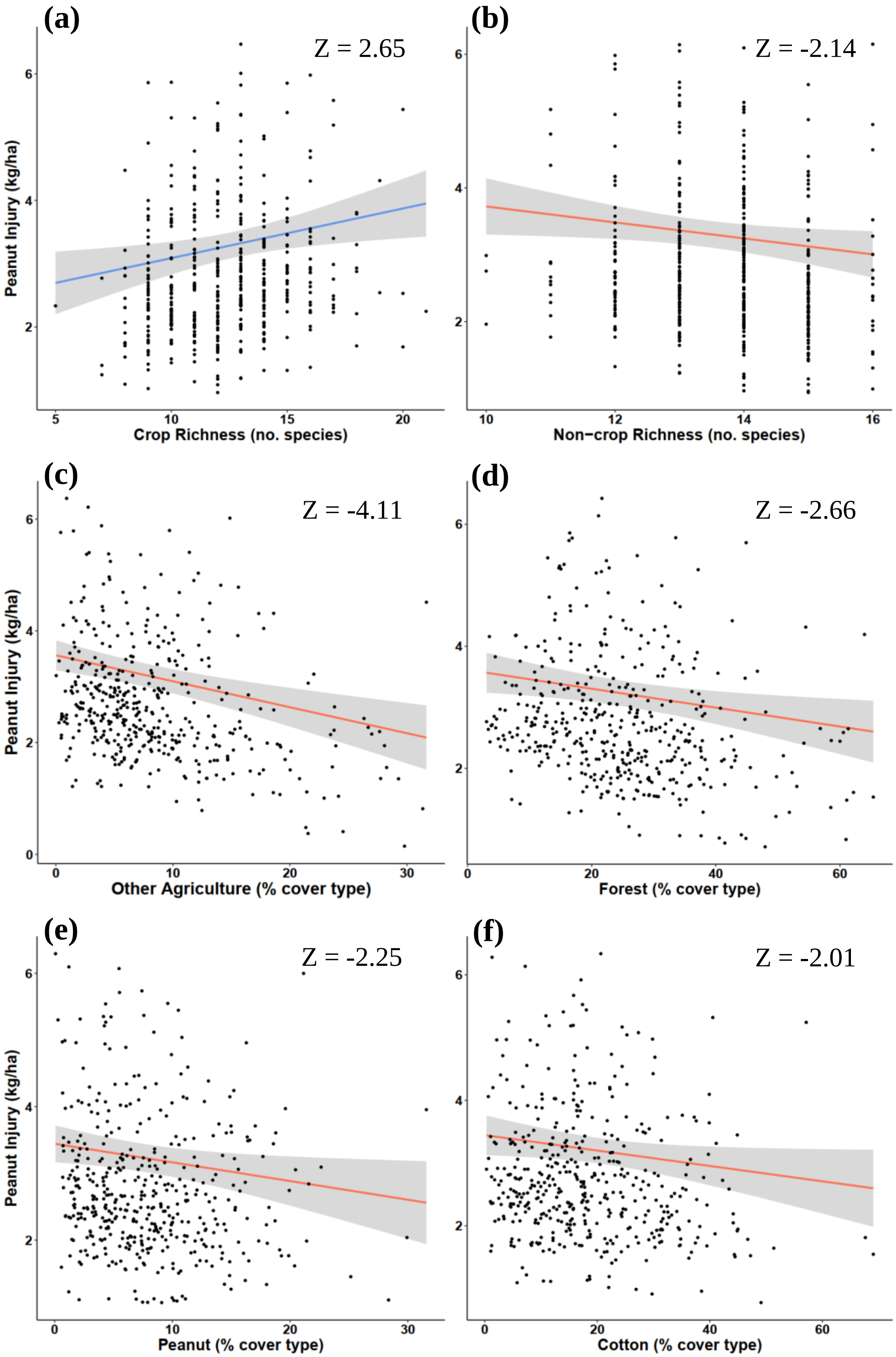
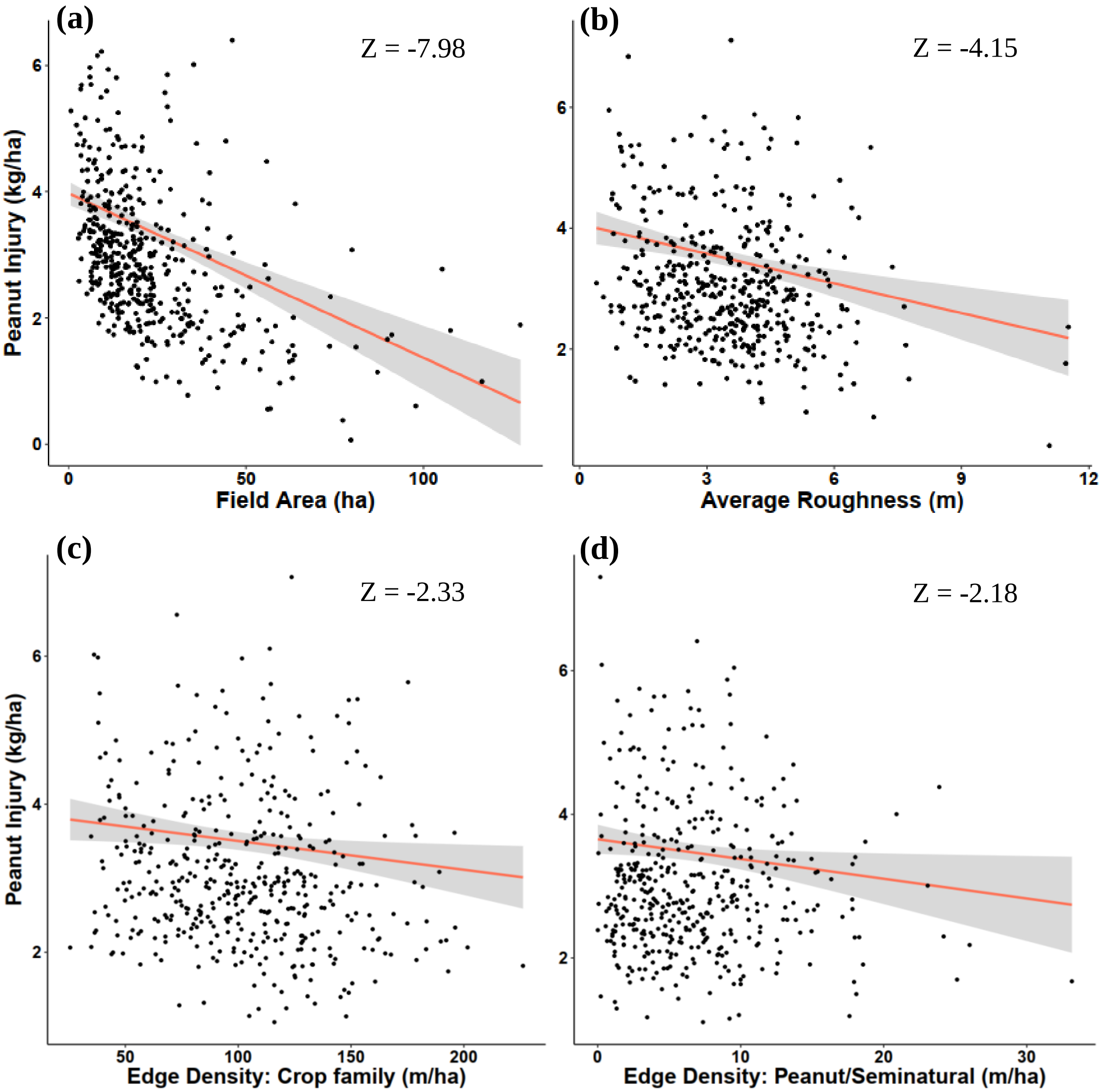


Fig. 3.1. Map of sample sites for landscape study. A total of 442 sites over 3 years were used to identify predictors of *Pangaeus bilineatus* injury to peanuts in Georgia, US.

