

PROSPECTS FOR NATURAL BIOLOGICAL CONTROL OF SUGARCANE APHID,  
*Melanaphis sacchari* (ZEHNTNER) (HEMIPTERA: APHIDIDAE) ON SORGHUM

by

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(Under direction of Professor JP Michaud - KSU and Jorge Braz Torres – UFRPE)

ABSTRACT

The sugarcane aphid, *Melanaphis sacchari* (Zehntner) (Hemiptera: Aphididae) is an important sugarcane and sorghum pest worldwide. To date, the economic impact of *M. sacchari* in Brazil is still restricted to sugarcane; however, both sorghum and sugarcane production have been expanding, especially in the Midwest region. Until recently, the sugarcane aphid was also restricted to sugarcane in the United States; however, outbreaks have been reported on sorghum fields since 2013. It is possible that in the near future we will face a similar host range expansion by *M. sacchari* in Brazil. The most abundant natural enemies observed attacking aphids in central Kansas are common in our agroecosystem; thus, the results of the present work could also prove valuable here. This work was undertaken to assist the development of sustainable pest management strategies for *M. sacchari* in the USA, focusing on biological control. A significant question in biological control is the extent to which indigenous natural enemies might be pre-adapted to exploit invasive species that constitute novel prey. We compared the recruitment of natural enemies to sorghum plants infested with either *M. sacchari*, a newly invasive aphid in USA, or *Schizaphis graminum* (Rondani) (Hemiptera: Aphididae), an established pest. Furthermore, we compared the developmental and reproductive performance of the four most abundant predator species observed in the field, feeding on both aphid species. Sorghum infested

with *M. sacchari* attracted the same guild of natural enemies as *S. graminum* and had similar biological control outcomes in the field. The four predator species; *Hippodamia convergens* Guérin-Ménéville (Coleoptera: Coccinellidae), *Coleomegilla maculata* De Geer (Coleoptera: Coccinellidae), *Chrysoperla carnea* Stephens (Neuroptera: Chrysopidae), *Orius insidiosus* (Say) (Hemiptera: Anthocoridae); exhibited similar performance on both prey species.

KEY WORDS: Introduced pest, predator-prey interaction, *Chrysoperla carnea*, *Coleomegilla maculata*, *Hippodamia convergens*, *Orius insidiosus*.

POTENCIAL DE CONTROLE BIOLÓGICO NATURAL DO PULGÃO DA CANA-DE-  
AÇÚCAR, *Melanaphis sacchari* (ZEHTNER) (HEMIPTERA: APHIDIDAE) EM SORGO

por

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(Sob Orientação dos professores JP Michaud - KSU e Jorge Braz Torres – UFRPE)

RESUMO

O pulgão-da-cana, *Melanaphis sacchari* (Zehntner) (Hemiptera: Aphididae) é uma importante praga da cana e do sorgo em todo o mundo. Até o momento, o impacto econômico de *M. sacchari* no Brasil ainda está restrito à cana; porém, tanto o cultivo de sorgo quanto o de cana têm se expandido, especialmente na região Centro-Oeste. Até recentemente, a ocorrência do pulgão-da-cana também era restrita à cana-de-açúcar nos Estados Unidos; no entanto, surtos têm sido relatados em campos de sorgo desde 2013. É possível que no futuro nós enfrentemos uma expansão semelhante da gama de hospedeiros por *M. sacchari* no Brasil. Os inimigos naturais mais abundantes observados atacando pulgões no Kansas são comuns em nosso agroecossistema; assim, os resultados do presente trabalho poderão ser válidos em nosso país. O trabalho foi realizado visando o desenvolvimento de práticas sustentáveis de manejo de pragas para *M. sacchari* com foco no controle biológico. Uma questão importante no controle biológico é o quanto inimigos naturais nativos podem ser pré-adaptados para explorar espécies invasivas, as quais constituem novas presas. Nós comparamos o recrutamento de inimigos naturais por plantas de sorgo infestadas com *M. sacchari*, espécie invasora no sorgo nos Estados Unidos, ou *Schizaphis graminum* (Rondani), uma praga estabelecida. Além disso, comparamos o desenvolvimento e reprodução das quatro espécies de predadores mais abundantes observados no

campo, quando alimentadas com *M. Sacchari* ou *S. graminum*. Plantas de sorgo infestadas com *M. sacchari* atraiu a mesma guilda de inimigos naturais que plantas infestadas com *S. graminum*, exibindo similar controle biológico no campo. As quatro espécies de predadores apresentaram desempenho semelhante em ambas as espécies de presas.

PALAVRAS-CHAVE: Praga exótica, interação predador-presa, *Chrysoperla carnea*, *Coleomegilla maculata*, *Hippodamia convergens*, *Orius insidiosus*.

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## DEDICATION

*I dedicate this thesis to my family, my brother and my sisters, especially to my parents for all support and encouragement given during my entire academic life. Hopefully one day I can make for my kids half of what you made for me. Love you!*



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# CHAPTER 1

## INTRODUCTION

### **Sorghum and sugarcane expansion in Brazil - implications on IPM**

Production of sorghum [*Sorghum bicolor* (L.) Moench] in Brazil began to expand in the 1970's, with an annual production of around 450,000 tons of grain over about 200,000 hectares from 1973 to 1977, of which 80% was concentrated in the states of Rio Grande do Sul and São Paulo (Sharffert *et al.* 1978). Currently, sorghum is cultivated practically all over the country; however, the contribution of each region has changed over the last 30 years. Whilst the South has seen an 80% reduction in area and production, the Midwest region has observed a 70% increase (Coelho *et al.* 2002). For example, in the last season, the cultivated area in Brazil exceeded 720,000 hectares, producing around 2,000,000 tons, with 70% of this area and 90% of the production concentrated in the states of Goiás, Minas Gerais and Mato Grosso (CONAB 2015a).

Another crop that has been expanded in the Midwest region is the sugarcane [*Saccharum officinarum* (L.)], especially in the state of Goiás (main sorghum producer in Brazil), which exhibited an increase of 34% in the cultivated area over the past five years (CONAB 2011, 2015b). The expansion of both crops in the same region might have some implications on the pest dynamics. Sorghum is usually an off-season crop, seeded around the time the sugarcane is being harvested in the Midwest (Teixeira *et al.* 1997); so, pests that are able to explore both host plants can have resources available throughout the year. The sugarcane borer (*Diatraea* spp.) and the lesser cornstalk borer [*Elasmopalpus lignosellus* (Zeller)] for example are species already known

to occur on both sorghum and sugarcane in Brazil and the sugarcane borer is one of the main causes of losses in sorghum production in the Midwest region (Coelho *et al.* 2002).

Another potential threat to sorghum associated to the sugarcane is the sugarcane aphid, *Melanaphis sacchari* (Zehntner) (Hemiptera: Aphididae). To date, the economic impact of *M. sacchari* in Brazil is still restricted to sugarcane, where it causes considerable damage only as a vector *Sugarcane yellow leaf virus* (ScYLV) (Gonçalves 2005). However, Lopes-da-silva *et al.* (2014) comparing the development and reproduction of sugarcane aphid in Brazil has already shown that sweet sorghum is more suitable than sugarcane for the population growth of this pest. Furthermore, it is one of the most important pests on sorghum in several countries, such as in China, Taiwan, Japan, India, South Africa and Botswana (Singh *et al.* 2004) and recently, in the United States (Villanueva *et al.* 2014, Armstrong *et al.* 2015, Elliott *et al.* 2015).

### **Sugarcane aphid host range and identification**

The sugarcane aphid has been reported to use host plants from five different genera (*Saccharum*, *Sorghum*, *Oryza*, *Panicum*, and *Pennisetum*) in the family Poaceae (Singh *et al.* 2004). It is an important pest on sugarcane [*Saccharum officinarum* (L.)] and sorghum [(*Sorghum bicolor* (L.) Moench)] (Nibouche *et al.* 2014), distributed throughout all countries where these crops are cultivated (Singh *et al.* 2004) and it is also considered the main vector of ScYLV (Schenck & Lehrer 2000, Singh *et al.* 2004, Paray *et al.* 2011). The sugarcane aphid was originally described as *Aphis sacchari* (Zehntner) and individuals from sugarcane and sorghum were considered different species, *M. sacchari* and *Melanaphis sorghi* (Theobald), respectively (Blackman & Eastop 2006). More recent work has shown that genetic variation in *M. sacchari* populations is not related to host plant use (Nibouche *et al.* 2014). The authors analyzed 57 samples from 42 localities in 15 countries and all clones appeared able to use the full range of

host plants, indicating that *M. sorghi* is a synonym for *M. sacchari*, and not a separate species. Aphids of the genera *Melanaphis* are small, with a broadly oval body and short cornicles (Blackman *et al.* 1990), with *M. sacchari* presenting a light yellow to gray color, with dark cornicles and tarsi that contrast with the remainder of the body (Brown *et al.* 2015). It can be distinguished from the greenbug, *Schizaphis graminum* (Rondani), by the absence of a longitudinal dark line that appears on the back of the latter species, and from all other aphids that commonly occur in sorghum by the short and dark cornicles (Brown *et al.* 2015).

### **Biology and damage of *Melanaphis sacchari***

Lopes-da-Silva *et al.* (2014) compared the suitability of sugarcane and sorghum for *M. sacchari* development and reproduction at  $24 \pm 1$  °C with 70% RH and 14:10 L:D photoperiod. The developmental period (from birth to adult) is around nine days on both sorghum and sugarcane, but the reproductive period is longer on sorghum than on sugarcane, with a larger number of nymphs produced. Females produced an average of 26 and 17 nymphs in 11 and seven days, with longevity of 20 and 17 days on sorghum and sugarcane, respectively. Reproduction is exclusively thelytokous (Nibouche *et al.* 2014, Brown *et al.* 2015) with only one record of sexual reproduction in India (David & Sandhu 1976). The nymphs and adults are usually wingless, the development of winged adults can be stimulated under stressful conditions, such as plant deterioration, excess of individuals in the colony or adverse environmental conditions (Brown *et al.* 2015). The population growth rate is highly temperature-dependent, being highest with the diurnal temperature variation from 11.4 to 30.0 °C in the morning and afternoon, respectively, and the population declines at 35 °C (Singh *et al.* 2004).

Plant damage can be direct, through the removal of sap, and indirect, by the excretion of honeydew and transmission of viruses (Brown *et al.* 2015, Singh *et al.* 2004). The aphids suck the

sap from plants, resulting in wilting/curling of leaves and chlorosis. Furthermore, the accumulation of honeydew and consequent growth of sooty mold can reduce the photosynthetic capacity of the plant, leading to reductions in growth and productivity (Singh *et al.* 2004). Infestations begin at the bottom of the plant, the colonies usually located on abaxial leaf surfaces; early or heavy infestations can kill the plants (Singh *et al.* 2004, Brown *et al.* 2015). Feeding typically results in reddish or purplish coloring of leaf tissue with subsequent necrosis, reduction of seed number and, under heavy aphid pressure, sterilization of seed heads (Chang & Fang 1984, van den Berg *et al.* 2003, Singh *et al.* 2004, Brown *et al.* 2015). Sorghum yield losses can vary from minor to severe depending, among other factors, on plant moisture stress, plant growth stage when the infestation occurs, and the intensity and duration of infestation (Singh *et al.* 2004). If the infestation occurs under drought conditions, the two factors together can have a synergistic interaction that intensifies yield reduction (Singh *et al.* 2004).

### **Management tools for *Melanaphis sacchari***

Management of the sugarcane aphid on sorghum requires the integration of various tactics, including host plant resistance, cultural control, biological control and, in extreme situations when outbreaks occur, insecticide sprays. Plant breeding for resistance to the sugarcane aphid seems to be the most studied approach (Singh *et al.* 2004, Armstrong *et al.* 2015, Brown *et al.* 2015). Several germplasm accessions, parental lines, experimental varieties and hybrids of sorghum have been reported as resistant to *M. sacchari* in different countries (Singh *et al.* 2004, Armstrong *et al.* 2015, Brown *et al.* 2015). Armstrong *et al.* (2015) classified the parental lines RTx2783, B11070, B11070, SC110 and SC170 as highly resistant to sugarcane aphid, as well as the hybrids AB11055-WF1-CS1/RTx436, and AB11055-WF1-CS1/RTx437. Early planting is another strategy that can help avoid early infestations (Brown *et al.* 2015). Despite a lack of studies on



interactions between the sugarcane aphid and weeds and native vegetation, the destruction of alternate host plants such as johnsongrass, *Sorghum halapense* (L.) Pers., before planting can reduce the risk of early infestation (Singh *et al.* 2004). For example, the johnsongrass, a common sub-tropical weed species, was observed supporting high numbers of sugarcane aphid while suffering only moderate chlorosis (Armstrong *et al.* 2015). It is considered to be a good wild host for *M. sacchari* and might serve as a reservoir of the pest early in the season if not controlled.

The impact of the biological control on sugarcane aphid populations in sorghum fields has not been well quantified and may have been underestimated. Singh *et al.* (2004) listed over 47 natural enemy species associated with *M. sacchari* in different countries, including pathogens, parasitoids and predators. Among these natural enemies, coccinellids, chrysopids and syrphids are the most frequently observed and the ones that probably contribute most *M. sacchari* mortality (Singh *et al.* 2004). In central Kansas, *Hippodamia convergens* Guérin-Ménéville is the most important coccinellid naturally controlling aphids on grain crops (Rice & Wilde 1988, Nechols & Harvey 1998, Michels *et al.* 2001). Parasitoids from the families Aphidiidae, Aphelinidae, Elasmidae and Braconidae have also been recorded attacking *M. sacchari*, but in general, at lower levels compared to predators (Singh *et al.* 2004). The sugarcane aphid may sometimes benefit from a symbiotic association (Singh *et al.* 2004). *Hamiltonella defensa* is a secondary endosymbiont known to protect aphids from parasitism by aphidiid wasps (Vorburger *et al.*, 2009). In regions where this wasp is a key mortality factor controlling aphids in sorghum, this symbiotic association could be an impediment to biological control.

