SPECIES COMPOSITION AND ACTIVITY OF THE NATURAL ENEMIES OF

SUGARCANE APHID (*Melanaphis sacchari*) ON SORGHUM

A Thesis

by

ERIN LYNETTE MAXSON

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Chair of Committee,   James B. Woolley
Co-Chair of Committee, Michael Brewer
Committee Member,   William Rooney
Head of Department,   David Ragsdale

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ABSTRACT

The sugarcane aphid (*Melanaphis sacchari*) is an emergent sorghum pest in the southern United States. The objectives of this study were to identify the natural enemy species that are feeding on the aphid in grain sorghum in Texas, track seasonal changes in aphid and natural enemy populations across sorghum hybrids that have differing levels of susceptibility to the aphid, and measure aphid suppression by natural enemies of different size classes. Aphids and natural enemies were sampled on multiple sorghum hybrids at two field locations in south and central Texas over two years. Additionally, aphid suppression by natural enemies of two size classes was evaluated using exclusion cages.

Aphids and natural enemies in both locations showed a trend of greater peak abundance on relatively more aphid-susceptible hybrids. At least 19 natural enemy species were present, consisting of parasitoids (*Aphelinus* sp. and *Lysiphlebus testaceipes*), lady beetles (Coccinellidae), hoverflies (Syrphidae), lacewings (Chrysopidae and Hemerobiidae), and minute pirate bugs (Anthocoridae). *Aphelinus* was heavily hyperparasitized by *Syrphophagus aphidivorus*. *Aphelinus* and Coccinellidae, the numerically dominant taxa, maintained high activity on resistant sorghum for a longer period than on susceptible sorghum. Natural enemy densities were similarly proportionate to aphid densities on both aphid-susceptible and aphid-resistant plants.

In the cage experiment, small lady beetles (Coccinellidae: Scymninae) were the only natural enemies to differ significantly in density between treatments. Scymninae density and Scymninae per aphid were greatest in closed standard mesh

cages. Scymninae density was greater on closed and open standard mesh than on fine mesh. Scymninae per aphid was higher on the resistant x susceptible hybrid. However, aphid density did not differ between cages or hybrids; therefore, aphid suppression was not detected. Problems and potential solutions involving cage integrity are discussed.

Overall, the existing community of aphidophagous insects in Texas has responded rapidly to the sugarcane aphid's introduction. Continuity in species composition and population trends across both counties and years indicates that the observed response is not an isolated event. The natural enemy complex was observed on both susceptible and resistant hybrids, suggesting that local natural enemies of the sugarcane aphid can complement host plant resistance.

DEDICATION

To my parents.

ACKNOWLEDGEMENTS

CONTRIBUTORS AND FUNDING SOURCES

NOMENCLATURE

Aphid = all uses of this word in the text and figures of this document refer specifically to the sugarcane aphid, *Melanaphis sacchari*, except where stated otherwise

Alatae = winged (alate) adult aphids

Apterae = wingless (apterous) adult aphids

Mummy = the dried, discolored husk of an aphid that has been killed by a parasitoid wasp larva, which the wasp uses as a pupation chamber

R; Res = a sorghum hybrid with partial resistance or tolerance to the sugarcane aphid

S; Sus = a sorghum hybrid susceptible to the sugarcane aphid

RxS = a sorghum hybrid obtained by crossing a maternal partially sugarcane-aphid-resistant inbred line with a paternal sugarcane-aphid-susceptible inbred line

RxR = a sorghum hybrid obtained by crossing two partially sugarcane aphid resistant or tolerant inbred lines

SxR = a sorghum hybrid obtained by crossing a maternal sugarcane-aphid-susceptible inbred line with a paternal partially sugarcane-aphid-resistant inbred line

SxS = a sorghum hybrid obtained by crossing two sugarcane aphid susceptible inbred lines