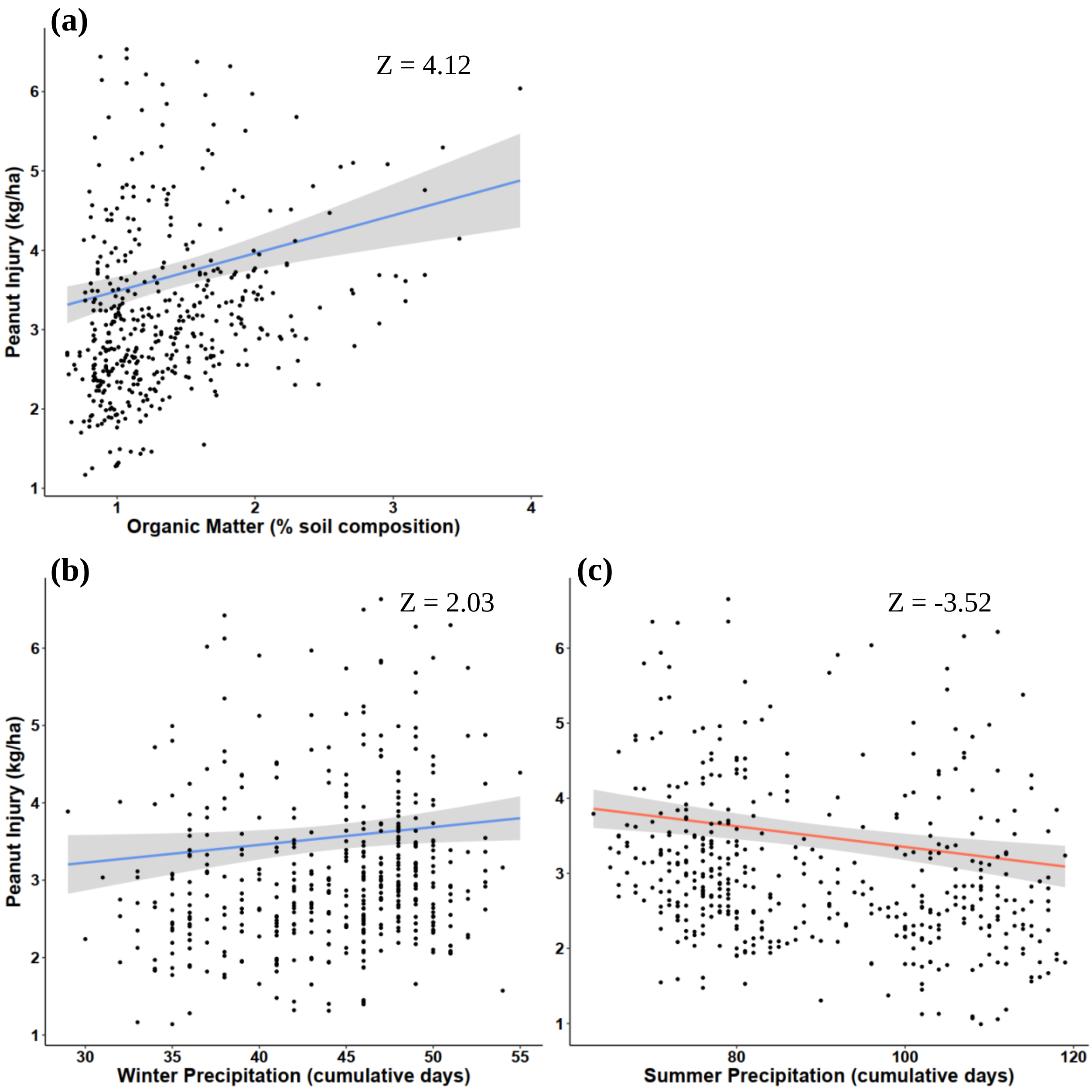


Figs. 3.2a-f. Partial residuals of peanut injury (kg/ha) with significant landscape composition variables at 1 km scale: a) crop richness has positive correlation with *P. bilineatus*, b) Non-crop

richness has negative correlation with *P. bilineatus* injury, c) Other agriculture has negative correlation with *P. bilineatus* injury, d) Forest has negative correlation with *P. bilineatus* injury, e) Peanut has negative correlation with *P. bilineatus* injury, and f) Cotton has negative correlation with *P. bilineatus* injury.



Figs. 3.3a-d. Partial residuals of peanut injury (kg/ha) with significant landscape configuration variables at 1.5 km scale: a) field area has negative correlation with *P. bilineatus* injury, b) average roughness has negative correlation with *P. bilineatus* injury, c) edge density of crop families has negative correlation with *P. bilineatus* injury, and d) edge density of peanut and seminatural habitat has negative correlation with *P. bilineatus* injury.



Figs. 3.4a-c. Partial residuals of peanut injury (kg/ha) with significant environmental variables at 1 km scale: a) soil organic matter has positive correlation with *P. bilineatus* injury, b) cumulative days of winter precipitation has positive correlation with *P. bilineatus* injury, and c) cumulative days of summer precipitation has negative correlation with *P. bilineatus* injury.

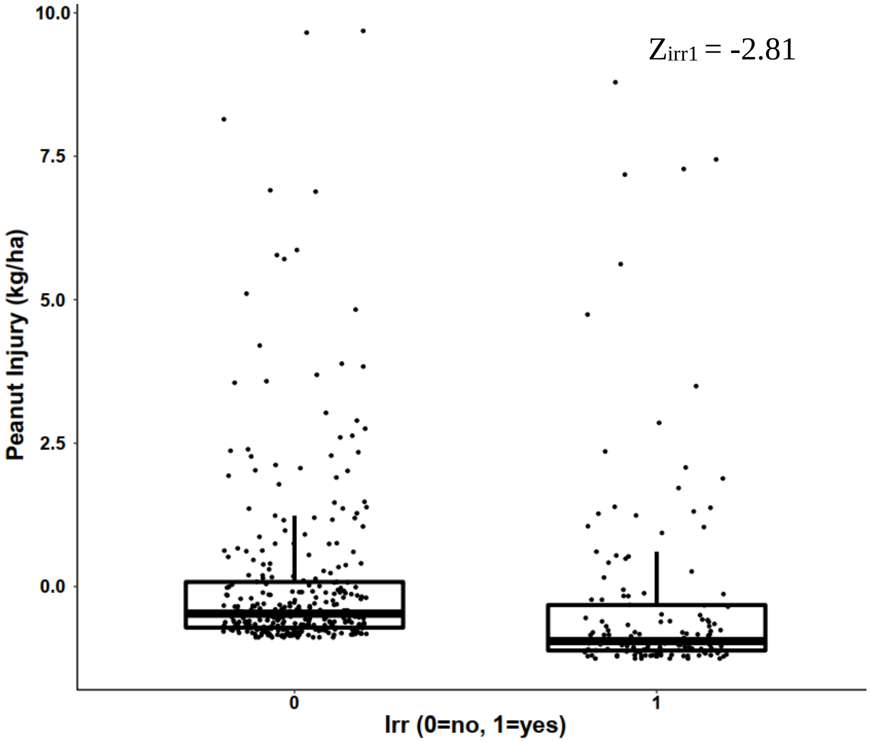


Fig. 3.5. Partial residuals of *P. bilineatus* peanut injury in non-irrigated (0) and irrigated (1) peanut fields in Georgia, US.

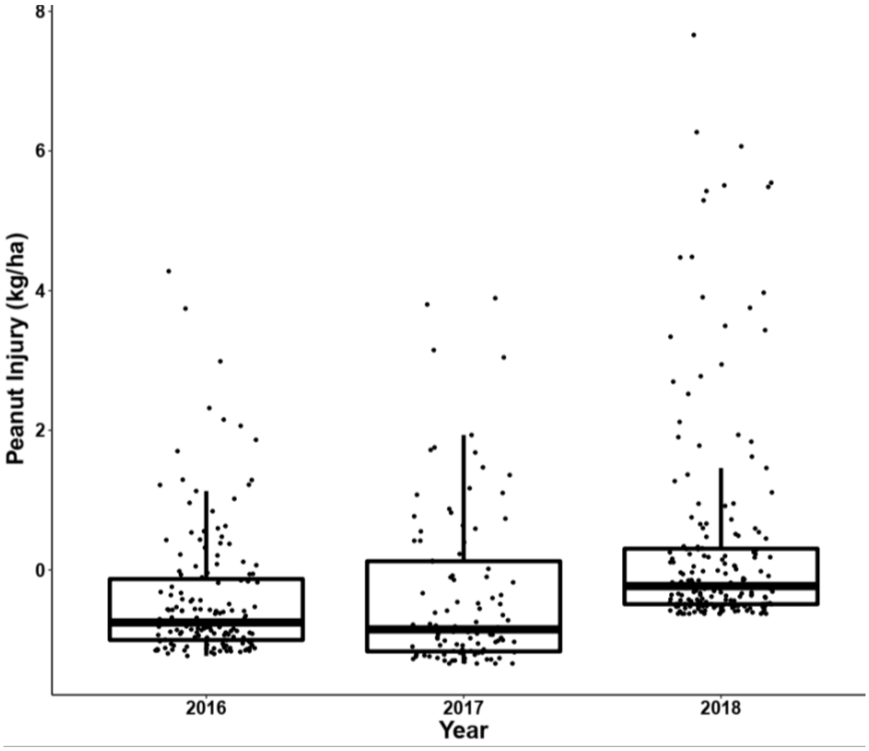
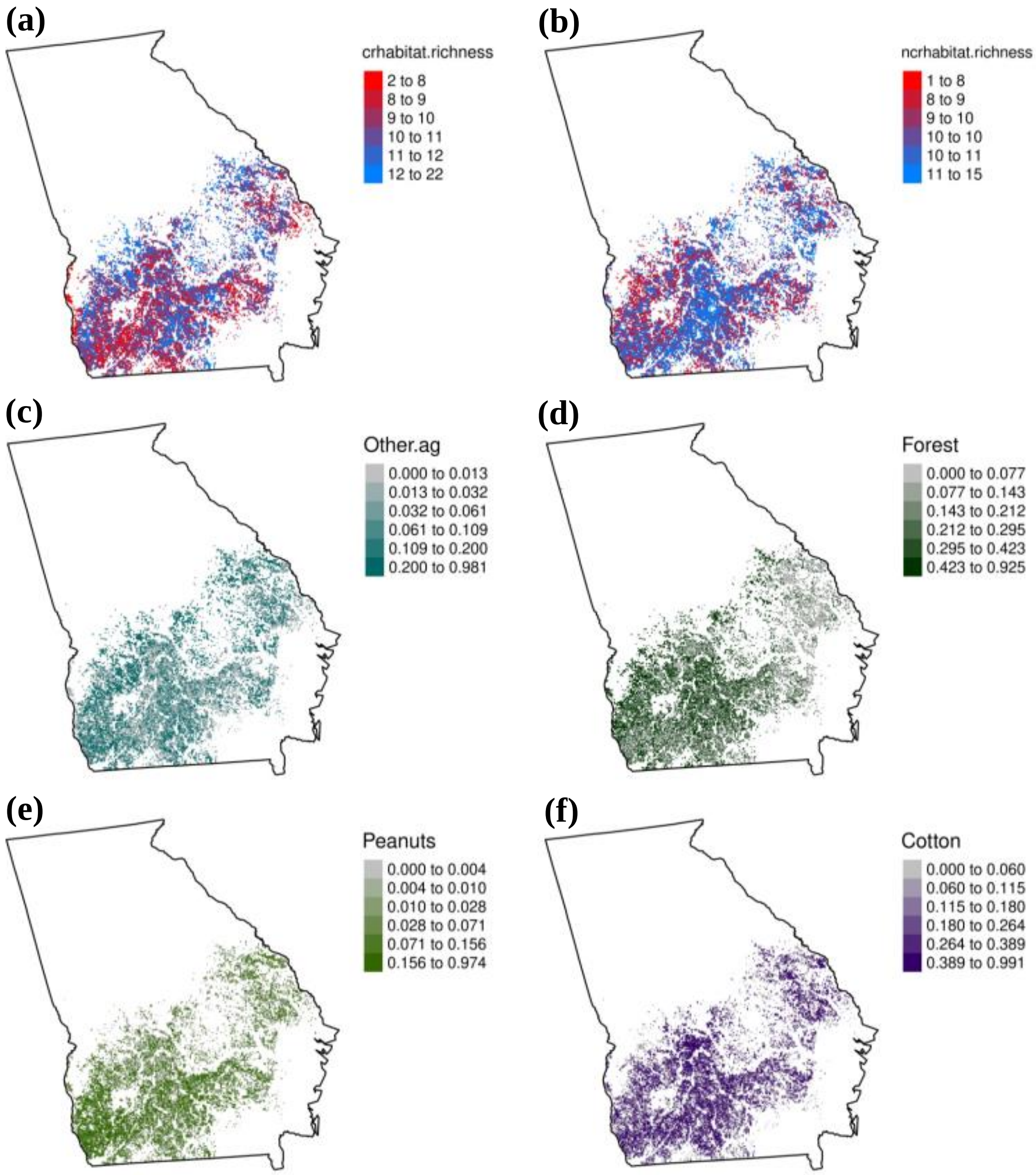
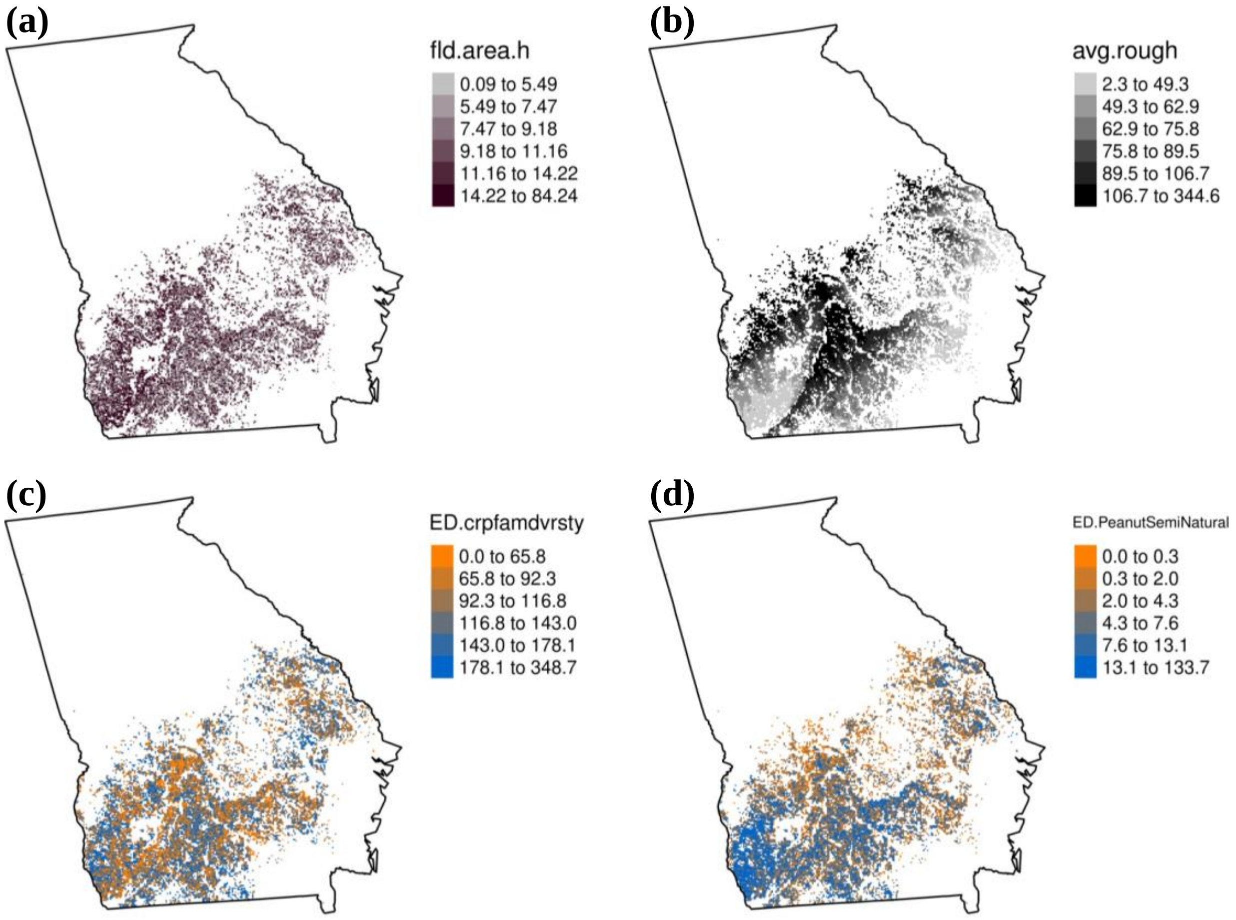