When aphid outbreaks occur on sorghum, chemical controls are an important tool to control infestations. Neonicotinoids have been recommended for seed treatment to prevent early infestation of seedlings when the risk of infestation is high (Brown *et al.* 2015). Insecticide sprays may be required during the growing season, but should be applied only when necessary. The

LSU AgCenter and Texas A&M Agrilife have set a preliminary threshold on boot/milk stage grain sorghum of 50 aphids per leaf colonizing 20 percent of plants in the field (Brown *et al.* 2015). The insecticides currently recommended for control of sugarcane aphid on sorghum in the United States are Transform 50 WG (75-115 mL/ha), Sivanto (210-315 mL/ha) and Chlorpyrifos (1.2-2.4 L/ha), which have 30-60 day pre-harvest intervals (Brown *et al.* 2015). The minimum volume recommended per area is 50-100 L per hectare, by air and by ground, respectively (Brown *et al.* 2015).

### **Current situation in the United States – Parallel between USA and Brazil**

In Brazil, aphids are generally not considered a problem in sorghum; currently, there are two aphid species known to attack sorghum, the greenbug [*Schizaphis graminum* (Rondani) (Hemiptera: Aphididae)] and the corn leaf aphid [*Rhopalosiphum maidis* (Fitch) (Hemiptera: Aphididae)], both are usually controlled by the action of natural enemies and rainfall (Coelho *et al.* 2002, Waquil *et al.* 2003). The presence of the corn leaf aphid is even considered beneficial, since it does not cause significant damage and acts as a food source, helping to sustain natural enemies in the area (Waquil *et al.* 2003). A similar situation could be observed in the United States before the invasion of the sugarcane aphid to sorghum fields. The sugarcane aphid was first reported in North America on sugarcane in Florida in 1977 (Mead 1978, Summers 1978, Denmark 1988), followed by Louisiana on the same crop (White *et al.* 2001). According to Nibouche *et al.* (2014), data strongly suggest that it was introduced to the continental USA from Hawaii, probably on infested ratoon material. The status of *M. sacchari* as an economic pest of sugarcane in the United States is still unclear, with only secondary damage recorded, such as the occurrence of sooty mold on the honeydew excreted by the aphid (White *et al.* 2001, Armstrong *et al.* 2015) and its role as the main vector of ScYLV (Schenck & Lehrer 2000, Singh *et al.* 2004, Akbar *et al.* 2009). However, during the summer of 2013, an outbreak of the sugarcane aphid was

detected in sorghum fields throughout the Rio Grande Valley, TX, and across the border into Tamaulipas, Mexico (Villanueva et al., 2014). In 2014, it expanded to the east and northeast, with sorghum infested in northern Texas, southern Oklahoma, Louisiana, Mississippi, Arkansas and Tennessee (Armstrong *et al.* 2015, Elliott *et al.* 2015).

The increased prevalence of the sugarcane aphid in sorghum in the United States provides a template that Brazil can use as a model, since this pest remains restricted to sugarcane in Brazil but may shift to sorghum as has been observed in the United States. This threat of a possible host range expansion by *M. sacchari* highlights the importance of the present study, which was undertaken to assist the development of sustainable pest management strategies for *M. sacchari* in the United States, providing results that could also prove valuable in Brazil.

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## CHAPTER 2

RECRUITMENT OF APHIDOPHAGOUS ARTHROPODS TO SORGHUM PLANTS  
INFESTED WITH *Melanaphis sacchari* (ZEHNTNER) AND *Schizaphis graminum* (RONDANI)  
(HEMIPTERA: APHIDIDAE)<sup>1</sup>

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<sup>1</sup>Colares, F., J.P. Michaud, C.L. Bain & J.B. Torres. 2015. Recruitment of aphidophagous arthropods to sorghum plants infested with *Melanaphis sacchari* (Zehntner) and *Schizaphis graminum* (Rondani) (Hemiptera: Aphididae). *Biological Control* 90: 16-24.

ABSTRACT – A significant question in biological control is the extent to which indigenous natural enemies might be pre-adapted to exploit invasive species that constitute novel prey. We observed the recruitment of natural enemies to aphid microcosms – sorghum plants infested with either *Melanaphis sacchari* (Zehntner), a newly invasive aphid, or *Schizaphis graminum* (Rondani), an established pest. The first cohort was monitored in open habitat along a tree line near parkland and urban plantings, and the second, within a sorghum monoculture. Both aphid species were eliminated by natural enemies within 13 days in the first cohort, but in the second, *M. sacchari* reached higher numbers than *S. graminum* and survived a week longer. Biological control was successful in both cases; neither aphid produced a generation of alates, nor did plants sustain significant damage. Syrphid larvae, primarily *Allograpta obliqua* (Say), caused most aphid mortality in the first cohort, whereas adult Coccinellidae, primarily *Hippodamia convergens* Guerin-Meneville, caused most mortality in the second. *Chrysoperla carnea* Stephens was present in both cohorts, flower flies and velvet mites only in the first, and flower bugs, in the second. *Aphelinus* sp. successfully parasitized both aphids, but *Lysiphlebus testaceipes* Cresson did not develop in *M. sacchari* due to their infection with the secondary endosymbiont *Hamiltonella defensa* Moran. Thus, sorghum infested with *M. sacchari* attracted the same guild of natural enemies as *S. graminum* and had similar biological control outcomes. The findings suggest that the capacity of indigenous aphidophagous guilds to respond to, and ultimately control, invasive aphid species may be underestimated.

KEY WORDS: *Allograpta obliqua*, *Aphelinus* sp., *Chrysoperla carnea*, *Coleomegilla maculata*, *Erythraeus aphidivorous*, *Hippodamia convergens*, *Lysiphlebus testaceipes*



RECRUTAMENTO DE ARTRÓPODOS AFIDÓFAGOS POR PLANTAS DE SORGO  
INFESTADAS COM *Melanaphis sacchari* (ZEHNTNER) E *Schizaphis graminum* (RONDANI)  
(HEMIPTERA: APHIDIDAE)

RESUMO – Uma importante questão no controle biológico é o quanto inimigos naturais nativos podem ser pré-adaptados para explorar espécies invasivas como novas presas. O recrutamento de inimigos naturais foi observado em plantas de sorgo infestadas com *Melanaphis sacchari* (Zehntner), espécie exótica no sorgo, ou *Schizaphis graminum* (Rondani), espécie já estabelecida. Um experimento foi feito em habitat aberto ao longo de uma linha de árvores próximo a um parque, e o outro, dentro de uma monocultura de sorgo. Ambas as espécies de pulgões foram controladas por inimigos naturais em 13 dias no primeiro experimento, mas no segundo, as colônias de *M. sacchari* atingiram números de indivíduos maiores do que *S. graminum* e sobreviveram uma semana a mais. O controle biológico foi bem sucedido em ambos os casos; nenhuma das espécies de pulgão produziu uma geração de alados. Larvas de sirfídeos, principalmente, *Allograpta obliqua* (Say), e adultos de Coccinellidae, principalmente *Hippodamia convergens* Guérin-Méneville, causaram maior mortalidade de pulgões no primeiro e segundo experimento, respectivamente. Sirfídeos e ácaros predadores ocorreram apenas no primeiro experimento e *Orius insidiosus* (Say), apenas no segundo. *Aphelinus* sp. parasitou com sucesso ambos os pulgões, enquanto *Lysiphlebus testaceipes* Cresson não se desenvolveu em *M. sacchari* devido à sua infecção com o endosimbionte *Hamiltonella defensa* Moran. Plantas infestadas com *M. sacchari* atraíram inimigos naturais e tiveram controle biológico similar a *S. graminum*. Os resultados sugerem que afidófagos nativos são atraídos para espécies invasoras e que o potencial de controle biológico pode estar sendo subestimado.

PALAVRAS CHAVE: *Allograpta obliqua*, *Aphelinus* sp., *Chrysoperla carnea*, *Coleomegilla maculata*, *Erythraeus aphidivorous*, *Hippodamia convergens*, *Lysiphlebus testaceipes*

## Introduction

Invasions of exotic agricultural pests have become more frequent with increasing international air travel and the globalization of commerce. Economically damaging outbreaks of invasive pests typically occur during the first few years after their introduction, often leading some to the conclusion that exotic natural enemies will be required to provide biological control. The implicit assumption is that existing guilds of natural enemies will be insufficient, either because they lack specific adaptations to exploit the new pest, or because key niches are unoccupied (e.g., no specialized parasitoid is present). While this may be true in some cases, the preadaptations of many indigenous predators and parasitoids to utilize a new prey/host may be often underestimated, or these species may simply require a period of evolutionary adaptation to achieve their full potential as biological control agents. Aphids are a case in point, as they are vulnerable insects that feed in exposed locations and suffer attack from a broad guild of natural enemies. The taxa that are primarily or exclusively aphidophagous (e.g., Coccinellidae, Syrphidae, Chrysopidae, Braconidae, Aphelinidae) are ubiquitous in agroecosystems worldwide, even though local species composition varies. The present study was conducted to test the hypothesis that natural enemies of aphids in cereal crops on the High Plains possess substantial preadaptations for exploiting a novel aphid pest.

The sugarcane aphid, *Melanaphis sacchari* (Zehntner) (Hemiptera: Aphididae) is a cosmopolitan pest of sugarcane and sorghum capable of attacking a relatively broad range of host plants in the family Poaceae with economic impacts that vary from benign to devastating (Singh *et al.* 2004). Originally described as *Aphis sacchari* (Zehntner) from specimens collected on sugarcane in Java, Indonesia (Zehntner 1897), it was first reported in North America on sugarcane in Belle Glade, Florida in 1977 (Mead 1978). Blackman & Eastop (2006) considered *Melanaphis sorghi* (Theobald) as a distinct species, but the morphological distinctions from *M. sacchari* are

ambiguous and recent analyses of population genetics revealed clones defined by geography, rather than by host plant utilization (Nibouche *et al.* 2014). Thus *M. sorghi* is likely a synonym, as argued by Remaudiere & Remaudiere (1997), and *M. sacchari* appears to have arrived in the USA on infested sugarcane material from Hawaii (Nibouche *et al.* 2014).

The aphid became problematic on sugarcane in Louisiana soon after its detection in 1999 (White *et al.* 2001), but was not recorded infesting grain sorghum, *Sorghum bicolor* L., until the summer of 2013 in Beaumont, TX, during which highly damaging populations developed in fields throughout the Rio Grande Valley and across the border into Tamaulipas, Mexico (Villanueva *et al.* 2014). In 2014, *M. sacchari* range expansion occurred to the east and northeast, with sorghum infested in northern Texas, southern Oklahoma, Louisiana, Mississippi, Arkansas and Tennessee. To date, there have been no reports of the aphid west of Interstate 35, a north–south highway that bisects Texas and Oklahoma, but this may simply reflect the prevailing wind patterns during peak periods of aphid flight in 2014.

The primary feature of *M. sacchari* that contributes to its pest status on sorghum is a very high reproductive rate – more than double that of greenbug, *Schizaphis graminum* Rondani, on susceptible sorghum cultivars at 23–24 °C (Colares, F.; unpublished observations). Feeding by *M. sacchari* does not damage sorghum plants as quickly as feeding by greenbug, but uncontrolled colonies eventually cause similar chlorosis and death of plant tissues, although this requires a heavier load of aphids feeding for a longer period. Whereas *S. graminum* can feed within the panicle up until flowering and cause some flower sterility, seed weight and quality is usually unaffected, even though yields may be reduced (Harvey & Hackerott 1974). In contrast, *M. sacchari* can continue feeding through the soft stages of grain fill, impacting both seed weight and quality (Chang & Fang 1984, van den Berg *et al.* 2003). In addition, the thermal tolerance of this particular *M. sacchari* population has not yet been tested, but if it is capable of development and

reproduction at temperatures exceeding 25 °C, this could contribute significantly to its pest status during hot summer conditions when high temperatures typically limit greenbug survival and reproduction (Pendleton *et al.* 2009). Another factor that could influence the pest status *M. sacchari* is its ability to utilize a wide range of wild and cultivated grasses, including barnyard grass, *Echinochloa crusgalli* (L.), Burmuda grass, *Cynodon dactylon* (L.) and Johnson grass, *Sorghum halepense* (L.) (Singh *et al.* 2004).

The literature suggests that a wide range of predators and parasitoids may contribute to biological control of *M. sacchari* throughout its geographic range. Singh *et al.* (2004) found 47 species of natural enemy reported to attack *M. sacchari*, with all major aphid natural enemy groups represented: Anthocoridae, Aphelinidae, Braconidae (Aphidiinae), Cecidomyiidae, Chamaemyiidae, Chrysopidae, Coccinellidae, Hemerobiidae, Lygaeidae, and Syrphidae. Anecdotal observations in 2014 indicate good initial recruitment of aphidophages to the first large *M. sacchari* infestations in south Texas (R. Villanueva, personal observations). However, there has been no effort yet to catalog the natural enemy species responding or to assess their rates of recruitment to *M. sacchari* in comparison to other aphids regularly infesting sorghum.

Over the past decade, our understanding of how natural enemies locate their herbivore prey by responding to induced plant volatiles has greatly improved (e.g., Takabayashi & Dicke 1996, Arimura *et al.* 2005, Turlings & Ton 2006). Adults of most aphid natural enemies orient to volatile compounds emitted by host plants in response to aphid feeding, (e.g., James *et al.* 2005, Sasso *et al.* 2009) and to odors of honeydew or aphid alarm pheromones (Hatano *et al.* 2008, Verheggen *et al.* 2008). Although many such compounds are ubiquitous across herbivore-plant associations, their activity is often dosage-dependent (e.g., Li *et al.* 2008). Furthermore, although variation among plant cultivars in emission profiles is well-recognized (e.g., Scutareanu *et al.* 2001, Kappers *et al.* 2011), the extent to which volatile profiles may vary among plants infested

with different aphid species is not yet known. If aphid natural enemies must evolve responses to novel signals following new aphid-host plant associations, this could explain the delay in establishment of biological control when aphids are newly invasive in a region. Notwithstanding this, indigenous aphidophagous guilds typically deliver biological control of invasive aphids in time, although this may only be recognized when introduced exotic natural enemies either fail to establish, or have little impact (Michaud 2002).