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

1. INTRODUCTION AND LITERATURE REVIEW

The sugarcane aphid, *Melanaphis sacchari* (Zehntner), is an emergent sorghum pest in the southern United States. The aphid was first reported on sorghum in 2013 in southeast Texas (Bowling et al. 2015a). Within the same year, the aphid was later recorded in Louisiana, Oklahoma, and Mississippi. In 2014, the sugarcane aphid was present in 12 states. As of fall 2015, the aphid had spread to a total of 17 states that together account for >95% of sorghum production in the US (USDA-NASS 2016). Its geographic range within the US extends south into Florida and Texas, north to Illinois and Kansas, and east-west from the lower east coast to the Great Plains (Fig. 1). The aphid has also been reported in the Caribbean Islands and throughout the sorghum-growing regions of Mexico (Bowling et al. 2016).
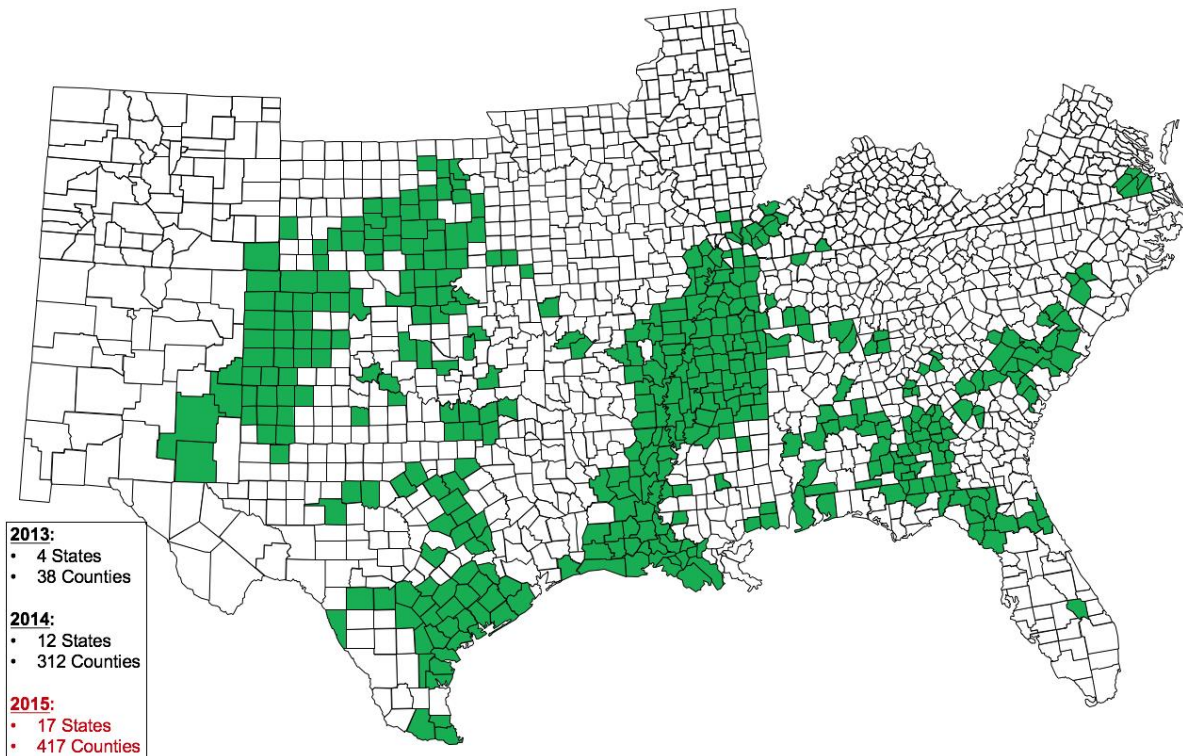
Fig. 1. 2015 range map of sugarcane aphid on sorghum in the US by county (reprinted from Bowling et al. 2016).

Cultivated sorghum, *Sorghum bicolor* (L.) Moench, is an annual, non-rhizomatous grass (Poaceae, syn. Gramineae) that is grown for grain and livestock forage (de Wet and Huckabay 1967). In the United States, grain sorghum is a multi-billion-dollar industry (Zapata et al. 2016). The state of Texas is the second-largest producer of sorghum in the US, comprising 2.7 million of the more than 7 million acres of US sorghum planted annually (Zapata et al. 2016; USDA-NASS 2016). In 2015, the value of Texas sorghum exceeded $700 million. Since its introduction, the sugarcane aphid has become a major threat to US sorghum production. Within the Lower Rio Grande Valley of Texas, where 11.5% of the state's sorghum is grown,

sugarcane aphids caused an estimated economic loss of $31.60 million from 2013

through 2015, and an average loss of $49.76 per acre between 2014 and 2015

(Zapata et al. 2016). Further research is needed to quantify the aphid's economic

damage throughout the rest of its US range; however, available data suggest that

yield losses of 10% to 50% are common (Villaneuva et al. 2014). In some fields with

no insecticide treatment, 100% yield loss has been reported (Zapata et al. 2016).

The sugarcane aphid is an established pest on sorghum and sugarcane

(*Saccharum officinarum* (L.)) throughout much of Asia, Africa, Australia, and Central

and South America (Singh et al. 2004; Zehntner 1897). The aphid was not recorded

in North America until 1977, when it was found on Florida sugarcane (Mead 1978).

The current distribution of the aphid on sugarcane within the United States is limited

to Hawaii, Florida, Texas, and Louisiana (Bowling et al. 2016; Singh et al. 2004).