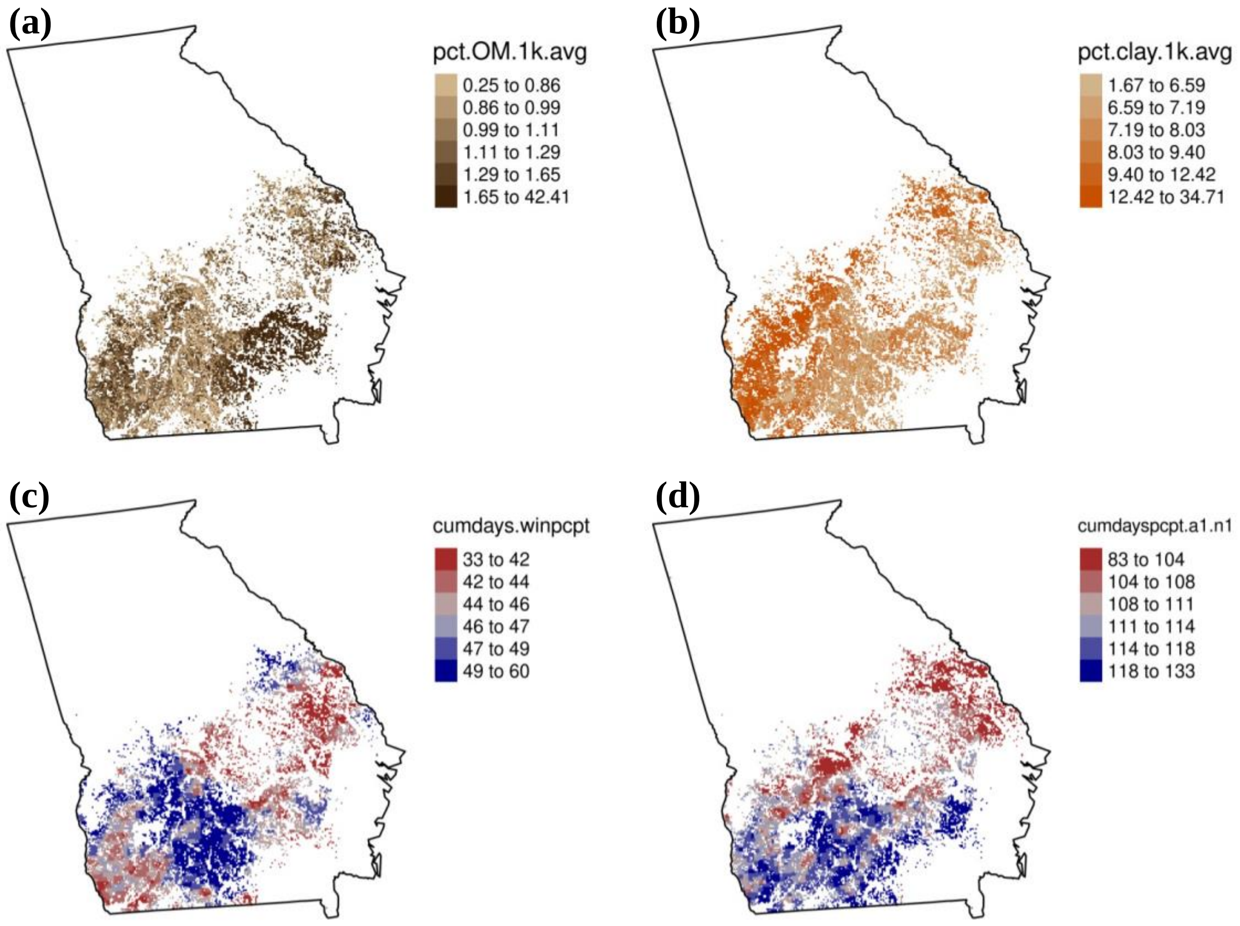
Fig. 3.6. Partial residuals of *P. bilineatus* peanut injury by year from all sites. Year 2016 had 155 sites, 100 in 2017, and 187 in 2018.