With the above considerations in mind, we designed a field experiment to compare the abundance and diversity of aphidophagous species recruited to potted plants of grain sorghum infested with either *M. sacchari* or *S. graminum*. We reasoned that, if *M. sacchari* infestation of sorghum elicits release of a volatile blend similar to that elicited by greenbug infestation, then the diversity and abundance of natural enemies attracted should be similar. However, if there are significant differences in recruitment of some species, but not others, it would suggest that different species may respond to different fractions of the volatile profile. Given that *M. sacchari* was not yet present in the study locality, the results provide an estimate of the extent to which aphidophagous insects on the High Plains are preadapted to discover and exploit *M. sacchari* on sorghum, and whether or not we can expect to eventually obtain levels of conservation biological control similar to those currently established for greenbug on this crop.

## **Material and Methods**

**Insect Colonies.** A colony of *S. graminum* ‘biotype I’, was established from material collected from sorghum in Hays, Kansas in 2013, whereas the *M. sacchari* colony was established from material obtained from the USDA-ARS laboratory in Stillwater, OK under a Material Transfer Agreement dated 27 May, 2014. Both species of aphids were reared on sorghum seedlings

cultivar P85Y40 (Pioneer Hi-Bred, Johnston, IA), which has no specific aphid-resistant traits. Seedlings were grown in dense rows in metal trays (60.0 x 45.0 x 8.0 cm) in a greenhouse under natural light and infested by dislodging aphids from infested plants onto the new tray. Once infested, trays were transferred to climate-controlled growth chambers set to  $23.0 \pm 1.0$  °C under continuous light.

**Experimental Setup.** Experiments were conducted during the summer of 2014 at the K-State Agricultural Research Station-Hays in Hays, KS (38°51`N, 99°20`W). To produce plants for the experiment, seeds of P85Y40 sorghum were planted in plastic flower pots (35.0 cm diam) filled with soil, about 10 seeds per pot. The pots were placed in a greenhouse under natural lighting at an ambient temperature of 24.0–26.0 °C and watered daily. Shortly after seedling emergence, plants were thinned to leave four per pot and pots were moved outdoors to an exposed location in full sunlight to ensure exposure to wind and rain under natural conditions. This was essential for the normal development of plants robust enough to sustain aphid infestation under outdoor conditions.

Once plants reached the whorl stage (30.0–40.0 cm all), pots were returned to the greenhouse to be infested with aphids. This was accomplished by clipping infested plants from the stock colonies of each aphid species and draping them over leaves of the potted plants. All plants were examined after 24 h and additional aphids were transferred, as needed, to ensure each plant had between 100 and 200 live aphids feeding. Each pot of four infested plants constituted a unit of sampling, hereafter referred to as a ‘microcosm’. After 48 h, all pots were transported to the field where they were placed in a line a minimum of 10 meters apart, with aphid species alternating. Two cohorts of aphid microcosms were followed; the first consisting of 26 pots ( $n = 13$  of each aphid species), and the second consisting of 28 pots ( $n = 14$  of each species). The first cohort was established in the field on 25 June and was distributed along the northern edge of a coniferous tree

line planted east–west in proximity to urban plantscapes, flower beds, mowed turf, and riparian parkland. This site was selected so that all pots would receive some afternoon shade and a degree of physical shelter, due to concerns about possible aphid mortality due to severe wind and rain events.

In contrast, the second cohort was established on 6 August in the middle of a 10 ha monoculture of forage sorghum with good herbicidal weed control, bordered by fields of mowed turf and wheat stubble. All pots were placed at least 15 m from the field border with the wheat stubble, with microcosms alternating in a north-west line in parallel with planted rows. In this case, the pots were partially dug in so that microcosm plants were similar in height to field plants at the start of observations and formed part of a continuous plant canopy. All pots were examined daily and watered as needed, with the exception of one day in each cohort when overnight rains made conditions too wet to count insects. On each observation day, the number of aphids on each plant was estimated and all eggs, larvae and adults of aphidophagous species were recorded. Because repeated observations of the same plants on successive days lead to many of the same insects being counted repeatedly, the only unbiased estimate of net ‘predator presence’ is provided by the sum of observations of each life stage of each species over all observation days, a measure expressed in ‘arthropod life stage days’. Pupae that formed on plants (and some late instar syrphid larvae) were collected and held in a growth chamber in the laboratory at 23 °C until emergence of adults so that species identity could be confirmed. Whenever an aphid colony was eliminated, the plant was destructively sampled to locate all remaining predator larvae and pupae, as these often occurred in concealed sites within the whorl or behind leaf sheaths.

**Statistical Analysis.** Arthropod counts were tallied as ‘arthropod life stage days’ on a per-pot basis, with the microcosm (group of four plants in one pot) as the experimental unit. Changes in the number of aphids per microcosm over time were analyzed graphically using polynomial

regression and trend lines were fit using the equation that yielded the most significant parameters and the highest  $r^2$  value. Fifth order equations provided lines of best fit in all cases except for *M. sacchari* microcosms in the first cohort, which were best described by a cubic function. Abundant natural enemy life stages were compared between aphid microcosms using repeated measures ANOVA and their daily means ( $\pm$ SE) are reported on a per-pot basis. When data was not normally distributed, it was subjected a Wilcoxon signed-rank test (SAS Institute 2001) to test for effects of aphid species. The total number of observations of each life stage of each natural enemy species are reported separately for each aphid type in each cohort.

## Results

In the first cohort, the last surviving microcosms of both aphid species effectively went extinct on the same day, but microcosms of *M. sacchari* in the second cohort persisted about a week longer than did those of *S. graminum* (Figs. 1A and 2A). Equations providing best fit to the data were as follows:

$$M. sacchari: Y = 1346.4 + 18.4x - 17.8x^2 + 0.6x^3; R^2 = 0.943$$

$$S. graminum: Y = 1079.5 - 930.1x + 470.2x^2 + 95.6x^3 + 8.2x^4 - 0.2x^5; R^2 = 0.920$$

The most abundant predators in the first cohort were syrphids (Table 1, Fig. 1B); of the 24 syrphid larvae and pupae reared out in the laboratory, 18 emerged as adults, of which 88.9% were *Allograpta obliqua* (Say) and 11.1% were *Syrphus* sp. Chrysopids were the next most abundant predator group (Table 1, Fig. 1C) and were assumed to be from the species complex of *Chrysoperla carnea* Stephens (Neuroptera: Chrysopidae), the only species normally collected in local grain crops. Larvae of these predators were directly observed consuming both aphid species on multiple occasions. There was no significant difference between aphid species in total numbers of syrphid egg days ( $F_{df = 1, 24} = 0.83, P = 0.371$ ), but microcosms of *M. sacchari* tallied more



syrphid larval days ( $F_{df=1,24} = 13.29$ ,  $P = 0.001$ ) and three times as many chrysopid egg days ( $Z = 2.13$ ,  $P = 0.033$ ) compared to those of *S. graminum*.

In the second cohort, adult Coccinellid were the most abundant predators, followed by *C. carnea* and *Orius insidiosus* (Say) (Hemiptera: Anthocoridae) ((Table 1, Figs. 2B and 2C). Equations providing best fit to the data were as follows:

$$M. sacchari: Y = 249.5 - 89.8x + 16.6x^2 + 0.79x^3 - 5.6x^4 + 6.5x^5; R^2 = 0.832$$

$$S. graminum: Y = 329.41 - 265.48x + 94.34x^2 - 13.66x^3 + 0.86x^4 - 0.02x^5; R^2 = 0.947$$

Of the 125 Coccinellid adults observed in both cohorts, 91.9% were *Hippodamia convergens* Guerin-Meneville, 4.1% were *Coccinella septempunctata* L., 3.3% were *Coleomegilla maculate* DeGeer and 0.8% were *Harmonia axyridis* Pallas. Microcosms of *S. graminum* and *M. sacchari* did not differ in numbers of coccinellid adult days ( $F_{df=1,26} = 0.93$ ,  $P = 0.344$ ), but there was a (borderline significant) tendency for *S. graminum* microcosms to register more anthocorid adult days ( $Z = 1.89$ ,  $P = 0.059$ ). Although *M. sacchari* microcosms registered almost twice as many chrysopid egg days as did *S. graminum* microcosms ( $F_{df=1,26} = 6.63$ ,  $P = 0.016$ ), the number of chrysopid larval days did not differ between aphid species ( $Z = 1.18$ ,  $P = 0.240$ ). Parasitism by *Aphelinus* sp. (Hymenoptera: Aphelinidae) was observed in both *S. graminum* and *M. sacchari* colonies (Table 1, Figs. 1D and 2D), with no difference between incidence of mummies in the first cohort ( $F_{df=1,24} = 0.04$ ,  $P = 0.516$ ), but with significantly more on greenbug colonies in the second one ( $Z = 0.98$ ,  $P = 0.329$ ). Mummies of *Lysiphlebus testaceipes* (Cresson) (Hymenoptera: Aphidiidae) were observed only on greenbug microcosms in both cohorts.

There were several minor precipitation events during each cohort (Fig. 3), but no weather severe enough to negatively impact aphid microcosms. Median ambient temperatures were slightly below seasonal norms, averaging  $24.1 \pm 0.8$  °C in the first cohort and  $26.4 \pm 0.4$  °C in the second, a significant difference ( $Z = 2.30$ ,  $P = 0.021$ ).

## Discussion

Despite being less than one kilometer apart, the two cohorts were placed in dramatically different habitats. The site of the first cohort was a habitat of much greater plant diversity, with nearby trees and flowers that were absent from the site of the second cohort. Another difference was that pots in the first cohort functioned as virtually isolated microcosms in open space, whereas in the second, they blended into a virtually continuous canopy of similar plants. Thus, the first cohort provided better foraging conditions for visually searching predators, and the second, greater opportunity for larval predators to arrive from (or disperse to) adjacent plants. It could be argued that some differences between cohorts were driven by seasonal changes in insect abundance because the two sets of observations were made in different time frames. However, all observations were made within a two month period of very similar, midsummer weather conditions, apart from slightly warmer median temperature in the second cohort. Thus, we infer that most of the clear-cut differences in insect observations between cohorts reflect local habitat effects rather than seasonal differences in abundance or activity levels. In comparison to the first cohort, the second lacked aphid flies, *Leucopis* sp., and velvet mites, *Erythraeus aphidivorus* Sundic (Sundic *et al.* 2015), only a single adult brown lacewing (Hemerobiidae) was observed, and syrphid abundance was orders of magnitude lower. The brown lacewing is sometimes found preying on aphids in wheat fields, but is also an arboreal species that exploits scales and other soft-bodied Hemiptera infesting cedars and pines (JPM, unpublished observations).

Microcosms of *M. sacchari* and *S. graminum* survived for similar periods in the first cohort, but those of *M. sacchari* survived about a week longer in the second (Figs. 1A and 2A). However, no microcosm of either species was successful in producing a generation of migrant alate (the occasional alate nymph was noted) and no serious plant damage occurred, so biological control of microcosms was successful for both aphid species in both cohorts. In the first cohort,

microcosms of *M. sacchari* had about twice as many aphids as those of *S. graminum* on the first observation date, which we considered indicative of higher reproduction by the former species during the 72 h infestation period; this was taken into account in preparation of the second cohort and similar numbers of aphids per microcosm were obtained for the two species on the first observation date. Laboratory observations indicated that the reproductive rate of *M. sacchari* at 23.5 °C was double that of *S. graminum* during the first week of adult life (FC, unpublished data); 4–5 nymphs per female, per day, as compared to 2–3 for greenbug. Because aphid reproductive rate tends to increase with temperature, the higher median temperature that prevailed during the second cohort may have further enhanced the reproductive advantage of *M. sacchari* relative to *S. graminum* and contributed to its higher peak abundance.

Adult syrphids are known to respond to specific host plants as well as aphid species (Almohamad *et al.* 2007), and their abundance in the first cohort clearly demonstrates good responses by *A. obliqua* to both aphid species infesting sorghum. The greater abundance of aphids in microcosms of *M. sacchari* compared to *S. graminum* throughout the first cohort may account for the higher levels of oviposition by both syrphids and chrysopids on the former. For example, oviposition by syrphid species on lettuce infested with *Nasonovia ribis-nigri* (Mosely) scales with aphid colony size (Nelson *et al.* 2012). Syrphids are proficient in discovering aphid colonies in their earliest stages of development, often before they are large enough to attract other predators (e.g., Kan 1988a,b). When syrphid larval counts were expressed as a percentage of egg observations, proportionally fewer larvae than expected were observed on *S. graminum* microcosms (48.2% versus 78.5%, Table 1). Syrphid larvae are notorious for egg cannibalism (e.g., Branquart *et al.* 1997, Belliure & Michaud 2001) and lower aphid densities in *S. graminum* microcosms relative to those of *M. sacchari* may have led to higher rates of egg cannibalism.

Notably, syrphid eggs and larvae were orders of magnitude lower in abundance in our second cohort compared to the first. Adult syrphids depend on flowers for nectar to fuel flight and pollen to mature eggs (Haslett 1989, Bugg *et al.* 2008), resources that were readily available in the locality of the first cohort, but absent from the vicinity of the second. For example, Meyer *et al.* (2009) found syrphid density strongly affected by the amount of pollen and nectar locally available for adults, as well as the presence of aphid-infested plants for larvae. Syrphids are notably rare in most field studies assessing aphid mortality in High Plains cereal crops (e.g., Rice & Wilde 1988, Nechols & Harvey 1998), very likely because of the scarcity of essential resources for adult females in large scale cereal monocultures with aggressive weed control. Another factor may be physical differences in habitat structure between the cohorts (microcosms in the open versus within a closed canopy); syrphids use both visual and chemical cues in locating aphid colonies and thus tend to prefer foraging in open habitats. For example, Michaud & Browning (1999) found six times as many syrphid eggs on colonies of brown citrus aphid, *Toxoptera citricida* (Kirkaldy), infesting heavily pruned citrus trees in open habitat compared to those infesting adjacent trees with dense canopies.

Chrysopids were the only major predator group to appear in similar numbers in both cohorts. Observations of chrysopid larvae were relatively few in consideration of the numbers of eggs observed. Larval chrysopids are also notorious cannibals (e.g., Duelli 1981, Bar & Gerling 1985, Mochizuki *et al.* 2006) and some emigration from microcosms may have occurred. However, larval observations in the first cohort corresponded to 1.5% and 3.1% of egg observations on *S. graminum* and *M. sacchari* microcosms, respectively, compared to 17.2% and 24.2% in the second cohort, where larval dispersal should have been easier. Consequently, we suspect more intraguild predation on chrysopid eggs and/or young larvae occurred in the first cohort. Larvae of *A. obliqua* were the dominant predators in the first cohort and thus prime

suspects as the IG predator; they were the first predator to oviposit and their eggs hatched very quickly. Similarly, larvae of a common European species, *Episyrphus balteatus* DeGeer (Diptera: Syrphidae) have been reported as intraguild predators of *C. carnea* larvae (Hindayana *et al.* 2001).