The sugarcane aphids that have spread throughout US sorghum since 2013 do not

readily colonize sugarcane (Zapata et al. 2016). Whether this host discontinuity is

the result of a host switch originating within the US, or a separate invasion by a

foreign biotype, is not yet clear (Bowling et al. 2016). However, genotyping with

microsatellite markers has shown one aphid biotype to be predominant on sorghum

in the US. This suggests that the outbreak has been caused largely by one asexual

clone (Harris-Shultz et al. 2017).

Several aspects of the sugarcane aphid's ecology have facilitated its rapid

spread through the sorghum-growing regions of North America. The sugarcane

aphid population in the US is composed of parthenogenetic, viviparous, anholocyclic

females capable of rapid, exponential population growth (Bowling et al. 2016; Chang

3

et al. 1982). Sexual, egg-producing, and male forms have been observed only in some populations of the sorghum aphid complex (*Melanaphis* sp., cited as "*sacchari/sorghi*") on sorghum in Mexico (Peña-Martinez et al. 2016). Adults are polymorphic and are either alate (winged) or apterous (wingless). Alate aphids are weak fliers but can travel great distances via wind-aided dispersal (Irwin and Thresh 1988). In climates with mild winters, aphids overwinter on remnant and ratoon sorghum (Bowling et al. 2016). Johnsongrass, *Sorghum halepense* (L.) Pers., also serves as a winter host of sugarcane aphid. This weedy plant is more cold-tolerant than sorghum (Bowling et al. 2016), is established across most of the United States (USDA-NRCS 2016), and readily hybridizes with cultivated sorghum (Howard 2004).

Alate aphids initially colonize leaves of sorghum during vegetative growth. As the aphid reproduces and new leaves emerge from the whorl, colony expansion typically develops from the lower to the upper part of the plant (Bowling et al. 2016; Villanueva et al. 2014). The majority of aphids are concentrated on the underside of leaves near the midrib. In very heavy infestations, aphids can spread to the stalk and grain sorghum head. A single heavily-infested plant can harbor 10,000-30,000 aphids (Bowling et al. 2016; Setokuchi 1977). The aphids develop and reproduce most rapidly under warm, dry conditions (Singh et al. 2004). Nymphs mature within 4-12 days (Chang et al. 1982), and each adult can produce 34-96 progeny over the course of its 2-5 week lifespan (Singh et al. 2004).

Sugarcane aphids damage sorghum crops in myriad ways. Heavy aphid infestation causes early leaf chlorosis and necrosis, delayed or aborted panicle development, rapid decline in overall plant health, and reduction in quantity and

nutritional quality of grain and forage yield (Villanueva et al. 2014; Singh et al. 2004). Sugarcane aphids also coat leaves with honeydew. Excess honeydew promotes the growth of black sooty mold (Ascomycete fungal complexes). While sooty mold does not damage plants directly, it can reduce net photosynthesis by blocking light penetration to leaves (Wood et al. 1988). Additionally, honeydew interferes with grain harvest (Villanueva et al. 2014). Honeydew-covered leaves and stalks can stick to sorghum heads, preventing harvesting machinery from separating and collecting the grain. Honeydew can also clog harvesters, necessitating time-consuming maintenance.

One means of preventing or reducing aphid damage is to cultivate sorghum varieties that have sugarcane aphid resistance in the form of antibiosis, antixenosis (non-preference), or tolerance (Brewer et al. 2016; Sharma 1993). Since 1980, dozens of sugarcane-aphid-resistant sorghum varieties have been reported from various countries, particularly India (Singh et al. 2004; Mbulwe et al. 2016). Morphological sources of sugarcane aphid resistance include reduction in size and number of leaves, increased wax production on abaxial leaf surfaces, and a taller stalk with greater distance between the bases of leaves (Armstrong et al. 2015). Chemical sources of resistance include herbivory-induced production of *p*-hydroxybenzaldehyde during the seedling stage. Changes in the nutrient profile of leaves—specifically, higher levels of phosphorus, polyphenols, and potassium—have antixenotic effects. Some cultivars show evidence of antibiosis: in no-choice tests, they are infested with 50%-80% fewer aphids compared to susceptible cultivars, and incur only moderate plant damage. Highly aphid-tolerant cultivars can

withstand feeding by moderate numbers of aphids (~800 aphids/plant) without economic damage (Armstrong et al. 2015; Villanueva et al. 2014). Resistance to other aphid species is not a reliable indicator of sugarcane aphid resistance. Some cultivars that resist greenbug (*Schizaphis graminum* (Rondani)), a widespread grain pest, are highly susceptible to the sugarcane aphid (Armstrong et al. 2015), while others express cross-resistance to both these aphids (Brewer, Rooney, pers. communication).