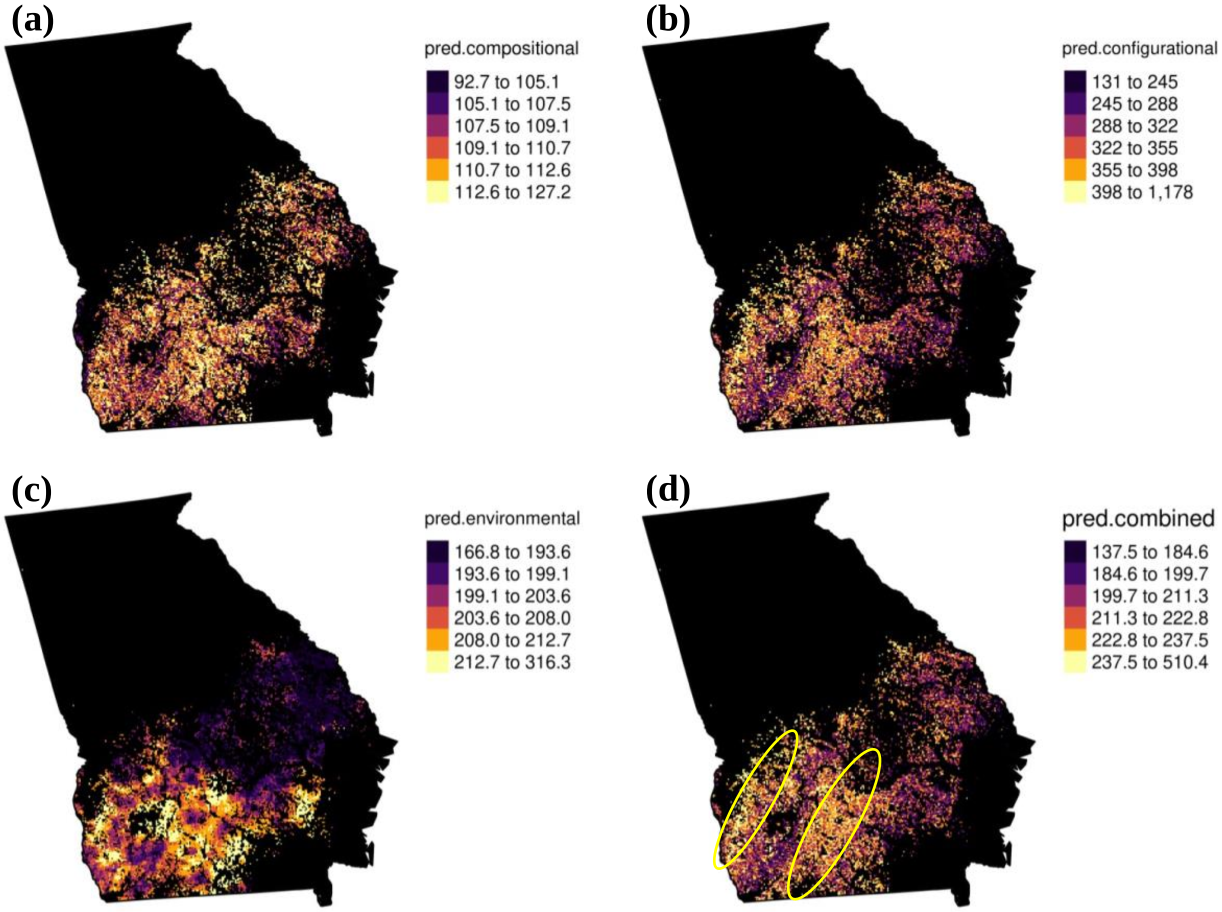
Figs. 3.7a-f. Predictive risk maps of *P. bilineatus* injury in Georgia, US, based on significant covariates of best fitting model. Legend represents scale of predicted risk of peanut injury in Kg/ha; a) crop habitat richness, b) non-crop habitat richness, c) other agriculture, d) forest, e) peanut, f) cotton.



Figs. 3.8a-d. Predictive risk maps of *P. bilineatus* injury in Georgia, US, based on significant covariates of best fitting model. Legend represents scale of predicted risk of peanut injury in Kg/ha; a) Field area, b) average roughness, c) edge density of contrasting crop families, d) edge density of peanut to seminatural habitat.



Figs. 3.9a-d. Predictive risk maps of *P. bilineatus* injury in Georgia, US, based on significant covariates of best fitting model. Legend represents scale of predicted risk of peanut injury in Kg/ha; a) percent soil organic material, b) percent clay, c) cumulative days of winter precipitation, d) cumulative days of summer precipitation.



Figs. 3.10a-d. Predictive risk maps of *P. bilineatus* injury in Georgia, US, based on significant covariates of best fitting model. Legend represents scale of predicted risk of peanut injury in Kg/ha; a) compositional covariates combined risk, b) configurational covariates combined risk, c) environmental covariates combined risk, d) compositional, configurational, and environmental covariates combined risk.

Appendix A. Area of land-cover types across peanut field sites for study years 2016, 2017 and 2018 at 1.5 km scale.

Major habitat types	Major land-cover types	Diversity land-cover types	Peanut sites % area	
			Range	Mean
Crops		Total	2.32—88.99	37.47
	Primary host	Peanut	0.08—31.57	7.92
	Rotational crops	Total	0.69—69.43	20.78
	(Cotton)	Total	0.32—69.05	18.27
		Cotton	0.32—68.57	18.13
		Dbl.Crop.WinWht.Cotton	0.00—13.31	0.14
	(Corn)	Total	0.00—27.02	2.52
		Corn	0.00—27.02	2.49
		Pop.or.Orn.Corn	0.00—6.51	0.01
		Sweet.Corn	0.00—0.49	0.00
		Dbl.Crop.Oats.Corn	0.00—1.54	0.01
		Dbl.Crop.WinWht.Corn	0.00—0.22	0.00
	Leguminous crops	Total	0.00—22.36	0.90
		Dry.Beans	0.00—1.59	0.00
		Peas	0.00—2.24	0.01
		Soybeans	0.00—19.88	0.76
		Alfalfa	0.00—0.40	0.00
		Dbl.Crop.Corn.Soybeans	0.00—0.04	0.00
		Dbl.Crop.WinWht.Soybeans	0.00—5.70	0.13
	Poaceous crops	Total	0.00—11.88	0.92
		Oats	0.00—4.93	0.11
		Rye	0.00—9.81	0.22
		Millet	0.00—6.95	0.14
		Sod.Grass.Seed	0.00—4.20	0.24
		Spring.Wheat	0.00—0.01	0.00
		Triticale	0.00—0.13	0.00
		Winter.Wheat	0.00—9.51	0.19

Major habitat types	Major land-cover types	Diversity land-cover types	Peanut sites % area	
			Range	Mean
		Dbl.Crop.Soybeans.Oats	0.00—2.54	0.01
		Dbl.Crop.WinWht.Sorghum	0.00—0.30	0.00
		Sorghum	0.00—4.48	0.05
	Woody crops	Total	0.01—39.64	3.00
		Apples	0.00—0.01	0.00
		Blueberries	0.00—19.44	0.41
		Caneberries	0.00—0.01	0.00
		Christmas.Trees	0.00—0.06	0.00
		Grapes	0.00—7.14	0.03
		Olives	0.00—0.56	0.00
		Other.Tree.Crops	0.00—0.03	0.00
		Peaches	0.00—9.04	0.04
		Pecans	0.00—37.09	2.52
	Other Agriculture	Total	0.00—27.06	3.95
		Broccoli	0.00—0.11	0.00
		Cabbage	0.00—1.09	0.00
		Canola	0.00—0.01	0.00
		Cantaloupes	0.00—1.86	0.01
		Celery	0.00—0.01	0.00
		Cucumbers	0.00—0.21	0.00
		Eggplants	0.00—0.32	0.00
		Greens	0.00—0.03	0.00
		Herbs	0.00—0.01	0.00
		Misc.Vegs & Fruits	0.00—0.01	0.00
		Onions	0.00—1.84	0.01
Crop	Other Agriculture	Other.Hay.Non.Alfalfa	0.00—27.06	3.83
		Peppers	0.00—0.66	0.00
		Squash	0.00—0.07	0.00
		Sunflower	0.00—0.34	0.00
		Sweet.Potatoes	0.00—0.36	0.00
		Tobacco	0.00—5.63	0.06
		Tomatoes	0.00—0.01	0.00
		Watermelons	0.00—2.12	0.03
		Total (+Leguminous, Poaceous, and Corn)	0.03—31.64	8.28

Major habitat types	Major land-cover types	Diversity land-cover types	Peanut sites % area	
			Range	Mean
Non-crop/ seminatural		Total	7.98—95.13	56.10
	Seminatural	Total	0.49—35.69	11.73
		Barren	0.00—2.36	0.05
		Clover/wildflowers	0.00—0.05	0.00
		Fallow/idle cropland	0.00—28.68	3.47
		Grass/pasture	0.12—13.95	2.32
		Shrubland	0.06—33.88	5.90
	Forest	Total	3.06—65.37	24.87
		Deciduous forest	0.00—36.34	4.04
		Evergreen forest	0.68—56.12	20.00
		Mixed forest	0.00—6.16	0.83
	Wetlands		0.43—56.77	19.50
		Herbaceous wetlands	0.01—14.69	1.24
		Woody wetlands	0.20—56.55	18.27
Other		Total	1.04—37.44	6.37
	Developed		0.36—36.86	5.21
		Developed open space	0.27—19.01	3.76
		Developed low intensity	0.00—14.60	1.19
		Developed med intensity	0.00—4.00	0.20
		Developed high intensity	0.00—2.49	0.06
	Water	Total	0.00—14.21	0.73
		Open water	0.00—14.21	0.73
		Aquaculture	0.00—0.08	0.00
	Unknown	Unknown	0.30—0.61	0.44
	Total			100.00

CHAPTER 5

PANGAEUS BILINEATUS BEHAVIORAL RESPONSE TO LIGHT-BASED STIMULI IN THE LABORATORY

Aigner, B.L. and M.R. Abney. To be submitted to the *Journal of Entomological Science*

Abstract

Peanut burrower bug, *Pangaeus bilineatus* Say, is a subterranean hemipteran pest of peanut, *Arachis hypogaea* L., in the southeast US. Light traps and pitfall traps are the only tools currently available for monitoring the insect's populations in the field. Optimizing light traps by using the most attractive light source for the pest while minimizing non-target capture would enhance trap efficacy and could provide a more useful tool for IPM. We evaluated the responses of male and female adult *P. bilineatus* to various wavelengths of light in a laboratory-based, two-choice bioassay. White compact fluorescent (CFL) bulbs (warm, bright, and white) attracted significantly more males and females than black CFL and black light-emitting diode (LED) bulbs. White CFL wavelengths attracted significantly more male and female adults in the laboratory and should be candidate bulbs to enhance *P. bilineatus* light trap efficacy in a field setting.