Based on prior observations, we expected coccinellids to be the primary source of aphid mortality in both cohorts, but this was true only in the second one. In central Kansas, adult coccinellids emerge from hibernation in early spring and have a single generation on aphids in wheat. The resulting adults leave the maturing wheat and enter reproductive diapause, which facilitates their survival through summer months when aphids are scarce (Michaud & Qureshi 2006). To break diapause, female *H. convergens* require ad libitum access to greenbugs for three to four days, but our aphid colonies did not appear to be sufficiently large to retain adult beetles within microcosms for such a period. Adults were quick to colonize the second cohort and rapidly inflicted heavy aphid mortality, likely reducing aphid numbers during the first 24 h before the first observations were made. These beetles were directly observed consuming the aphids, but abandoned the plants as aphid densities declined, without laying eggs and without completely eliminating the aphids (Fig. 2B), permitting some resurgence in aphid numbers (Fig. 2A). The greater resurgence of *M. sacchari* in the second cohort, despite being initially reduced to similar densities as *S. graminum*, likely reflects its higher reproductive rate.

All immature coccinellids recorded in the first cohort resulted from a single female *H. axyridis* that laid two egg masses totaling 89 eggs on plants in one microcosm. This species is largely arboreal, as evidenced by its contributions to aphid biological control in pine trees (McClure 1987), pecans (Teddars & Schaefer 1994), apple (Brown & Miller 1998), and citrus (Michaud 1999) and is not normally associated with cereal aphids, except in the vicinity of trees, as in this case. The virtual absence of *H. convergens* from the first cohort (only one adult observed) may have been a location effect – substantial distance from potential reservoirs of *H.*

*convergens* – combined with early discovery of the aphids by other predator groups, especially syrphids. Coccinellid females are known to avoid oviposition on plants contaminated by conspecific larval residues (Ruzicka 2002), heterospecific larval residues (Ruzicka 2006), and even those of other aphidophagous insects (Ruzicka 2001), including possibly syrphid larvae (Alhmedi *et al.* 2010). Although avoidance of syrphid larvae and their residues has not been shown for *H. convergens*, females of this species avoid oviposition on plants contaminated with residues of conspecific larvae and those of *C. maculata* (Michaud & Jyoti 2007). It is also possible that voracious feeding by syrphids quickly reduced aphid numbers below levels sufficient to attract beetles from any distance or retain those that did arrive.

Aphelinid and aphidiid parasitoids were observed in both aphid cohorts, but only *Aphelinus* sp. mummies formed on *M. sacchari*, although both occurred on *S. graminum*. In laboratory trials, we found that the two aphid species were attacked equally by female *L. testaceipes*, but no parasitoid larvae developed in any of the *M. sacchari* nymphs that were stung ( $n > 100$ ). Two trials were conducted with *L. testaceipes* sourced from two different collections. Subsequently, two separate DNA extractions from the aphids tested positive for *Hamiltonella defensa* (C. Vorburger, pers. comm.), a secondary endosymbiont known to protect aphids from parasitism by aphidiid wasps (Vorburger *et al.* 2009). Genotype x genotype interactions exist between strains of *H. defensa* and those of aphidiid parasitoids, resulting in varying levels of protection in infected aphids (Rouchet & Vorburger 2012). We conclude that this particular *H. defensa* strain is highly effective in protecting *M. sacchari* against our local strain of *L. testaceipes*, and is thus could be an impediment to biological control in regions where this wasp is a key mortality factor controlling aphids in sorghum, probably anywhere south of the Kansas–Oklahoma border (Jones *et al.* 2007). However, *H. defensa* can be acquired and lost among aphid clones, and parasitoid populations can evolve to overcome the resistance of particular strains

(Rouchet & Vorburger 2014), so it will be important to determine if infected clones are widely distributed, and if virulence against *H. defensa* exists in *L. testaceipes* populations. Complicating matters is the fact that the parasitoids themselves serve as vectors of the symbiont (Gehrer & Vorburger 2012). Mummies of *L. testaceipes* are common on *M. sacchari* in south Texas and appear to have normal emergence (R. Villanueva, pers. comm.), so infection with *H. defensa* does not appear to be universal among invasive clones of *M. sacchari* in the USA.

Some comment is warranted on differences in detectability among the various natural enemy species, and among life stages, within the sampling regime employed. Each microcosm was examined by two people for about 15 min daily, sufficient time to approximate aphid numbers and count natural enemies with reasonable accuracy. Sessile life stages (e.g., eggs, mummies, etc.) and larval stages that never leave aphid colonies (i.e., syrphids) have high detectability in this protocol, whereas more motile and/or secretive larvae (e.g., chrysopids and hemerobiids) are likely to be less detectable. Similarly, any predators that were exclusively nocturnal foragers would not have been detected. It was also not possible to sample any insects hiding deep in the whorl of the plant without doing so destructively, so this was done only on the last sampling date. Adult predators are highly active and may not spend much longer on an aphid colony than it takes to oviposit, reducing their detectability relative to immature stages. Adult syrphids and chrysopids do not consume aphids and eggs provide reliable evidence of their presence, so the issue is of little consequence for these groups. However, the actual numbers of adult *H. convergens* arriving at microcosms in the second cohort would have been substantially underestimated in 15 min of observation and oviposition by females does not occur prior to 3–4 days of aphid consumption. We inferred that our microcosms in the second cohort were not large enough, nor abundant enough, to withstand the functional response of *H. convergens* females, or cause them to break their reproductive diapause.

We conclude that all the major groups of aphidophagous species inhabiting High Plains cereal crops are preadapted to respond to, and exploit, sugarcane aphid, although the higher reproductive rate of *M. sacchari* relative to *S. graminum* may render conservation biological control of the former species more problematic. The identification and incorporation of plant resistance traits into sorghum cultivars that diminish the aphid's reproductive rate would therefore be a valuable approach to complement biological control of *M. sacchari*. The results of this study suggest that the ability of indigenous aphidophagous guilds to respond to, and ultimately control, invasive aphid species may be often underestimated, which would explain why effective biological control of invasive aphids typically evolves in time, even when classical programs fail to establish exotic natural enemies.

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Table 1. Summary of total natural enemy occurrence on two cohorts of two aphid species (*Schizaphis graminum* and *Melanaphis sacchari*) established on potted sorghum plants in microcosms (pots each containing four plants) and observed daily until aphid colonies were eliminated. Values are total numbers of arthropod-days, by life stage, observed on all microcosms of each aphid species. The two cohorts were observed in different locations over different time frames (see text for details) in Hays, KS, in the summer of 2014.

Natural enemies	Life stage	Cohort 1		Cohort 2	
		<i>S. graminum</i> n = 13	<i>M. sacchari</i> n = 13	<i>S. graminum</i> n = 14	<i>M. sacchari</i> n = 14
Anthocoridae	adults	0	0	39	11
	nymphs	0	0	104	22
Aphelinidae	adults	0	4	8	4
	mummies	22	41	771	319
Aphidiidae	adults	2	5	0	0
	mummies	47	0	225	0
Chamaemyiidae	larvae	0	24	0	0
Chrysopidae	adults	3	12	10	34
	eggs	194	609	204	401
	larvae	3	19	35	97
Coccinellidae	adults	0	3	58	64
	egg masses	0	8	2	97
	larvae	0	77	2	24
Erythraeidae	nymphs	12	5	0	0
Hemerobiidae	adults	0	0	0	1
	eggs	0	181	0	0
Nabidae	adults	0	1	3	2
Syrphidae	adults	5	9	0	0
	eggs	1308	1626	13	37
	larvae	631	1278	33	60
	pupae	0	5	0	1

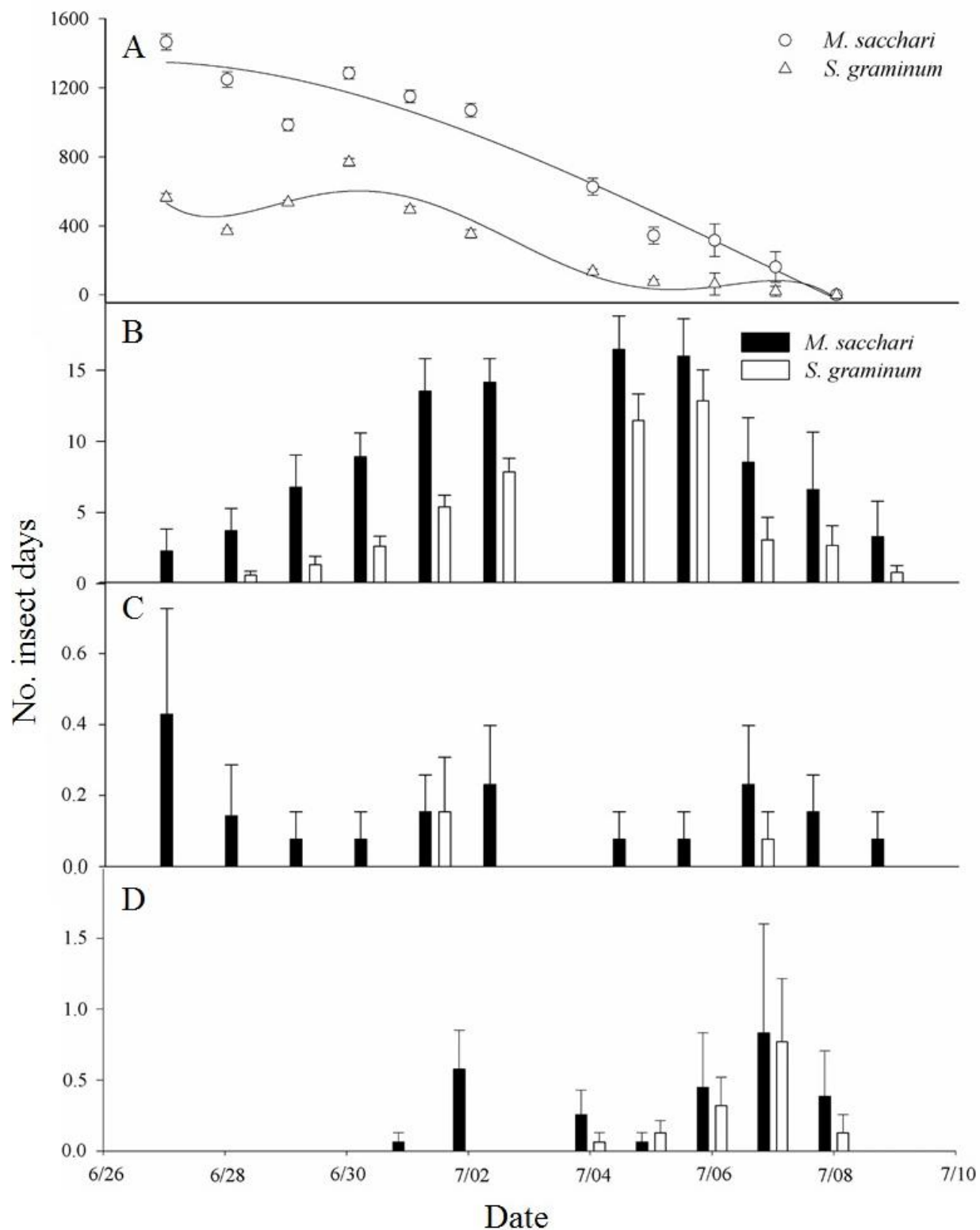


Figure 1. Mean (+SE) daily counts of aphids (A), syrphid larvae (B), chrysopid larvae (C) and aphelinid mummies (D) observed per microcosm (pot of four sorghum plants infested with either *Melanaphis sacchari* or *Schizaphis graminum*), per sampling date, during the first cohort. *M. sacchari*:  $Y = 1346.4 + 18.4x - 17.8x^2 + 0.6x^3$ ;  $R^2 = 0.943$ ; *S. graminum*:  $Y = 1079.5 - 930.1x + 470.2x^2 + 95.6x^3 + 8.2x^4 - 0.2x^5$ ;  $R^2 = 0.920$ .