Peanut burrower bug, *Pangaeus bilineatus* Say, is a hemipteran that is native to the US, spends much of its life in the soil, and is an economically important pest of peanut, *Arachis hypogaea* L., in the Southeast US (Aigner et al. 2021). It feeds directly on peanut seeds with piercing-sucking mouthparts causing substantial reduction in quality and value (Chapin et al. 2006; Mbata et al. 2013; Aigner et al. 2021). Its range in the US covers most southern and eastern states (Froeschner 1960; Aigner et al. 2021). The US state of Georgia produces about 50% of all peanuts grown in the country (USDA – NASS; Aigner et al. 2021). Since 2010, the pest has been an annual economic concern for peanut farmers in Georgia, Alabama, Florida, and South Carolina and, given its cryptic nature and a lack of available management tools, presents numerous management challenges. The cancellation of all food tolerances for chlorpyrifos in February of 2022 (Hites 2021) – the only insecticide registered for use in peanut with proven efficacy against *P. bilineatus* – exacerbates these challenges and emphasizes the need for new and improved integrated pest management (IPM) tactics and strategies.

Effective pest monitoring is the foundation of IPM, as it provides information about the presence and abundance of pest populations that is required for accurate and timely management decisions. Light traps are relatively simple monitoring tools that are often used for studies on population dynamics of insect species that exhibit positive phototaxis, and light traps have been used to monitor the distribution and seasonal abundance of *P. bilineatus* in Texas and Georgia (Highland and Lummus 1986, Aigner et al. 2021). Though the efficacy of light traps for collecting *P. bilineatus* was first shown by Highland and Lummus in 1986, there were no subsequent efforts to assess the insect's response to different wavelengths of light or to develop a more efficient light trap. Different insect orders are known to vary in their response to different wavelengths of light (Van Grunsven et al. 2014). Identifying a light source that is optimally attractive to the target insect while reducing unwanted bycatch would improve the quality of research data while lowering the costs associated with sorting large volumes of mixed species insect samples.

Given its cryptic, subterranean life history, directly assessing *P. bilineatus* populations in the field is difficult, and light traps could be a more efficient and cost-effective monitoring tool for IPM decision making than direct scouting. Currently, a lack of knowledge regarding economic injury levels and absence of thresholds for *P. bilineatus* in peanut, combined with only limited understanding of how trap capture relates to absolute pest abundance, reduces the utility of light traps for pest management decision making in commercial production systems (Aigner et al. 2021). Nevertheless, light traps have played an important role in IPM programs for a number of insect pests (Kim et al. 2019), and traps with improved energy efficiency and catch efficiency could play an important role in future IPM for *P. bilineatus*.

The availability of low-cost, high efficiency compact fluorescent bulbs (CFLs) and more recently light-emitting diodes (LED) has led to their use in newer light trap designs (Cohnstaedt et al. 2008). In spite of potential benefits of LED technology, some studies have shown that LEDs are not as attractive to insects as incandescent or fluorescent light sources (Kim et al. 2019, Justice and Justice 2016). In the work presented here, bioassays were conducted to assess the phototactic response of male and female adult *P. bilineatus* to various wavelengths of light emitted from CFL and LED bulbs in the laboratory and identify candidate light sources for future testing in the field.

Materials and Methods

Insect rearing.

The experiment was conducted using insects from a laboratory colony of *P. bilineatus* maintained at the University of Georgia in Tifton, GA. The colony was established with nymphs and adults collected in November 2016 from a field in Terrell County, Georgia, US (31.7929°, -84.3241°). Adults were also collected from Emanuel County, GA (32.60534°, -82.26979°) and added to the colony in December 2018. Both collection sites had been planted to peanut the summer prior to being made.

Plastic food storage containers (27.5 × 25.5 × 19.5 cm; MPN: FG631200CLR; Rubbermaid Commercial Products Inc., Winchester, VA, USA) were filled with approximately 10 L of sandy loam soil collected at the UGA Lang-Rigdon Farm (31.511239°, -83.549084°) in Tifton, GA. Containers were covered with a plastic lid. A single 16 × 8.5 cm hole was cut into the center of each lid to provide ventilation, and 1.5 mm cloth mesh screen was hot-glued over the hole. Bugs in the laboratory colony were fed raw, untreated peanut seed (3 – 7% moisture content), and soil in rearing containers was wetted with approximately 100 mL filtered tap water (Model Number: 56151-03; 3M Purification Inc., Meriden, CT, USA) twice per week (every 4th day or as needed). Soil moisture was maintained at 10-15% volumetric water content (VWC) as averaged over four measurements per container with a Field Scout TDR (time domain reflectometry) 300 Soil Moisture Probe (12.2 cm probe length; Model Number: 6430FS; Spectrum Technologies, Inc., Aurora, IL, USA). Roughly 1 peanut seed/6.5 cm^2 was placed on the soil surface in each container. Containers were kept in an insect rearing room maintained at 28°C ± 1.1°C, 14: 10 L: D cycle, and 55% ± 10% RH. WatchDog Data Loggers (1000/2000 Series Spectrum Technologies, Inc., Aurora, IL.) were used to monitor temperature and relative humidity in the rearing room. Populations were allowed to grow in each container for about 1.5 months before using adult bugs for bioassays. This ensured enough time for a new generation to appear so that healthy and relatively young adult bugs were used for Bioassays.

Three days prior to beginning bioassays, 3 rearing containers were moved into each of two controlled environment chambers (Model Number: I36LLVLC8; Percival Scientific Inc., Perry, IA, USA). It was shown that *P. bilineatus* adults were collected most frequently in light traps in the field within 3hr of sunset (Aigner et al. 2021). Therefore, the day (33 ± 1°C) and night (22 ± 1°C) temperature and light settings (14: 10, L:D) of the two environment chambers were adjusted and staggered such that the dark cycle began at either 11:00 am or 5:00 pm. Experiments conducted in the morning hours were run using bugs from the chamber with the dark cycle set to begin at 11:00 am, and experiments conducted in the afternoon were run using bugs from the chamber with the dark cycle set to begin at 5:00 pm. In each case, 10 bugs were collected from containers in the environment chamber using soft forceps; they were placed together in a Petri dish and allowed to rest for 10 minutes in a dark room prior to initiation of each replication of the experiment.

Light sources.

The relative spectral irradiance by wavelength of each light source was measured using a S2000 Miniature Fiber Optic Spectrometer and Ocean Optics SpectraSuite software (Ocean Optics, Inc., Dunedin, FL, USA). Each measurement was taken from a single scan over an integration time of .1 sec. The relative spectral irradiance by wavelength of each light source are shown in figs. 4.3a-k. Light sources used for the experiments included a black CFL (Mfg. #: 05645; Sunlite Mfg.; Brooklyn NY, USA), black LED (Mfg. #: GVLA19BK; Wal Mart, Inc.; Bentonville, AK, USA), warm white CFL (Mfr. Model: EL/mdTQ 23W T2 5k; Signify NA Corp.; Somerset, NJ, USA), bright white CFL (Mfr. Model: EL/mdTQ 23W T2 2.7k; Signify NA Corp.; Somerset, NJ, USA), cool white CFL (Mfr. Model: EL/mdTQ 23W T2 4.1k; Signify NA Corp.; Somerset, NJ, USA), and an adjustable wavelength LED bulb (ASIN: B07DLSNNDS; Lumiman, China). Specifications for each light bulb or bulb setting (in the case of the adjustable LED) are displayed in Table 4.1. The relative spectral irradiance and specifications of fluorescent circline light tubes (PN: GEL33774; General Electric Co., Cleveland, OH, USA) previously used in the field are displayed in table 4.1 and figs. 4.3 l-n, respectively.

Apparatus.

A light-based Y-tube apparatus was constructed from black foam core board (5 mm thick; Model #: PAC5554; Pacon Mfg.; Leland, NC, USA), clear poly-carbonate tubing (19 mm ID; 22mm OD; BULK-PT-PC-19; W.W. Grainger, Inc.; Macon, GA, USA), and PVC pipe fittings, and was adapted from the design described by Leskey et al. (2015). A fixture was constructed to allow light from two bulbs to be simultaneously, yet independently, directed through two gooseneck fiber optic cable attachments into one or the other side of the light-based Y-tube (Figs. 4.1a-b).

Experimental Design.

All experiments were conducted in the dark in a 3.7 m^2 windowless room at the University of Georgia Peanut Entomology laboratory in Tifton, GA with a temperature and relative humidity of 74.9 ± 1.4°F, and 37.8 ± 4.3%, respectively.

Each treatment replicate of the two-choice bioassay paired one of the experimental bulbs with either a black CFL or black LED light bulb. For each rep, 10 bugs were placed in the release chamber of the Y- tube and given 30 mins to make a choice. A 1 cm diameter hole at the lower surface of each polycarbonate tube located 14 cm from the release chamber allowed bugs that had made a choice to fall from the tube into a collection chamber. After 30 min, the Y-tube cover was removed, the number of bugs in the collection chambers was recorded, and the bugs were discarded. The relative location of the two bulbs was reversed after each rep to control for potential directional bias. Some bugs did not make a choice after 30 mins, and each treatment was replicated until at least 30 bugs made a choice.

Statistical Analysis.

Data were analyzed in R 4.0 (R Core Team 2020). Chi-square goodness of fit and tests of independence were used to identify treatment differences of count proportions within sex and between sex respectively. All analyses were conducted with a significance level of .05.

Results and Discussion

Significantly more males chose bright white LED light when tested against black CFL light ($X^2(1, n = 37) = 4.57$, $p = 0.0326$), and males chose black CFL light significantly more than green LED light ($X^2(1, n = 33) = 6.82$, $p = 0.0090$) (Table 4.1). In assays that included the black LED bulb, significantly more males chose warm white CFL ($X^2(1, n = 50) = 6.48$, $p = 0.0109$), cool white CFL ($X^2(1, n = 32) = 4.50$, $p = 0.0339$), and red LED light ($X^2(1, n = 30) = 4.80$, $p = 0.0285$) (Table 4.1). No other statistically significant differences in male response were observed.

In assays comparing black CFL light to cool white CFL light, significantly more females chose cool white CFL light ($X^2(1, n = 31) = 3.90$, $p = 0.0482$) (Table 4.1). In assays that included black LED light, significantly more females chose warm white CFL ($X^2(1, n = 35) =$

4.83, $p = 0.0280$) and cool white LED lights ($X^2(1, n = 34) = 7.53$, $p = 0.0061$) (Table 4.1). No other statistically significant differences in female response were observed.

Significant differences in phototaxis by sex were observed for comparisons of black LED light against cool white ($X^2(1, N = 73) = 5.50$, $p = 0.0191$; Table 4.3) and warm white LED lights ($X^2(1, N = 68) = 4.60$, $p = 0.0320$; Table 4.3). Males were more likely to choose black LED light, whereas females were more likely to choose warm white and cool white LED lights (Table 4.3; Fig 4.4a,b). The reason for these disparities is unknown, but reports of sex-based differences in phototaxis within an insect species are rare. Nevertheless, sexual dimorphism of insect eye structure with receptors of different spectral sensitivity is not uncommon (Briscoe and Chittka 2001).

Previous studies reported capture of *P. bilineatus* adults in light traps equipped with fluorescent bulbs producing white and black light (Highland and Lummus 1986; Abney and Aigner 2018), and black lights have been the standard of many insect trap designs for decades (Harding et al. 1966). Conventional fluorescent black light bulbs used in traps for research studies on *P. bilineatus* in Georgia resulted in collections with high biomass of non-target species (personal observation). This non-target bycatch decreasesoverall efficiency and increases the cost of research by increasing processing time. Concerns have also been raised about the indiscriminate attraction of insects to light sources in the environment, and the potential negative ecological effects this might have (Nabli et. al 1999). A significant reduction in the abundance and diversity of non-target insects collected in light traps was observed in GA when conventional white fluorescent light bulbs were used in place of conventional black fluorescent light bulbs in *P. bilineatus* population dynamics research (personal observation). Though *P. bilineatus* was collected in traps with either bulb, the relative phototactic response of the target insect to the two light sources is unknown.

The increased energy efficiency of CFL and LED bulbs compared to incandescent and conventional fluorescent bulbs could significantly decrease energy costs, extend run times, and facilitate the use of light traps at remote locations. Nevertheless, this will only be possible if the more efficient bulbs are adequately attractive to the target pest. The results presented here show that *P. bilineatus* exhibits positive phototaxis to UV and white light from CFL and LED sources. Overall, there were only minor differences in the attractiveness of paired light sources, but white light from LEDs or CFLs was generally more attractive than the UV light sources. This suggests that white lights could be expected to collect similar (or greater) numbers of *P. bilineatus* in the field while potentially reducing non-target captures compared with UV light. Field studies should be conducted to validate the findings reported here.

Acknowledgements

We thank the Georgia Peanut Commission, National Peanut Board, and USDA for funding this project. A special thanks to Zeke Culbreath for providing assistance in completing bioassays.

References

Abney, M.R., and B.L. Aigner. 2018. Spatial abundance and temporal flight activity of peanut burrower bug, *Pangaeus bilineatus*, in Georgia. Abstract. In *50th Proceedings of the American Peanut Research and Education Society, Inc.* Annual Meeting. July 10 – 12, 2018. Williamsburg, VA.

Aigner, B.L., M.S. Crossley, and M.R. Abney. 2021. Biology and Management of Peanut Burrower Bug (Hemiptera: Cydnidae) in Southeast US Peanut. *J. Integr. Pest Manag.* 12(1): 29; 1-8.

Briscoe, A.D., and L. Chittka. 2001. The evolution of color vision in insects. *Annu. Rev. Entomol.* 46(1): 471-510.

Chapin, J.W., and J.S. Thomas. 2003. Burrower bugs (Heteroptera: Cydnidae) in peanut: seasonal species abundance, tillage effects, grade reduction effects, insecticide efficacy, and management. *J. Econ. Entomol.* 96(4): 1142-1152.

Cohnstaedt, L.E.E., J.I. Gillen, and L.E. Munstermann. 2008. Light-emitting diode technology improves insect trapping. *J. Am. Mosq. Control Assoc.* 24(2): 331.

Harding Jr, W.C., J.G. Hartsock, and G.G. Rohwer. 1966. Blacklight trap standards for general insect surveys. *Bull. Ent. Soc. Amer.* 12(1): 31-32.

Highland, B.H., and P.F. Lummus. 1986. Use of light traps to monitor flight activity of the burrowing bug, *Pangaeus bilineatus* (Hemiptera: Cydnidae), and associated field infestations in peanuts. *J. Econ. Entomol.* 79(2): 523-526.

Hites, R.A. 2021. The rise and fall of chlorpyrifos in the United States. *Environ. Sci. Technol.* 55(3): 1354-1358.

Justice, M.J., and T.C. Justice. 2016. Attraction of Insects to Incandescent, Compact Fluorescent, Halogen, and Led Lamps in a Light Trap: Implications for Light Pollution and Urban Ecologies. *Entomol. News.* 125(5): 315-326, (1 March 2016). https://doi.org/10.3157/021.125.0502

Kim, K.N., Q.Y. Huang, and C.L. Lei. 2019. Advances in insect phototaxis and application to pest management: a review. *Pest Manag. Sci.* 75(12): 3135-3143.

Leskey, T.C., D.H. Lee, D.M. Glenn, and W.R. Morrison. 2015. Behavioral responses of the invasive Halyomorpha halys (Stål) (Hemiptera: Pentatomidae) to light-based stimuli in the laboratory and field. *J. Insect Behav.* 28: 674-692.

Park, J.H., and H.S. Lee. 2017. Phototactic behavioral response of agricultural insects and stored-product insects to light-emitting diodes (LEDs). *Appl. Biol. Chem.* 60: 137-144.

R Core Team. 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Van Grunsven, R.H.A., M. Donners, K. Boekee, I. Tichelaar, K.G. van Geffen, D. Groenendijk, F. Berendse, E.M. Veenendaal. 2014. Spectral composition of light sources and insect phototaxis, with an evaluation of existing spectral response models. *J. Insect Conserv*. 18: 225 – 231.

Table 4.1. Specifications of light bulbs used in *P. bilineatus* wavelength response bioassays.

	Bulb type	Wattage	Lumens	K (color temp)
(a)	Warm white CFL	23	1600	2700
(b)	Bright white CFL	23	1600	5000
(c)	Cool white CFL	23	1600	4100
(d)	Black CFL	20	15	2700
(e)	Black LED	7.5	800	2700
(f)	Red LED	7.5	800	5000
(g)	Blue LED	7.5	800	5000
(h)	Green LED	7.5	800	5000
(i)	Bright white LED	7.5	800	5000
(j)	Cool white LED	7.5	800	4100
(k)	Warm white LED	7.5	800	2700
(l)	Cool white fluorescent	22	1100	4100
(m)	Warm white fluorescent	22	1400	3000
(n)	Black circline fluorescent	22	NA	NA

Table. 4.2. Results of chi-square goodness of fit tests to identify significant differences of treatment decisions within sex for each treatment combination tested. An asterisk (*) denotes a significant p-value.

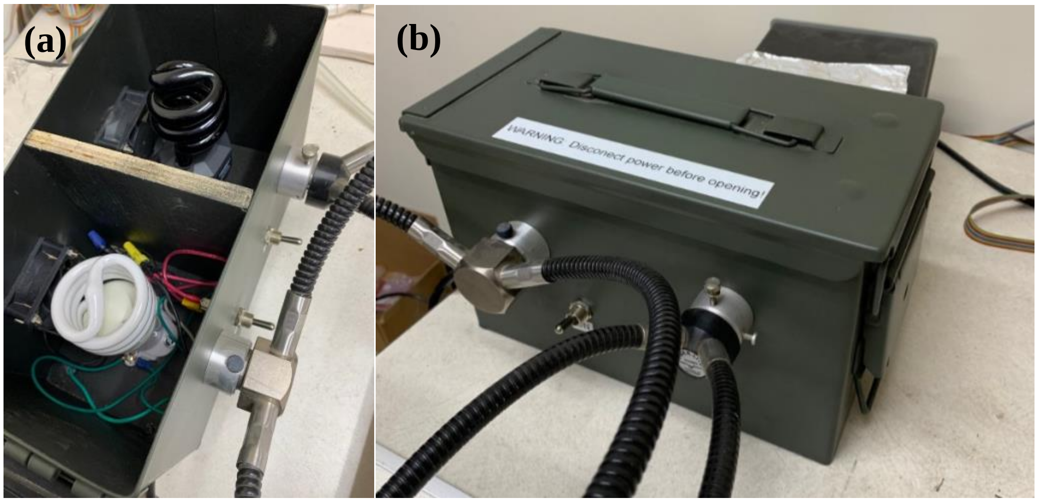
Bulb Type		**Males**				**Females**			
		X^2	*p*	*n*	*df*	X^2	*p*	*n*	*df*
Black CFL	WW CFL	0.50	0.4795	32	1	0.03	0.8618	33	1
	CW CFL	1.48	0.2230	33	1	3.90	**0.0482***	31	1
	BW CFL	0.53	0.4652	30	1	1.20	0.2733	30	1
	BW LED	4.57	**0.0326***	37	1	0.00	1.00	30	1
	WW LED	2.13	0.1441	30	1	0.81	0.3692	31	1
	CW LED	1.20	0.2733	30	1	0.50	0.4795	32	1
	Blue LED	3.67	0.0555	33	1	1.20	0.2733	30	1
	Green LED	6.82	**0.0090***	33	1	0.03	0.8618	33	1
	Red LED	2.61	0.1060	31	1	0.53	0.4652	30	1
Black LED	Blk CFL	0.13	0.7150	30	1	0.29	0.5900	31	1
	WW CFL	6.48	**0.0109***	50	1	4.83	**0.0280***	35	1
	CW CFL	4.50	**0.0339***	32	1	3.00	0.0833	27	1
	BW CFL	1.68	0.1944	38	1	1.88	0.1701	34	1
	BW LED	3.67	0.0555	33	1	0.29	0.5900	31	1
	WW LED	3.79	0.0516	38	1	2.13	0.1441	30	1
	CW LED	0.64	0.4200	39	1	7.53	**0.0061***	34	1
	Blue LED	3.46	0.0623	35	1	0.13	0.7150	30	1