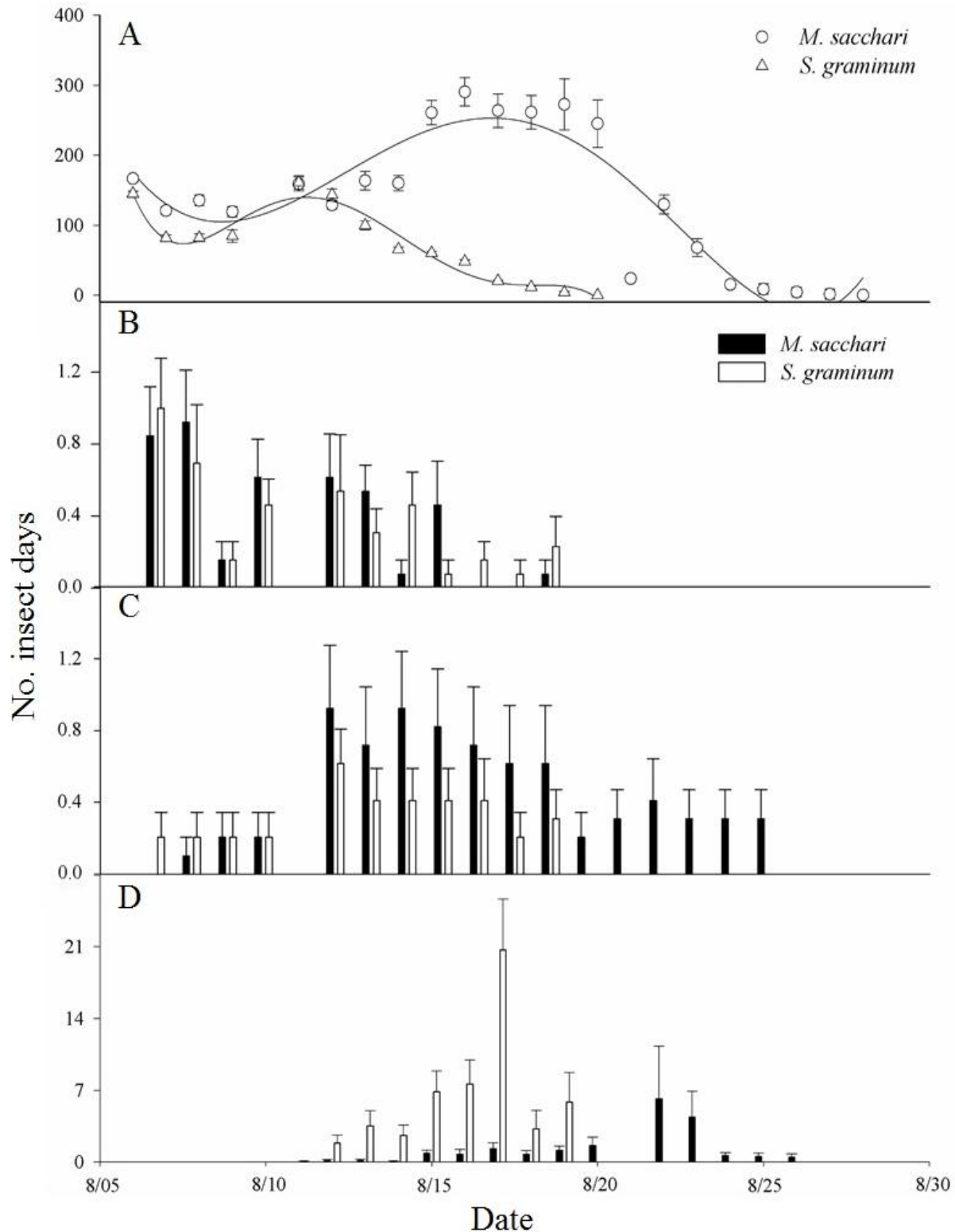


Figure 2. Mean ( $\pm$ SE) daily counts of aphids (A), coccinellid adults (B), chrysid larvae (C) and aphelinid mummies (D) observed per microcosm (pot of four sorghum plants infested with either *Melanaphis sacchari* or *Schizaphis graminum*), per sampling date, during the second cohort. *M. sacchari*:  $Y = 249.5 - 89.8x + 16.6x^2 + 0.79x^3 - 5.6x^4 + 6.5x^5$ ;  $R^2 = 0.832$ ; *S. graminum*:  $Y = 329.41 - 265.48x + 94.34x^2 - 13.66x^3 + 0.86x^4 - 0.02x^5$ ;  $R^2 = 0.947$ .

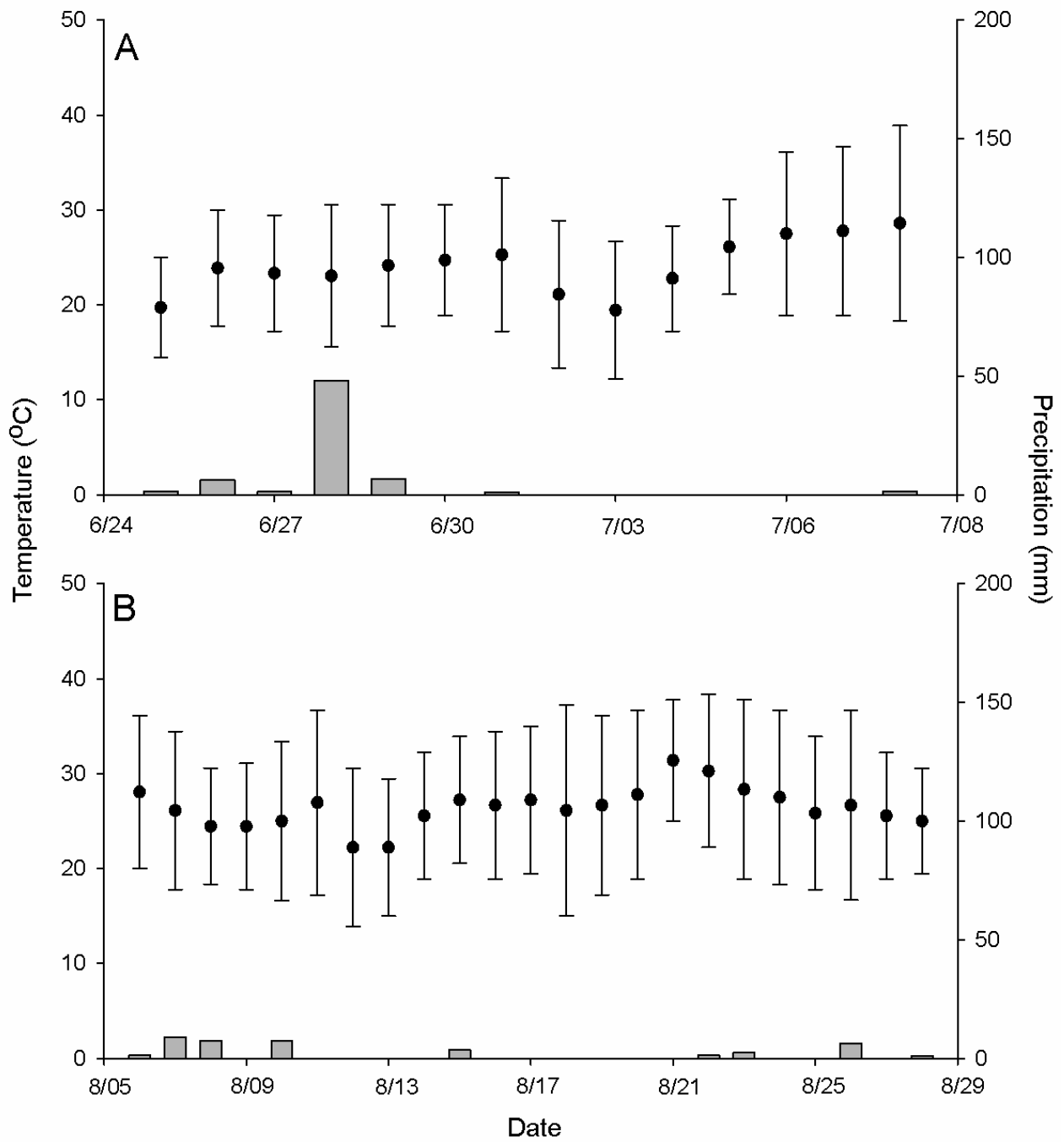


Figure 3. Median ( $\pm$ maximum and minimum) daily temperatures and precipitation during the first (A) and second (B) aphid cohorts.



## CHAPTER 3

### INDIGENOUS APHID PREDATORS SHOW HIGH LEVELS OF PREADAPTATION TO A NOVEL PREY, *Melanaphis sacchari* (ZEHNTER) (HEMIPTERA: APHIDIDAE)<sup>1</sup>

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<sup>1</sup>Colares, F., J.P. Michaud, C.L. Bain & J.B. Torres. 2015. Indigenous aphid predators show high levels of preadaptation to a novel prey, *Melanaphis sacchari* (Hemiptera: Aphididae). Published in Journal of Economic Entomology 108: 2546–2555.

ABSTRACT– The biological performance of four key cereal aphid predators, *Hippodamia convergens* Guerin-Meneville, *Coleomegilla maculata* DeGeer, *Chrysoperla carnea* Stephens, and *Orius Insidiosus* Say, on the sugarcane aphid *Melanaphis sacchari* (Zehntner) was compared with the performance on the greenbug *Schizaphis graminum* Rondani, and the insect rearing prey item *Ephestia kuehniella* Zeller eggs. Differences were generally greater between the *E. kuehniella* eggs and aphids than between the two aphid species. Immature survival to adulthood was high for all predators, irrespective of prey, except for *O. insidiosus* nymphs which had survival on *E. kuehniella* > *S. graminum* > *M. sacchari*. Developmental times differed slightly across preys, with *S. graminum* yielding the fastest development of *H. convergens*, and *E. kuehniella* the fastest development of *C. carnea* and *O. insidiosus*. The *S. graminum* diet also yielded the largest adults of *H. convergens*, whereas the largest adults of other predatory species were all obtained on *E. kuehniella*. Fecundity and egg viability were similarly high on both aphids for *H. convergens* and *C. maculata* females, whereas on *E. kuehniella*, 50% of the former entered reproductive diapause and the latter species had reduced fecundity. Reproductive success of *C. carnea* was *S. graminum* = *M. sacchari* > *E. kuehniella*, whereas in *O. insidiosus* it was similar among treatments, although female infertility ranged from 25–37.5%. We concluded that all the predators studied are preadapted to exploit sugarcane aphid on grain sorghum and have excellent potential to provide sustainable biological control of this newly invasive pest.

KEY WORDS: *Chrysoperla carnea*, *Coleomegilla maculata*, development, *Ephestia kuehniella*, greenbug, *Hippodamia convergens*, *Orius insidiosus*, reproduction, sugarcane aphid

PREDADORES NATIVOS DE PULGÕES DEMONSTRAM ALTO NÍVEL PRE-  
ADAPTAÇÃO A NOVA PRESA, *Melanaphis sacchari* (ZEHNTNER) (HEMIPTERA:  
APHIDIDAE)

RESUMO - O desempenho biológico de quatro dos principais predadores de pulgões, *Hippodamia convergens* Guérin-Ménéville, *Coleomegilla maculata* DeGeer, *Chrysoperla carnea* Stephens, e *Orius insidiosus* Say, em pulgão-da-cana *Melanaphis sacchari* (Zehntner) foi comparado com o desempenho no pulgão-verde *Schizaphis graminum* Rondani, e em ovos de *Ephestia kuehniella* Zeller. As diferenças foram maiores entre os ovos de *E. kuehniella* e os pulgões que entre as duas espécies de pulgão como presas. A sobrevivência da fase imatura foi alta para todos os predadores, com exceção de ninfas de *O. insidiosus*, que tiveram a sobrevivência em *E. kuehniella* > *S. graminum* > *M. sacchari*. O desenvolvimento diferiu entre presas sendo mais rápido em *S. graminum* para *H. convergens* e em ovos de *E. kuehniella* para *C. carnea* e *O. insidiosus*. Adultos de *H. convergens* foram maiores em *S. graminum*, enquanto que para as demais espécies predadoras foram maiores em *E. kuehniella*. Fecundidade e viabilidade dos ovos foram alta para *H. convergens* e *C. maculata* em ambos os pulgões, enquanto que 50% de *H. convergens* entrou em diapausa reprodutiva e *C. maculata* tiveram redução da fecundidade em *E. kuehniella*. O sucesso reprodutivo de *C. carnea* foi *S. graminum* = *M. sacchari* > *E. kuehniella*, enquanto em *O. insidiosus* foi semelhante entre os tratamentos, embora a infertilidade variou entre 25-37,5%. Concluiu-se que todos os predadores estudados são pré-adaptados para explorar o pulgão-da-cana no sorgo, e têm potencial para fornecer controle biológico desta praga.

PALAVRAS-CHAVE: *Chrysoperla carnea*, *Coleomegilla maculata*, desenvolvimento, *Ephestia kuehniella*, *Hippodamia convergens*, *Orius insidiosus*, pulgão-verde, pulgão da cana-de-açúcar, reprodução

## Introduction

The sugarcane aphid, *Melanaphis sacchari* (Zehntner), is a pest of cosmopolitan distribution that was first reported in Florida, United States, in 1977 on sugarcane (Summers 1978, Denmark 1988). In 1999, it was discovered infesting sugarcane in southern regions of Louisiana (White *et al.* 2001). In 2013, the first infestations of grain sorghum were reported from the gulf coast of Texas and, by the fall of 2014, the aphid had spread north and east to infest grain and forage sorghum across eleven states, as far as Kansas and Tennessee in the north, and Georgia and Florida in the southeast.

Over the past century, agriculture in the continental USA has suffered many similar invasions by exotic aphid species that provide relevant historical perspectives. The recurrent pattern is one of three chronological stages: 1) the epidemic stage: large aphid populations have devastating impact over a wide geographic region of crop production; 2) the attenuation stage: infestations gradually recede into periodic, intermittent outbreaks that are more spatially limited – geographic range may contract; 3) the endemic stage: natural biological control, exerted by a complex of resident natural enemies, becomes broadly established – economic infestations become rare and usually limited to situations where natural enemy populations are disrupted by either climatic or cultural impacts. The establishment of biological control is often assisted by plant resistance traits bred into crop varieties, particularly in cereals and row crops, since antibiosis typically lowers rates of aphid development and/or reproduction (Fuentes-Contreras & Niemeyer 1998, Qureshi *et al.* 2006, van Emden 2007), despite some minor impacts on the quality of aphids as prey or hosts (e.g., Starks *et al.* 1972, Reed *et al.* 1992, Shannag & Obeidat 2008). There is scant evidence, if any, to suggest that introduced exotic biocontrol agents have ever made a measurable contribution to sustainable biological control of an invasive aphid, although virtually every aphid invasion in North America has been met with this approach (Table 1). In virtually all

cases, control was eventually delivered by the collective contributions of existing guilds of aphid natural enemies. From an ecological perspective, aphid natural enemies are ubiquitous in agroecosystems – aphidophagy is rarely, if ever, an empty niche. From an evolutionary standpoint, it is probably much easier for a locally adapted predator/parasitoid to broaden its diet to include a novel prey/host than it is for an exotic species to evolve the myriad adaptations necessary for establishment in a novel geographic region, even if a familiar prey is present there.

The period required for new aphid invasions to recede to endemic levels can vary from a few years to decades. In the case of the invasion of Florida citrus by *Toxoptera citricida* (Kirkaldy), excellent levels of biological control were provided by resident natural enemies in about five years, an unusually short period, and despite the failure of efforts to establish exotic parasitoids (Michaud 2002a). Notably, there were no intensive efforts to control *T. citricida* with insecticides; although important as a disease vector, the aphid does little direct damage to trees and most citrus producers were conscious of the risk of disrupting biological control of other pests. In comparison, the Russian wheat aphid, *Diuraphis noxia* (Mordvilko), arrived in the Great Plains in 1986 and required about ten years to recede to non-economic levels throughout most of its former range; total control costs and the acreage treated with insecticides could be observed declining in the early 1990's (Morrison & Pairs 1999). One of the most persistent aphid problems in North American cereals has been the greenbug, *S. graminum*, following its host shift from wheat to sorghum. Although the shift occurred in 1968 (Harvey & Hackerott 1969), it was not until thirty years later that greenbug problems in the central Great Plains diminished to the point that screening of new cultivars for greenbug resistance could be largely abandoned. Screening for resistance to *M. sacchari* in sorghum cultivars has just begun, but several sources of resistance to greenbug showing promising activity against *M. sacchari* (Armstrong et al. 2015).