Bulb Type		**Males**				**Females**			
		X^2	***p***	***n***	***df***	***X^2***	***p***	***n***	***df***
Black LED	Green LED	0.12	0.7316	34	1	0.13	0.7237	32	1
	Red LED	4.80	**0.0285***	30	1	0.00	1.00	34	1
	Black LED	0.00	1.00	30	1	0.00	1.00	22	1

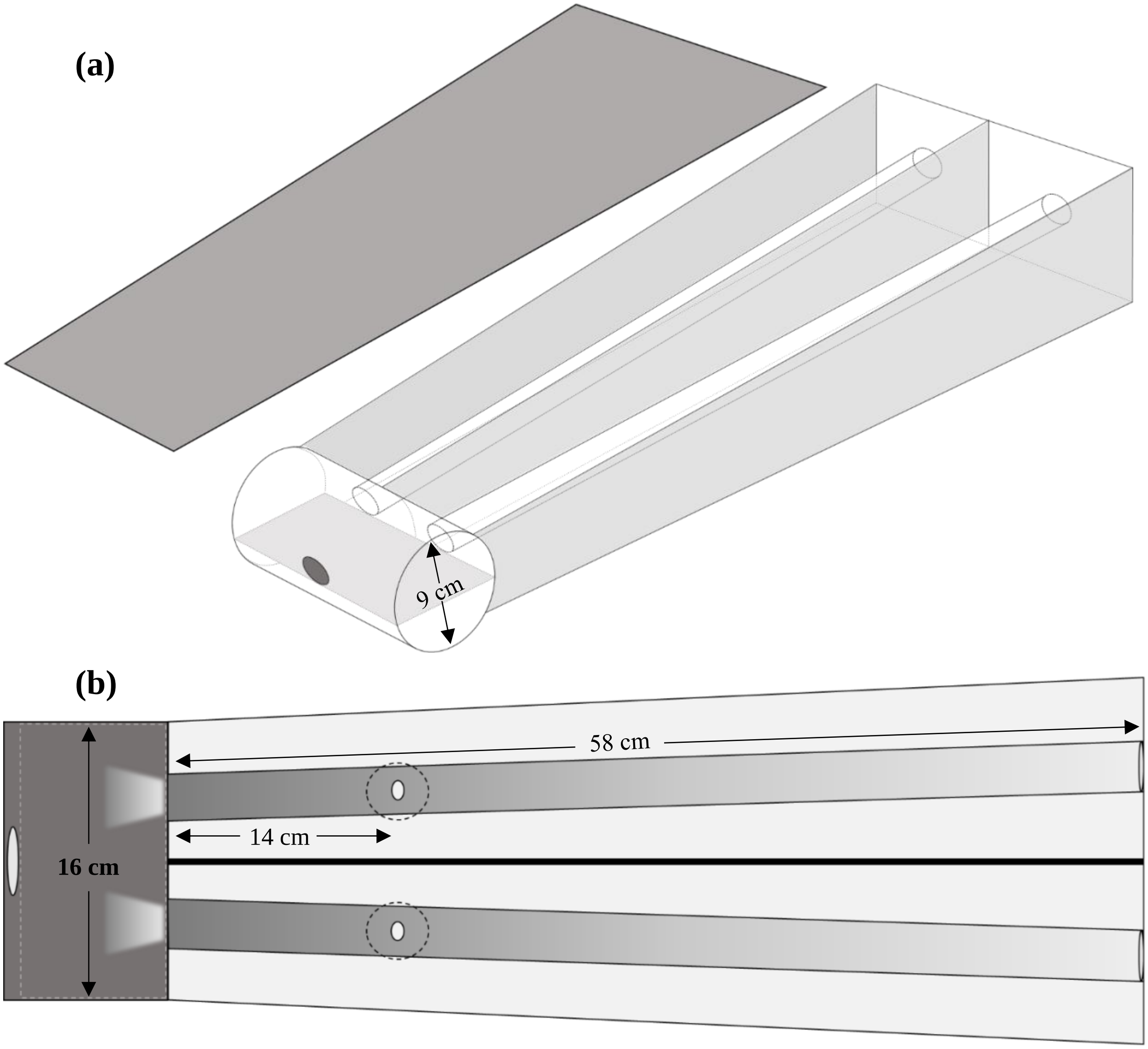
Table 4.3. Results of chi-square tests of independence to identify significant differences of treatment decisions between sex for each treatment combination tested. An asterisk (*) denotes a significant p-value.

			Males vs. Females		
Bulb type		***X* ^{2}**	***p***	***N***	***df***
Black CFL	WW CFL	0.0180	0.8934	65	1
	CW CFL	0.1115	0.7384	64	1
	BW CFL	0.00	1.00	60	1
	BW LED	1.4577	0.2273	67	1
	WW LED	2.0068	0.1566	61	1
	CW LED	0.0017	0.9669	62	1
	Blue LED	0.0823	0.7742	63	1
	Green LED	3.1096	0.0778	66	1
	Red LED	0.1334	0.7149	61	1
Black LED	Blk CFL	0.00	1.00	61	1
	WW CFL	0.00	1.00	85	1
	CW CFL	3.18e-31	1.00	59	1
	BW CFL	0.00	1.00	72	1
	BW LED	2.1936	0.1386	64	1
	WW LED	4.5982	**0.0320***	68	1
	CW LED	5.4951	**0.0191***	73	1
	Blue LED	1.6765	0.1954	65	1

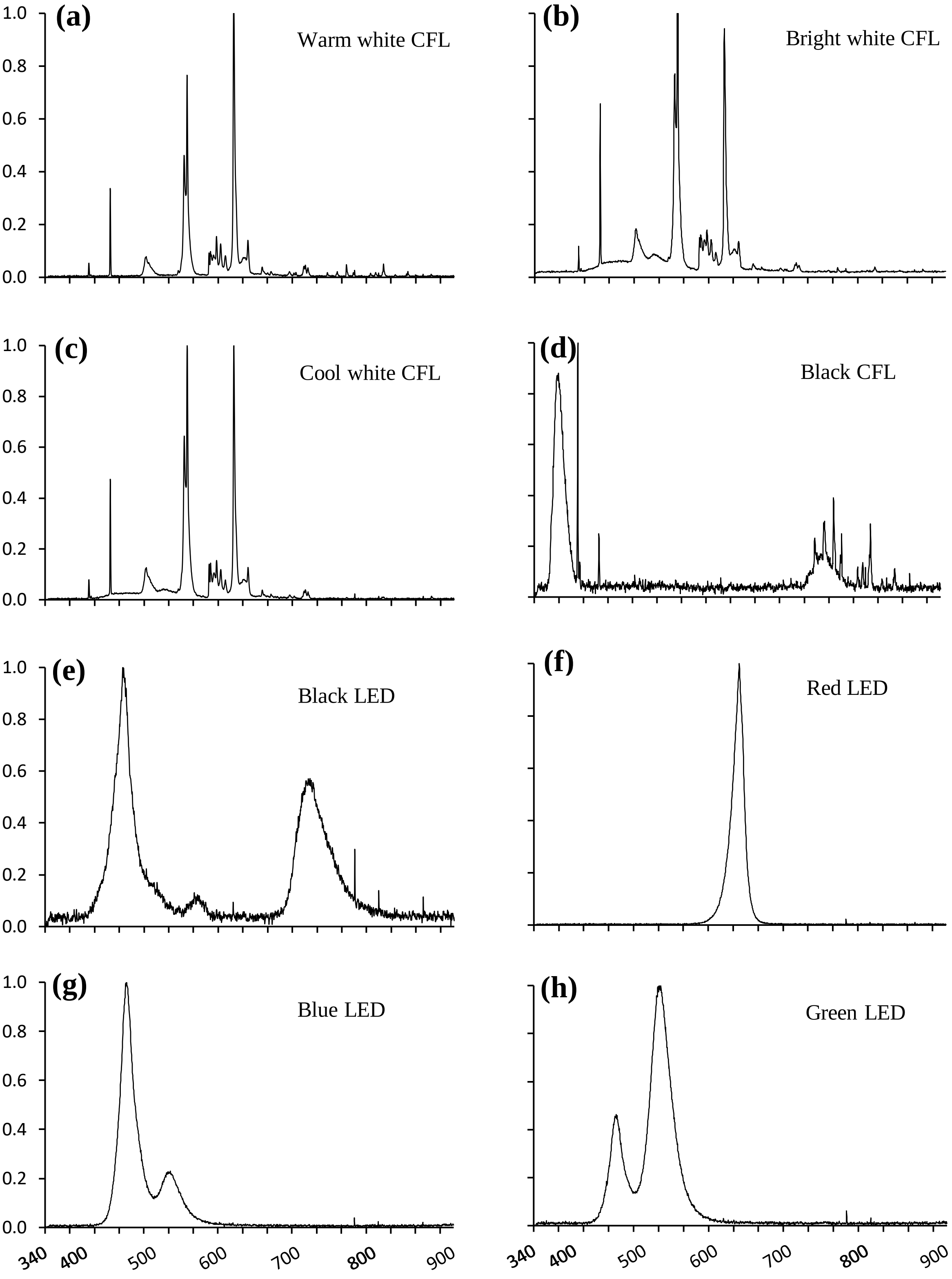
		Males vs. Females			
Bulb type		X^2	***p***	***N***	***df***
Black LED	Green LED	0.0607	0.8055	66	1
	Red LED	1.8788	0.1705	65	1
	Black LED	0.00	1.00	52	1



Figs. 4.1a-b. Interior (a) and exterior (b) of dual light fixture used for *P. bilineatus* wavelength response bioassays.

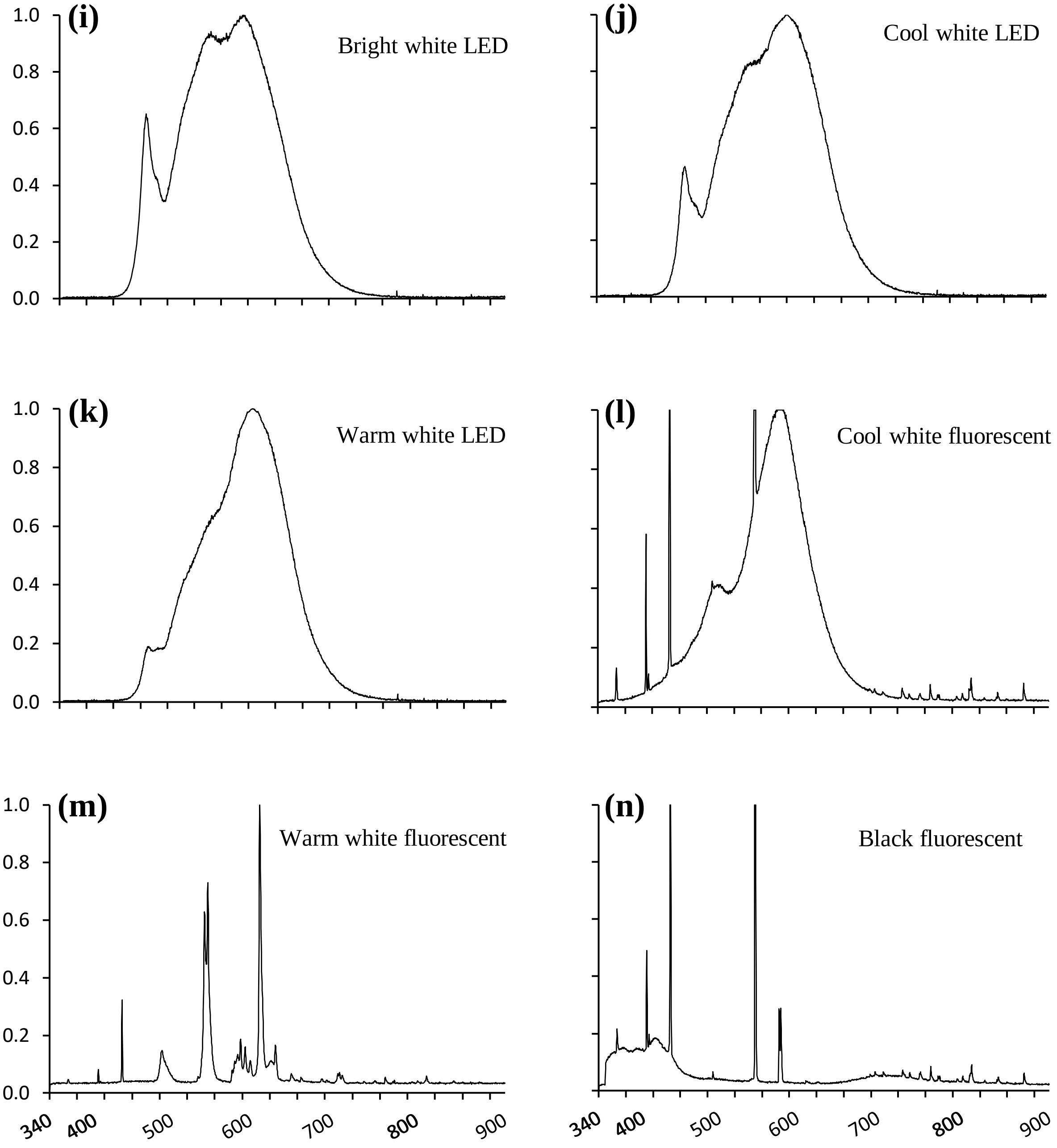


Figs. 4.2a-b. Illustrations (not to scale) of test chamber used for *P. bilineatus* wavelength response bioassays: a) oblique view of test chamber with lid, b) overhead view of test chamber. Holes were drilled into the bottom of each treatment tube (14 cm from stage area) to trap bugs after they made a choice.

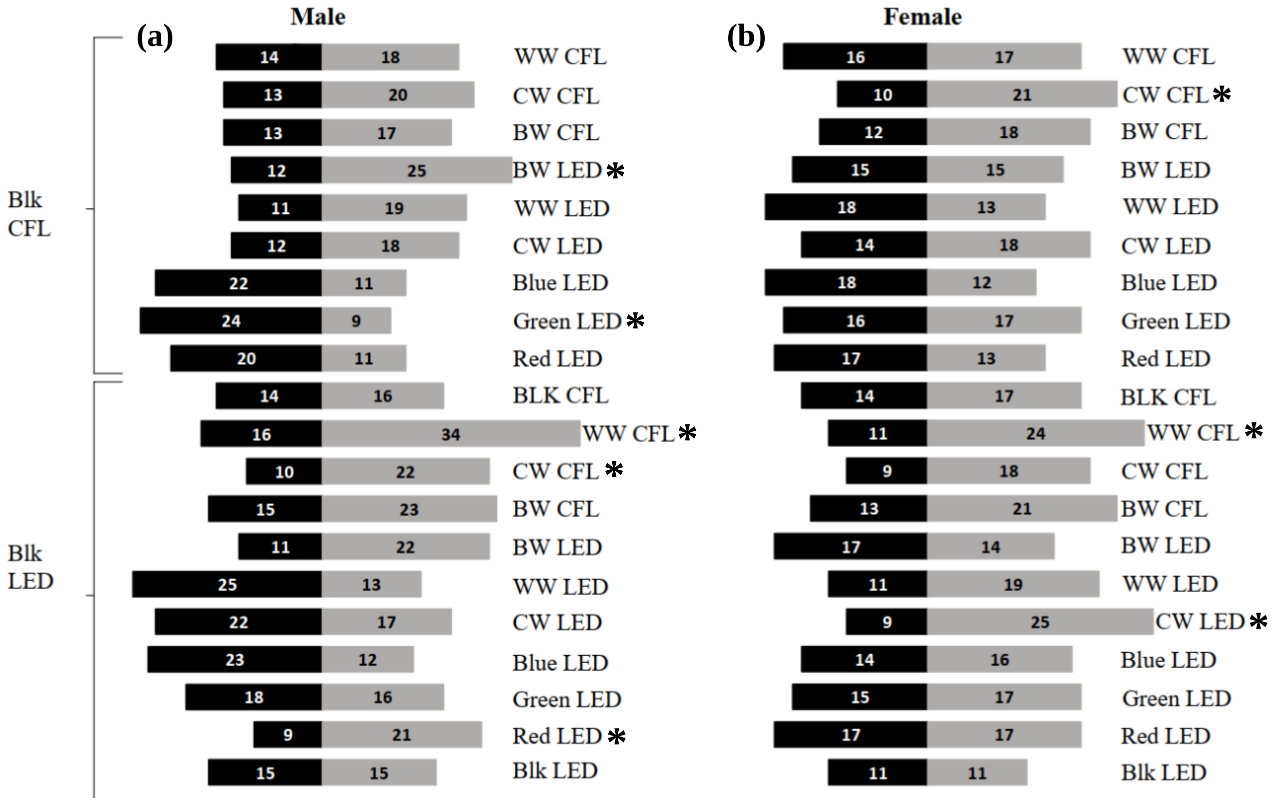


Figs. 4.3a-h. Relative spectral irradiance (*y*; a.u.) by wavelength (*x*; nm) of light treatments

used in *P. bilineatus* wavelength response bioassays. Treatments include: (a) warm white CFL, (b) bright white CFL, (c) cool white CFL, (d) black CFL, (e) black LED, (f) red LED, (g) blue LED, and (h) green LED.



Figs. 4.3i-n. Relative spectral irradiance (*y*; a.u.) by wavelength (*x*; nm) of light treatments used in *P. bilineatus* wavelength response bioassays. Additional treatments include: (i) bright white LED, (j) cool white LED, and (k) warm white LED. Relative spectral irradiance of circline fluorescent light tubes currently used in the field include: (l) cool white fluorescent, (m) warm white fluorescent, and (n) black fluorescent.



Figs. 4.4a-b. Counts of *P. bilineatus* male (a) and female (b) responses to wavelength choice bioassays. All treatments were tested against either black CFL or black LED light bulbs. An asterisk (*) symbol denotes significant differences in chi-square results.

CHAPTER 6

SUMMARY

The breadth of novel information in this dissertation provides a foundation for future research towards the development of an IPM strategy for managing *P. bilineatus* in peanut. The expansion of knowledge regarding *P. bilineatus* biology, behavioral ecology, landscape ecology, abiotic environmental influences, and adult response to specific wavelengths of light all contribute vital information that is required to enhance our ability to manage this pest.

The results presented in Chapter 3 experiments reveal the reproductive and population growth potential of *P. bilineatus* under constant conditions. These studies provide a framework for future studies to identify optimal conditions for reproduction and development. Modeling the reproductive potential of field populations could improve targeting of management tactics by informing when populations are most likely to burgeon to economically important levels. Additionally, the ability to predict life stage by head capsule width allows for sampling populations and accurate assessment of seasonal population dynamics. This ability to predict occurrence of new generations and/or cohorts of vulnerable life stages is fundamental to peanut farmers' timing of potential chemical, cultural, and/or biological interventions to prevent later generations from damaging maturing peanut seed. The experiments of *P. bilineatus* behavioral ecology were the only curiosity driven aspect of this research project, and should springboard further investigation into female adults' role in nymph development.

Influences of landscape complexity (composition and configuration) and abiotic environmental factors on *P. bilineatus* peanut injury are described in Chapter 4. Understanding these effects is critical to recognize why *P. bilineatus* proliferates in some areas of the agricultural landscape and not others. With the risk model presented here, we can predict high risk areas where the pest is most likely to occur. Although management tools remain limited, farmers may avoid planting peanut in high risk fields or designate “high risk” peanuts for separation from “low risk” peanuts at harvest. This could prevent contamination of high quality product and further financial loss.

Finally, optimization of light traps is an important step towards improving our monitoring capability for *P. bilineatus*. Adults’ positive response to wavelengths emitted from white compact fluorescent light bulbs provide a basis for future field testing of these light sources found to be effective in the laboratory. Improving the capture capability of light traps can support more accurate estimation of field populations, and increase trap efficiency (e.g., reduce processing time, energy demand, etc.) and versatility (e.g., use at remote locations).

Although there is much to learn of *P. bilineatus* biology, ecology, and management, this work encompasses significant developments towards the ultimate goal of managing this pest and reducing the economic burden it imposes to peanut farmers.