The epidemic phase of an aphid invasion is typically met with heavy insecticide applications, which often results in the evolution of insecticide resistance in the target aphid (e.g., Teetes *et al.* 1975, Zhu *et al.* 2000) and probably delays the evolution of natural biological control by eliminating the most pre-adapted individuals in predator populations, i.e., those that are first to respond to the new aphid prey. When biological control is slow to evolve, whether due to cultural impediments or other factors, new aphid biotypes may evolve virulence to resistant varieties when these are relied upon as a cornerstone of management (e.g., Porter *et al.* 1982, Harvey *et al.* 1991, Burd & Porter 2006, Puterka *et al.* 2007). Whenever biological control is slow to evolve, it is often assumed that the existing guild of natural enemies is somehow deficient and that the addition of exotic species is therefore necessary to achieve acceptable levels of control (Michaud 2002b). However, in nearly every case where efforts failed to establish exotic species against invasive aphids, natural control was eventually delivered by locally-adapted, resident species. For example, the invasion of the American midwest by the soybean aphid, *Aphis glycines* (Matsumura), began in 2001 and required about 10 years to reach the attenuation phase, with economic outbreaks now becoming less frequent and more limited in scale, despite the failure of an extensive program to establish the exotic parasitoid *Binodoxys communis* (Gahan) (Ragsdale *et al.* 2011). In the case of the Russian wheat aphid, there was a contribution of plant resistance on its management, but the establishment of natural biological control was delayed for about ten years following its arrival in North America, probably due to heavy insecticide use. In the interim, more than 15.5 million insects belonging to 24 exotic natural enemies were reared and released in possibly the largest program of its kind (Prokrym *et al.* 1998). Most of these species were not only poorly-matched to the challenging environmental conditions prevailing on the High Plains, they were also redundant to many locally-adapted, congeneric species that eventually proved to be the key biocontrol agents (see Nechols & Harvey 1998). Thus, historical evidence

suggests that local populations of beneficial arthropods may require a number of generations to adapt to a newly invasive aphid, and that this process may be impeded or delayed by certain cultural practices in the cropping system, especially insecticide use.

The case histories listed in Table 1, especially those pertaining to cereal crops, share many commonalities that can inform us as we address the problem of the *M. sacchari* invasion which is already being met by an onslaught of established, locally-adapted, aphidophagous arthropods. A recent field study conducted in central Kansas, prior to the natural arrival of *M. sacchari* in the region, demonstrated that a similar diversity of natural enemies recruited to sorghum plants infested with either *M. sacchari* or *S. graminum* at similar rates (Colares *et al.* 2015). Having observed a good response by aphid predators to *M. sacchari* in the field, it remained to evaluate the relative suitability of the aphid as a primary food source for these species, and thus their potential for numerical response.

*Hippodamia convergens* Guerin-Meneville (Coleoptera: Coccinellidae) is one of the most important predators of cereal aphids on the High Plains where it has had a major role in controlling Russian wheat aphid (Nechols & Harvey 1998, Michels *et al.* 2001) and greenbug on both wheat and sorghum (Rice & Wilde 1988, Michels *et al.* 2001). It is the most abundant of coccinellids in Kansas field crops and eminently well adapted to survive the frequently severe weather conditions on the High Plains. Adults can utilize plant resources to stay hydrated during periods of drought (Michaud & Qureshi 2005), survive long periods of prey scarcity in reproductive diapause (Michaud & Qureshi 2006), and hibernate successfully through prairie winters with little snow cover. Another indigenous aphid predator is *Coleomegilla maculata* DeGeer, although both larvae and adults have a much higher water demand than *H. convergens* (Michaud & Grant 2005, Moscardini *et al.* 2015), which tends to limit its abundance in arid environments such as the High Plains. Lacewings of the *Chrysoperla carnea* Stephens species

complex (Neuroptera: Chrysopidae) are other predators that contribute significantly to aphid population control in cereals across the High Plains region (e.g., Hesler *et al.* 2000, Messina & Sorenson 2001). The minute pirate bug, *Orius insidiosus* Say (Hemiptera: Anthocoridae), is a ubiquitous omnivore that, although not an aphid specialist, is often highly abundant in grain sorghum and readily consumes aphids. All four species were observed preying upon *M. sacchari*-infested sorghum plants under field conditions (Colares *et al.* 2015). Therefore, we hypothesized that they would all express good levels of preadaptation to utilize *M. sacchari* as prey, as reflected in fast development, good adult body size, and high fecundity and egg fertility. To test this, we compared the developmental and reproductive performance of these four species on *M. sacchari*, comparing to *S. graminum*, and eggs of the Mediterranean flour moth, *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae), a factitious diet that can be superior to aphids for many aphidophagous species, by virtue of higher protein and lipid content (Specty *et al.* 2003, Michaud & Jyoti 2007).

## Material and Methods

**Insect Colonies.** A colony of *S. graminum* was established from material collected from infested sorghum in Hays, KS, in 2014. A sample of apterous *M. sacchari* was obtained from the USDA-ARS laboratory in Stillwater, OK, from a laboratory colony that had been established from a collection from infested sorghum in Beaumont, TX in 2013. Both species of aphids were reared on sorghum seedlings cultivar P85Y40 (Pioneer Hi-Bred, Johnston, IA), which has no specific aphid-resistant traits. Sorghum seedlings were grown in metal trays in a greenhouse under natural light, supplemented with metal halide lights in a 16:8/L:D photoperiod. Following infestation with aphids, the trays were placed in a growth chamber set to  $23.0 \pm 1.0$  °C under continuous light. Adults of all predator species were collected from corn, sorghum, and sunflower fields at the Agricultural Research Center – Hays during the summer months of 2014. A stock colony of



each species was established in a climate-controlled growth chamber set to  $24 \pm 1$  °C and a 16:8/L:D photoperiod.

For breeding, pairs of adults of *H. convergens* and *C. maculata* were isolated in ventilated polystyrene vials (145 ml) and fed greenbugs ad libitum on excised shoots of their host plants. Egg clusters were collected daily and held under the same physical conditions as the adults until eclosion of larvae. Larvae of both species were transferred to plastic Petri dishes (5.5 cm diam), five per dish, and fed eggs of *E. kuehniella* with water provided on a small cube of sponge, both replaced every other day.

Adults of *C. carnea* were held in 2.5 L polyethylene containers covered with an organdy mesh screen and fed a mixture of Wheast and honey (1:1) with water provided on a cotton roll, both replaced every other day. Eggs were collected daily by clipping their stems with scissors and isolating them in plastic Petri dishes (as above) until larvae eclosed. Larvae were reared in isolation in the same Petri dishes fed on eggs of *E. kuehniella* with water provided on a small cube of sponge, both replaced every other day.

Adults of *O. insidiosus* were held in a 1 L glass mason jar covered with organdy mesh, fed eggs of *E. kuehniella*, and provided water on a cotton roll, both replaced every other day. Sunflower stems cut from greenhouse-grown seedlings were used as an oviposition substrate. The ends of excised stems were sealed with paraffin wax to delay desiccation. The stems were replaced daily and held in plastic Petri dishes (15.0 cm diam) until nymphs eclosed. Nymphs were reared in the same Petri dishes, ten per dish, fed on eggs of *E. kuehniella* with water provided on a small cube of sponge, both replaced every other day.

**Experiments.** All experiments were conducted under the same physical conditions as the stock predator colonies. Feeding treatments consisted of three preys: *M. sacchari*, *S. graminum*, and eggs of *E. kuehniella* (control). The predator populations had no prior exposure to *M. sacchari* as

this species was not yet present in central Kansas in 2014, whereas *S. graminum* was assumed to be a prey commonly utilized by all species. Previous laboratory studies had confirmed the suitability of *E. kuehniella* eggs for supporting the development of all species and the reproduction of *C. maculata* and *O. insidiosus*. In contrast, *H. convergens* adults require aphid prey for reliably stimulate oviposition (Michaud & Qureshi 2005). Because larval and adult predators may differ in nutritional requirements (Michaud 2005), and larval and adult diets can interact in their effects on adult performance (Michaud & Jyoti 2007), we assessed the suitability of each prey species for larval development and adult reproduction independently, with the exception of *C. carnea* which is predatory only in the larval stage. For reproduction assays, all insects were reared to adulthood on the factitious diet (*E. kuehniella* eggs) and then assigned to a prey treatment so that we could measure aphid suitability for reproductive performance without any carry-over effects of larval diet.

**Development Assays.** Neonate larvae ( $12 \pm 4$  h old) of *H. convergens* and *C. maculata* (n = 20 per treatment) were isolated in ventilated polystyrene vials (as above) and assigned to one of the three monotypic diet treatments. Water was provided on a small cube of sponge to insects receiving the *E. kuehniella* eggs, since the eggs are much lower in water compared to aphids (Michaud & Grant 2005). Insects receiving aphids were provided an excised sorghum seedling infested with their respective aphid species and a clean sorghum seedling was included in each replicate of the *E. kuehniella* treatment to control for presence of plant material in the other treatments. All materials were replaced daily in each replicate.

Neonate larvae of *C. carnea* ( $12 \pm 4$  h old) were isolated in plastic Petri dishes (as above) and assigned to one of the three diet treatments (n = 60 per treatment). The large number of replicates was employed so that sufficient numbers of insects could be reared for both development and reproduction assays, given that all adults feed on the same diet of wheat and

honey (1:1). Provisioning of food to developing larvae was the same as for the coccinellid species.

For *O. insidiosus*, neonate nymphs ( $12 \pm 4$  h old) were placed in ventilated polystyrene vials, ten nymphs per vial, and the vials each assigned to one of the three diet treatments (n = 20 vials per treatment). This design was selected because we anticipated significant nymph mortality in this species. Each vial was considered a replicate and the bugs were fed daily in the same manner as the other species.

We recorded the duration of larval development and pupation separately for all holometabolous species, and the numbers pupating and emerging successfully as adults. In the case of *O. insidiosus*, duration of nymphal development was tallied as the mean for all individuals molting to adult successfully in each replicate.

**Reproduction Assays.** A total of 150 larvae each of *H. convergens* and *C. maculata* were reared on eggs of *E. kuehniella* + water until they pupated and emerged as adults. Newly emerged beetles (< 24 h old) were isolated in polystyrene vials (as above) and assigned to one of the three diet treatments. Provisioning of food was identical to the larval development assays. Five days post-emergence, male and female beetles from the same treatments were paired for mating. Males were removed after 24h and held separately in plastic Petri dishes (5 cm diam) where they were fed the same diet as their respective mate. In order to maintain fertility, females were each reunited with the same male for a 24h period every five days throughout the course of the experiment. Egg clusters were collected daily until ten days of oviposition for each female, or until beetles were 30 days old (*H. convergens*) or 60 days old (*C. maculata*). Reproduction is significantly slower in the latter species.

Since *C. carnea* are predatory only during the larval stage, prey suitability for adult reproduction in this species was assayed using the standard adult diet after rearing on each of three prey

species. Adults obtained from the larval development experiment were sexed and paired within 24 h of emergence. Each pair was transferred to a ventilated polystyrene vial and provisioned *ad libitum* with honey and wheat (1:1) and water on a small cube of sponge, both replaced every other day. All eggs were collected daily and isolated in Petri dishes (as above) until females died. The eggs were held for six days under the same environmental conditions as the adults and the number hatching, recorded.

Newly eclosed nymphs of *O. insidiosus* were reared in Petri dishes (as above) on eggs of *E. kuehniella* with water on a sponge cube, both replaced every other day, until they molted to adults. The adults were then sexed and pairs were established in ventilated polystyrene vials (145 ml) each containing a sunflower stem as an oviposition substrate. Pairs were then assigned to one of the three feeding treatments (*E. kuehniella*, n = 16; *S. graminum*, n = 16; *M. sacchari*, n = 17) and provisioned in the same manner as larval development assay, with all materials replaced daily. Following removal from replicates, sunflower stems were held in plastic Petri dishes (15.0 cm diam) for eight days under the same physical conditions as the adults. The numbers of intact and everted opercula were then counted to determine the numbers of eggs laid (fecundity), and the latter value subtracted from the former to determine egg viability. Assays were terminated when the female died.

**Statistical Analysis.** Immature survival data (except for *O. insidiosus*, which were reared in groups of 10) and proportions of infertile females were compared among treatments pairwise using a 2-tailed test of proportions, weighted by sample size (StatSoft 2000). All other data were subjected to normality and homogeneity of variance testing before analysis by one-way ANOVA followed by the Bonferroni test ( $\alpha = 0.05$ ) to separate means (SAS Institute 2001).

## Results

**Development and Reproduction of *Hippodamia convergens*.** Larval survival was 100% in the sugarcane aphid treatment and 95% in the greenbug and *E. kuehniella* treatments (one larval death in each). The *S. graminum* diet produced the fastest larval development and *E. kuehniella* the slowest, with *M. sacchari* intermediate ( $F_{2, 55} = 22.46$ ,  $P < 0.001$ ), and treatment differences in total developmental time followed the same pattern ( $F_{2, 55} = 46.75$ ,  $P < 0.001$ , Fig. 1A). Pupation was faster on the *M. sacchari* than on the *E. kuehniella*, with the *S. graminum* treatment intermediate and not significantly different from others ( $F_{2, 55} = 5.3$ ,  $P = 0.008$ ). Both male ( $F_{2, 25} = 14.09$ ,  $P < 0.001$ ) and female ( $F_{2, 27} = 20.27$ ,  $P < 0.001$ ) adults were larger on *S. graminum* than on either the *M. sacchari* or *E. kuehniella* diets, which did not differ (Fig. 2A).

One pair in the *S. graminum* treatment was infertile (no eggs hatched) and one female in the *M. sacchari* treatment died before producing 10 clutches; these replicates were excluded from analysis. Only 9/20 pairs laid eggs in the *E. kuehniella* treatment and, of these, only three produced 10 clutches. Given the large number of females entering reproductive diapause, as expected, this treatment was excluded from analysis. There were no significant differences between the two aphid diets in the number of days required to produce 10 clutches ( $F_{1, 36} = 0.20$ ,  $P = 0.659$ ), 10-day fecundity ( $F_{1, 36} = 2.23$ ,  $P = 0.144$ ), or proportion of eggs hatching ( $F_{1, 36} = 0.90$ ,  $P = 0.765$ ). Females took an average of  $11.6 \pm 0.18$  days to lay 10 clutches for a total of  $326.7 \pm 11.10$  eggs, of which  $72.2 \pm 3.6\%$  hatched.

**Development and Reproduction of *Coleomegilla maculata*.** Survival of larvae ( $n = 20$  per treatment) was high in all three treatments, with two larvae dying on greenbug, one on sugarcane aphid, and three on *E. kuehniella* (pairwise, 2-tailed test of proportions weighted by sample size,  $P > 0.05$  in all cases). There was no significant effect of treatment on larval developmental time ( $F_{2, 51} = 0.78$ ,  $P = 0.466$ ), pupation time ( $F_{2, 51} = 1.37$ ,  $P = 0.263$ ), or total developmental time ( $F_{2, 51} =$

0.52,  $P = 0.600$ ; Fig. 1B). In contrast to *H. convergens*, the *E. kuehniella* produced the largest males ( $F_{2, 19} = 9.23$ ,  $P = 0.0016$ ) and females ( $F_{2, 29} = 27.67$ ,  $P < 0.0001$ ), with adults on the aphid treatments not significantly different from each other (Fig. 2B).

Two females in the greenbug treatment and four in the sugarcane aphid treatment either died before producing 10 clutches, or did not produce 10 clutches within the 60 days observation period; these females were excluded from analysis. Females on the *E. kuehniella* took almost twice as long, on average, to produce 10 clutches when compared with either of the aphid treatments, and produced significantly fewer eggs, although egg fertility did not differ as a function of treatment (Table 2).

**Development and Reproduction of *Chrysoperla carnea*.** Survival of larvae was similar across diets ( $\chi^2 = 0.59$ ,  $df = 2$ ,  $P = 0.745$ ), with 85, 95, and 82% of larvae ( $n = 60$  per treatment) emerging as adults on greenbug, sugarcane aphid and *E. kuehniella*, respectively. However, diet treatment significantly affected larval developmental time ( $F_{2, 155} = 88.76$ ,  $P < 0.001$ ), pupation time ( $F_{2, 155} = 36.55$ ,  $P < 0.001$ ) and total developmental time ( $F_{2, 155} = 114.59$ ,  $P < 0.001$ ), which was *E. kuehniella* < *S. graminum* < *M. sacchari* (Fig. 1C). The adult fresh mass of both males ( $F_{2, 68} = 68.30$ ,  $P < 0.001$ ) and females ( $F_{2, 84} = 57.31$ ,  $P < 0.001$ ) was greater on *E. kuehniella* eggs than on either aphid treatments (Fig. 2C).

Thirty percent of females (6/20) did not lay eggs in the *M. sacchari* treatment, significantly more ( $P = 0.019$ ) than in the *E. kuehniella* treatment (0/17). Females in the *S. graminum* treatment were intermediate in sterility (2/18 = 11.1%) and not significantly different either the *M. sacchari* treatment ( $P = 0.14$ ) or the *E. kuehniella* treatment ( $P = 0.17$ ). Infertile pairs were excluded from analysis of reproductive performance. Although feeding larvae on *E. kuehniella* resulted in the shortest preoviposition period, the highest fecundity, and the most oviposition days, egg viability was very low compared to either aphid treatments (Table 3). There was no

effect of diet treatment on the longevity of males ( $F_{2,44} = 0.9$ ,  $P = 0.895$ ) or females ( $F_{2,44} = 0.1$ ,  $P = 0.422$ ) which averaged  $15.8 \pm 0.7$  and  $19.8 \pm 0.7$  days, respectively.

**Development and Reproduction of *Orius insidiosus*.** Nymphal survival on the three preys was *E. kuehniella* (mean  $\pm$  SE =  $59.5 \pm 6.1\%$ ) > greenbug ( $38.5 \pm 4.4\%$ ) > sugarcane aphid ( $22.5 \pm 2.9\%$ ) ( $F_{2,57} = 15.75$ ,  $P < 0.001$ ). Therefore, developmental parameters were measured on different numbers of insects in the three treatments ( $n = 125$ ,  $77$  and  $45$  for *E. kuehniella*, *S. graminum* and *M. sacchari*, respectively). The sex ratio averaged  $58.6 \pm 4.1\%$  female and did not vary among treatments ( $F_{2,55} = 0.10$ ,  $P = 0.902$ ). The fastest nymphal development was obtained on *E. kuehniella* eggs, with neither aphid diet significantly different from the other ( $F_{2,55} = 21.93$ ,  $P < 0.001$ ; Fig. 1D). Both males ( $F_{2,42} = 25.13$ ,  $P < 0.001$ ) and females ( $F_{2,55} = 44.11$ ,  $P < 0.001$ ) were larger when reared on *E. kuehniella* eggs than on aphids (Fig. 2D).

Considerable numbers of *O. insidiosus* females were infertile on each of the monotypic diets provided: *S. graminum*, 4/16 pairs (25.0%); *M. sacchari*, 6/17 pairs (35.3%); *E. kuehniella*, 6/16 pairs (37.5%), with no significant difference among treatments (2-tailed test of proportions,  $P > 0.05$  in all cases). Females that did not lay eggs were excluded from analysis of reproductive performance. There was no effect of adult diet on pre-oviposition period ( $F_{2,30} = 2.1$ ,  $P = 0.140$ ), number of oviposition days ( $F_{2,30} = 0.29$ ,  $P = 0.754$ ), fecundity ( $F_{2,30} = 0.66$ ,  $P = 0.524$ ) or egg viability ( $F_{2,30} = 1.84$ ,  $P = 0.176$ ). Females had a (mean  $\pm$  SE) preoviposition period of  $9.8 \pm 0.96$  days and laid an average of  $32.7 \pm 7.66$  eggs in  $8.3 \pm 1.46$  days of which  $44.0\% \pm 6.49$  hatched. The longevity of males ( $F_{2,46} = 1.61$ ,  $P = 0.211$ ) and females ( $F_{2,46} = 0.69$ ,  $P = 0.506$ ) were also similar, irrespective of diet treatment, and averaged  $16.9 \pm 2.6$  and  $27.2 \pm 3.9$  days, respectively.

## Discussion

In general, the performance and life history of predators was quite similar whether feeding on the new prey *M. sacchari* or on the established one *S. graminum*. All four natural enemy species were able to develop and reproduce successfully on all three preys, with generally more differences in suitability evident between the *E. kuehniella* eggs and the aphid diets than between the two aphid species.

Although *H. convergens* obtained slightly faster development and larger body mass on greenbug than on sugarcane aphid, both aphid species can be considered 'adequate' prey (sensu Michaud 2005) for both larvae and adults of this species. Similarly, reproductive performance did not vary between aphid diets. Although Vargas *et al.* (2012a) found that fecundity can be reduced in very small *H. convergens* females, there is generally little, if any, correlation between body size and fecundity in this species (Michaud & Qureshi 2006), likely because smaller females tend to produce smaller eggs (Vargas *et al.*, 2012b). In contrast, only 45% of females fed *E. kuehniella* eggs began oviposition during the experiment, and the remainder entered reproductive diapause. This diapause is a key adaptation of *H. convergens* on the High Plains because it aids adult survival through summer months when aphids can be scarce for long periods; diapause is only broken when females are able to feed *ad libitum* on aphids for 3-4 days (Michaud & Qureshi 2006). Considering that *H. convergens* has been associated with greenbug on wheat since the crop was first grown extensively on the High Plains (Hunter 1909), these species have a long history of association. The beetle also followed the host range expansion of greenbug to sorghum in the late 1960's (Kring & Gilstrap 1984) and remains one of the most common predator of *S. graminum* and other cereal aphids on the High Plains (e.g., Rice & Wilde 1988, Nechols & Harvey 1998). Given that *M. sacchari* is nearly equivalent to greenbug in suitability as prey for



*H. convergens*, we anticipate that the latter species will emerge as a key biocontrol agent of this new pest.

Larvae of *C. maculata* survived well on all diets, and there were no significant differences in developmental time among diet treatments. Although *E. kuehniella* as a larval diet yielded the largest *C. maculata* adults, females fed *E. kuehniella* laid fewer eggs and took almost twice as long to produce 10 clutches. *C. maculata* is considered one of the most generalist of aphidophagous coccinellids (Hodek & Honěk 1996) and is able to complete development feeding only on pollen (Michaud & Grant 2004, Lundgren & Weidenmann 2004, Weber & Lundgren 2011). Although eggs of various moth species are often natural prey, they are not likely to be consumed exclusive of other prey, and our stock colony had four generations on this diet before use in experiments, which may have led to some transgenerational nutritional depletion. Also, changes in diet between larval and adult stages can improve reproductive performance if either diet has nutritional limitations (Michaud & Jyoti 2007), but no change in diet occurred in the *E. kuehniella* treatment, which could have disadvantaged these insects relative to the other treatments that were reared on *E. kuehniella*, but then fed aphids as adults. This indigenous species is similar to *H. convergens* in having a long association with *S. graminum* (e.g., Fenton & Fisher 1940), although it has a much higher water demand which may limit its numbers in arid regions of the High Plains (Michaud & Grant 2004, Moscardini *et al.* 2015). Nevertheless, the fact that the novel prey *M. sacchari* is nearly equivalent in suitability to *S. graminum* for *C. maculata* development and reproduction is auspicious for its eventual biological control.

For *C. carnea*, *E. kuehniella* eggs provided the fastest immature development and the largest adults, with development on *S. graminum* slightly faster than on *M. sacchari*, but no difference between aphid diets in adult fresh weights. Although *E. kuehniella* eggs also yielded the shortest pre-oviposition period and the highest fecundity, egg viability was substantially lower

than on either aphid diets. Once again, we suspect that some transgenerational nutrient depletion was possible, as the stock colony had been reared on *E. kuehniella* for four generations prior to use in experiments. This could also explain why fecundity was relatively low across the whole experiment compared to earlier observations of the same population on the *E. kuehniella* larval diet (Gontijo *et al.* 2014). Once more, the comparative equivalence of the two aphid as prey in terms of supporting development and reproduction suggests that *C. carnea* will be capable of both functional and numerical responses to the *M. sacchari* invasion and is preadapted to contribute to its biological control.

Nymphs of *O. insidiosus* fed *E. kuehniella* had the highest survival, the fastest development and molted into the heaviest adults, with both aphid species proving inferior in these regards. However, adult reproductive performance on *E. kuehniella* eggs was similar to that on the aphid diets, although there were significant numbers of infertile females in all treatments. Part of the apparent discrepancy between immature and adult performance may be due to the very small size of *O. insidiosus*, especially in early nymphal stages. It is well recognized that success in predation is influenced by the predator: prey size ratio; the smaller the predator relative to the prey, the longer the handling time and the higher the risk of injury (Sabelis 1992, Hirvonen & Ranta 1996, Magalhães *et al.* 2005). Early instar nymphs of *O. insidiosus* are probably limited to attacking only the smaller aphid instars, which may have initially reduced their foraging efficiency in the arenas relative to adults. *Orius insidiosus* is also omnivorous, especially in immature stages, and we have no way to evaluate the relative suitability of the sorghum seedlings as a plant food source, a factor known to influence life history parameters (Bush *et al.*, 1993). Although *O. insidiosus* are often observed eating aphids in sorghum and other small grain crops (Kring & Gilstrap 1984, Salas-Ariza *et al.* 1999, Colares *et al.* 2015), they feed also on thrips and mites, and probably do not feed on aphids exclusively (e.g., Messelink *et al.* 2013, Wang *et al.*

2014). A mixed diet may also yield better predator performance than a monotypic one, even when behavioral costs are involved (Messelink *et al.* 2008, Marques *et al.* 2015). Even if *O. insidiosus* have poor reproductive success on *M. sacchari*, they may still contribute substantial biocontrol impact if they colonize the crop in large numbers and reproduce on other prey.

In conclusion, the results of the present work indicate that these four indigenous aphid predators, already known to be key factors regulating the populations of other grain aphids, are all preadapted to utilize *M. sacchari* as a principle prey. Our data suggest that this aphid is broadly suitable prey for aphid predators in general, as evidenced in other studies (Singh *et al.* 2004), which bodes well for its eventual biological control by indigenous natural enemies within its new range. There is no evidence of an 'empty niche' that might justify introduction of an exotic natural enemy. Given recent field observations that sorghum infested with *M. sacchari* attracts a similar diversity and abundance of aphid natural enemies as sorghum infested with *S. graminum* (Colares *et al.* 2015), we can conclude that these predators not only have good biological performance on the novel prey, they actively orient to it in the field. Therefore, we anticipate that these natural enemies will eventually provide an acceptable level of natural biological control of *M. sacchari*, as they currently do for many other damaging aphid species in cereal crops. Some insecticide applications will be required to preserve yield in the face of *M. sacchari* outbreaks during the initial years of its invasion, but materials should be chosen based on their selectivity to aphid natural enemies. Similarly, cultural measures can be promoted that conserve beneficial species, such as leaving less productive portions of fields unsprayed to serve as natural enemy refuges.

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Table 1. A list of some aphid invasions in the continental USA indicating deployments of classical biological control (CBC) and host plant resistance (HPR) as management tactics.

Aphid species	Year discovered	Crop affected	CBC attempted	HPR developed	References
<i>Acyrtosiphum pisum</i> (Harris)	late 1800s	<i>Medicago sativa</i> L. (+)	yes	yes	1, 2, 3
<i>Myzocallis coryli</i> (Goetze)	late 1800s	<i>Corylus americana</i> Walter	yes	no	4
<i>Therioaphis maculata</i> (Buckton)	1954	<i>Medicago sativa</i> L.	yes	yes	5, 6
<i>Acyrtosiphum kondoi</i> Shinji	1974	<i>Medicago sativa</i> L.	yes	yes	7, 8
<i>Schizaphis graminum</i> * (Rondani)	1968	<i>Sorghum bicolor</i> L.	yes	yes	9, 10, 11
<i>Brachycorynella asparagi</i> (Mordvilko)	1984	<i>Asparagus officinalis</i> L.	yes	no	12, 13
<i>Diuraphis noxia</i> (Mordvilko)	1987	<i>Triticum aestivum</i> L. (+)	yes	yes	14
<i>Toxoptera citricida</i> (Kirkaldy)	1996	<i>Citrus</i> spp.	yes	no	15
<i>Aphis glycines</i> Matsumura	2001	<i>Glycines max</i> L.	yes	yes	16
<i>Nasonovia ribisnigri</i> (Mosely)	1961	<i>Lactuca sativa</i> L.	no	yes	17

\*Host shift from wheat. (+) indicates economic damage to other crops.

<sup>1</sup>Halfhill *et al.* 1972; <sup>2</sup>Davis 1915; <sup>3</sup>Painter & Grandfield 1935; <sup>4</sup>Aliniaze & Messing 1995; <sup>5</sup>Harvey & Hackerott 1956; <sup>6</sup>van den Bosch, R. 1957; <sup>7</sup>Stern *et al.* 1980; <sup>8</sup>Gonzalez *et al.* 1995; <sup>9</sup>Harvey & Hackerott 1956; <sup>10</sup>Jackson *et al.* 1971; <sup>11</sup>Hackerott *et al.* 1969; <sup>12</sup>Castle *et al.* 1987; <sup>13</sup>Daane *et al.* 1995; <sup>14</sup>Nechols & Harvey 1998; <sup>15</sup>Michaud 1999; <sup>16</sup>Ragsdale *et al.* 2011; <sup>17</sup>Macnay 1961.

Table 2. Mean ( $\pm$  SE) reproductive parameters for *Coleomegilla maculata* when reared on an *ad libitum* diet of *Ephestia kuehniella* eggs and then switched to one of three adult diets (greenbugs, sugarcane aphids, or *E. kuehniella* eggs). Means followed by the same letter were not significantly different within columns (Bonferroni,  $\alpha = 0.05$ ).

Adult diet	No. days for 10 clutches	10-d fecundity (no. eggs)	Egg viability (% hatching)
<i>S. graminum</i>	16.1 $\pm$ 1.5 b	182.1 $\pm$ 12.4 a	83.8 $\pm$ 3.4 a
<i>M. sacchari</i>	19.2 $\pm$ 1.9 b	165.7 $\pm$ 10.0 a	85.6 $\pm$ 2.6 a
<i>E. kuehniella</i>	29.9 $\pm$ 3.6 a	131.4 $\pm$ 7.6 b	86.5 $\pm$ 2.3 a
<i>F</i>	8.34	6.28	0.24
df	1,43	1,43	1,43
<i>P</i>	0.001	0.004	0.785

Table 3. Mean ( $\pm$  SE) reproductive parameters for pairs of *Chrysoperla carnea* when reared on an *ad libitum* diet of either *Schizaphis graminum*, *Melanaphis sacchari*, or eggs of *Ephestia kuehniella* and provisioned with the same adult diet of wheat and honey. Means followed by the same letter in the column were not significantly different from others for a given dependent variable (Bonferroni,  $\alpha = 0.05$ ).

	Preoviposition	No. oviposition	Fecundity	Egg viability
Larval diet	period (d)	days	(no. eggs)	(% hatching)
<i>S. graminum</i>	6.1 $\pm$ 0.4 a	4.9 $\pm$ 0.4 ab	31.8 $\pm$ 3.7 b	80.9 $\pm$ 6.4 a
<i>M. sacchari</i>	6.5 $\pm$ 0.4 a	4.5 $\pm$ 0.7 b	25.8 $\pm$ 5.5 b	73.3 $\pm$ 7.5 a
<i>E. kuehniella</i>	4.4 $\pm$ 0.5 b	6.8 $\pm$ 0.6 a	54.2 $\pm$ 5.3 a	14.4 $\pm$ 4.9 b
<i>F</i>	13.6	4.5	9.5	35.9
df	2,44	2,44	2,44	2,44
<i>P</i>	< 0.001	0.017	< 0.001	< 0.001

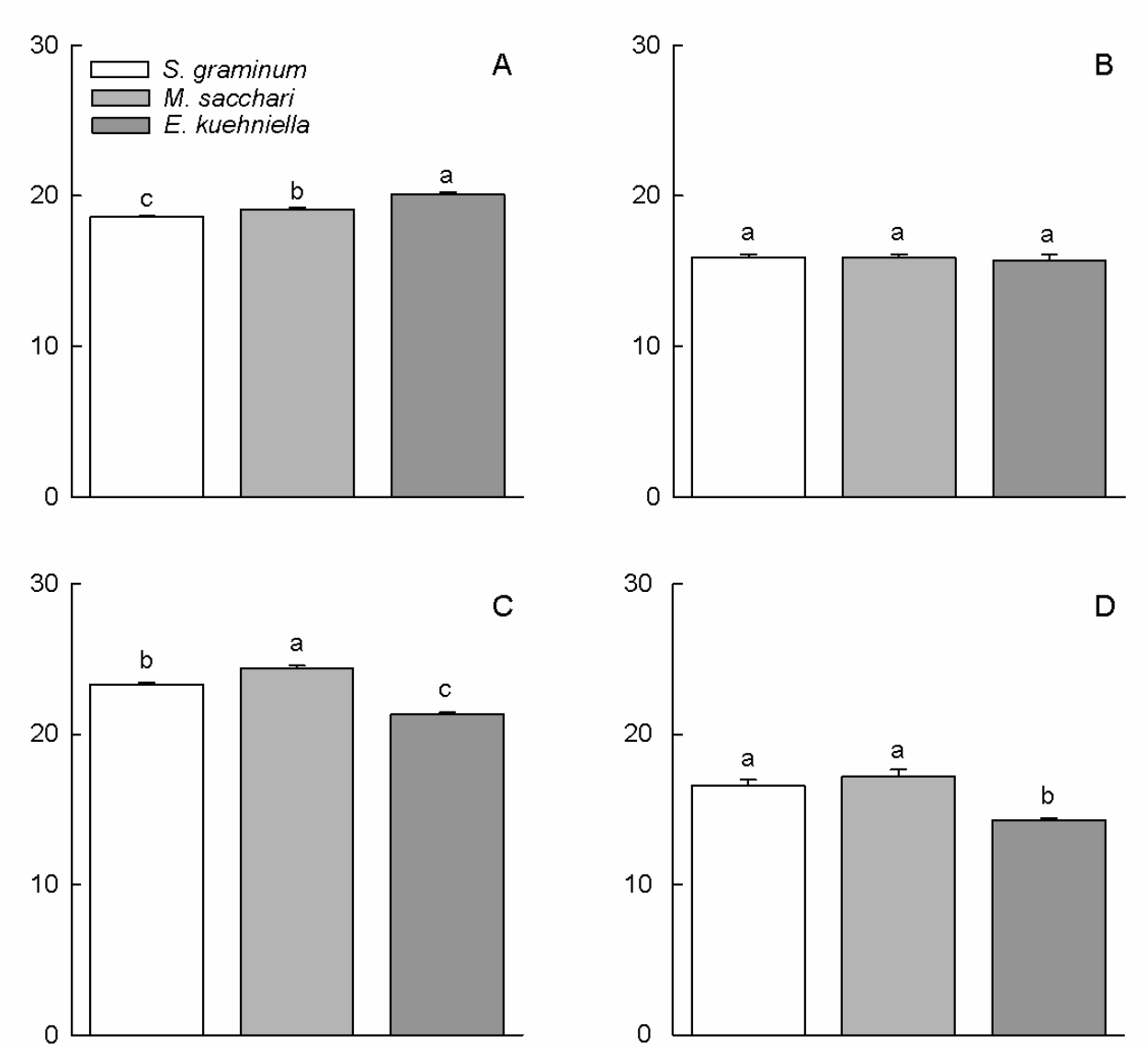


Fig. 1. Mean (+ SE) total developmental times (days) for *Hippodamia convergens* (A), *Coleomegilla maculata* (B), *Chrysoperla carnea* (C) and *Orius insidiosus* (D), reared on each of three diets (*Schizaphis graminum*, *Melanaphis sacchari*, or eggs of *Ephestia kuehniella*). For each predator species, columns bearing the same letters were not significantly different from others (ANOVA followed by Bonferroni,  $\alpha = 0.05$ ).

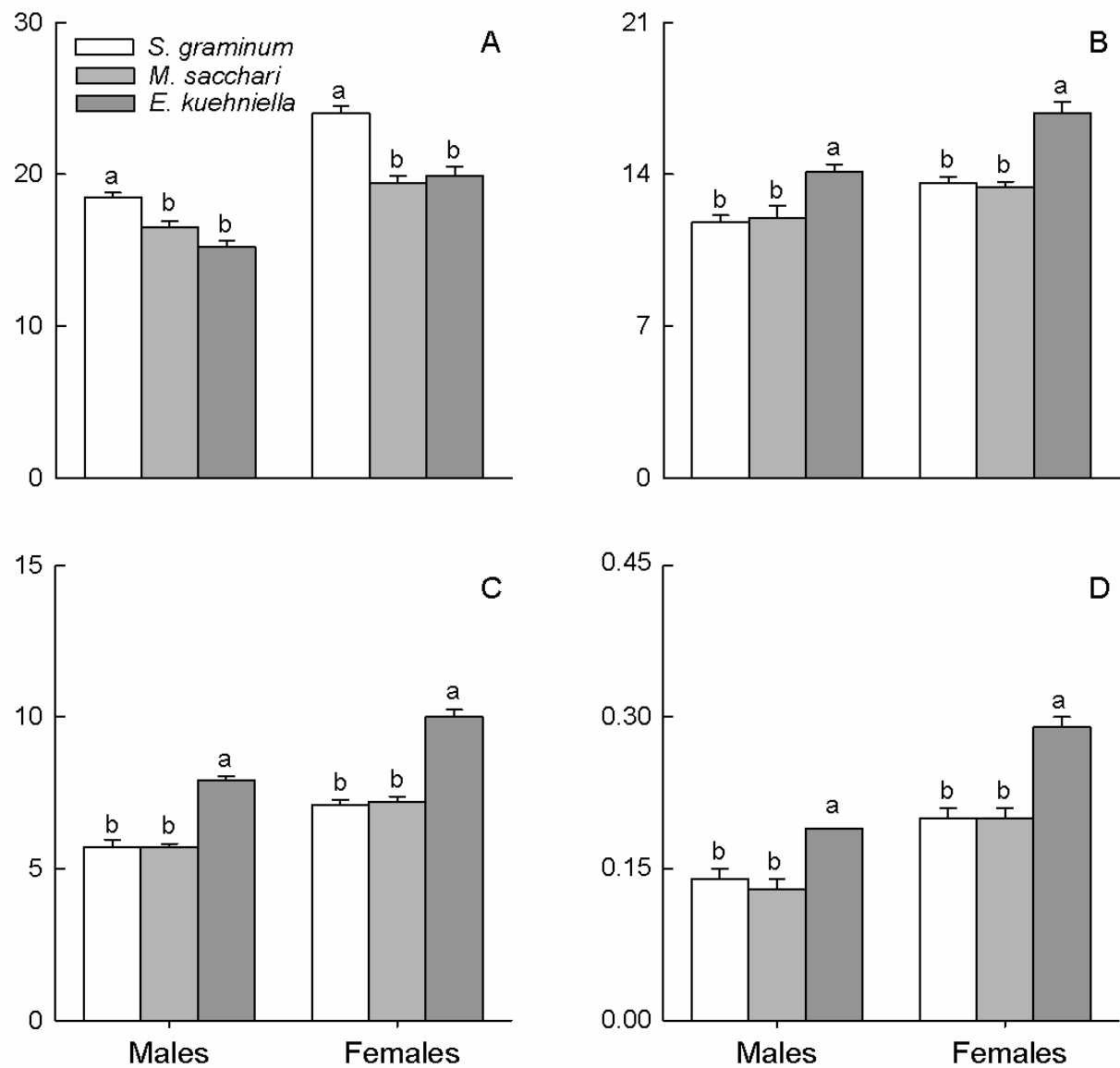


Fig. 2. Mean (+ SE) fresh body weight (mg) for male and female *Hippodamia convergens* (A), *Coleomegilla maculata* (B), *Chrysoperla carnea* (C) and *Orius insidiosus* (D), reared on each of three diets (*Schizaphis graminum*, *Melanaphis sacchari*, or eggs of *Ephestia kuehniella*). For each predator species and gender, bars bearing the same letters were not significantly different from others (ANOVA followed by Bonferroni,  $\alpha = 0.05$ ).

## CHAPTER 4

### FINAL CONSIDERATIONS

The occurrence of *M. sacchari* in Brazil is still restricted to sugarcane; however, both sorghum and sugarcane cultivated areas have been extended, especially in the Midwest region. The present work will be helpful, allowing us to anticipate some strategies to manage the sugarcane aphid in sorghum if a similar host range expansion by *M. sacchari* is observed in Brazil in the future.

The results obtained in this work indicate that the natural enemies will eventually provide an acceptable level of natural biological control of *M. sacchari* in the United States, as they currently do for *S. graminum* and many other damaging aphid species in cereal crops. We observed that sorghum infested with *M. sacchari* attracted the same guild of natural enemies as *S. graminum* and had similar biological control outcomes in the field. Furthermore, four key cereal aphid predators exhibited a similar biological performance feeding on both the sugarcane aphid and the greenbug in laboratory trials.

The most abundant natural enemies observed attacking *M. sacchari* in central Kansas were *H. convergens*, *C. maculata*, *C. carnea*, *O. insidiosus*, *A. obliqua*, *Aphelinus* sp., all of which are common in our agroecosystem in Brazil. Thus, we can expect a good performance of the indigenous natural enemies in Brazil on the sugarcane aphid if we face a similar host expansion and outbreaks on sorghum fields in the future. Therefore, the adoption of measures that conserve these beneficial species would be recommended, what means for example that if insecticide

applications are required to control outbreaks during the initial years, materials should be chosen based on their selectivity for the target aphid.