The sugarcane aphid has many natural enemies that may complement host plant resistance by acting as biological controls. Natural enemies that recruit to an aphid colony early in that colony's growth cycle can limit population growth, prevent the production of alates, and ultimately eliminate the aphids before the infestation can cause significant damage to the plant (Colares et al. 2015a). A diverse assemblage of insects feed on the sugarcane aphid, including lady beetles (Coleoptera: Coccinellidae), hoverfly larvae (Diptera: Syrphidae), predatory gall midge larvae (Diptera: Cecidomyiidae), aphid fly larvae (Diptera: Chamaemyiidae), green lacewing larvae (Neuroptera: Chrysopidae), brown lacewing larvae (Neuroptera: Hemerobiidae), minute pirate bugs (Hemiptera: Anthocoridae), predatory seed bugs (Hemiptera: Lygaeidae), damsel bugs (Hemiptera: Nabidae), and parasitoid wasps (Hymenoptera: Aphelinidae; Hymenoptera: Braconidae) (Colares et al. 2015a, Singh et al. 2004). The Aphelinidae, Braconidae (Aphidiinae), Chrysopidae, Coccinellidae, and Syrphidae are particularly important aphid specialists that occur in agroecosystems worldwide. All five of these taxa prey on sorghum-associated sugarcane aphids in the US; however, across much of the

aphid's US range, the species composition of associated natural enemies has yet to be comprehensively surveyed.

To develop effective and cost-efficient integrated pest management strategies for the sugarcane aphid on sorghum, it is beneficial to identify the species community of aphid predators and parasitoids, and to evaluate their response to this invasive aphid on its new association with sorghum in North America. Natural enemies that are already native or naturalized within sorghum-producing regions of North America can complement conventional control measures—such as aphid-resistant cultivars or appropriate insecticides—at little or no additional cost. Natural enemies that are able to attack sugarcane aphid colonies early in the season, while aphid densities are low, can suppress aphid populations by maintaining the aphid mortality rate above the aphid population growth rate (Obrycki et al. 2009; Rutledge and O'Neil 2005; Hindayana et al. 2001; Messina and Sorenson 2001; Obrycki and Kring 1998; Dean and Wilding 1973). In addition to causing aphid mortality through predation, natural enemies also disrupt aphid colonies indirectly through non-consumptive effects. A colony disturbed by predators or parasitoids will engage in defensive behaviors, which divert aphids' time and energy away from foraging and reproducing (Rutledge and O'Neil 2005). Suppressing aphid population growth prevents aphids from overwhelming their host plants, thereby preventing yield loss. Surveying local natural enemies over time may help to elucidate which native or naturalized species have potential to contribute to the regulation of sugarcane aphids on sorghum.

2. SPECIES COMPOSITION AND ACTIVITY OF THE NATURAL ENEMIES OF SUGARCANE APHID (*Melanaphis sacchari*) ON SORGHUM

2.1. Overview

The sugarcane aphid (*Melanaphis sacchari*) is an emergent sorghum pest in the southern United States. This study was designed to identify which natural enemy species are feeding on the aphid in sorghum cropping systems in Texas, and to track the seasonal population trends of the aphid and its natural enemies on sorghum hybrids that differ in susceptibility to the aphid. Aphid-susceptible and partially aphid-resistant sorghum hybrids were planted in a randomized complete block pattern. From 2015 through 2016, aphids and natural enemies were sampled weekly on sorghum at two field sites in Texas in Nueces County and Burleson County.

In 2015, aphids and natural enemies at both field locations had greater peak abundance on the susceptible hybrid than on the resistant hybrid. Peak abundance of most natural enemies tended to lag behind that of aphids by one to two weeks. Natural enemy taxa observed at both field sites included two parasitoid wasp species (Hymenoptera: Aphelinidae: *Aphelinus* sp. *varipes* group; Braconidae: *Lysiphlebus testaceipes*), ten lady beetle species (Coleoptera: Coccinellidae), three hoverfly species (Diptera: Syrphidae), five green lacewing species (Neuroptera: Chrysopidae), brown lacewings (Neuroptera: Hemerobiidae: *Hemerobius*), and minute pirate bugs (Hemiptera: Anthocoridae: *Orius insidiosus*). *Aphelinus* and Coccinellidae were the numerically dominant natural enemy groups across

treatments, followed by lacewings (of which Chrysopidae were more abundant than Hemerobiidae) and hoverflies. Aphids mummified by *Aphelinus* were hyperparasitized by *Syrphophagus aphidivorus* at a rate of approximately 90%. *Aphelinus* and Coccinellidae maintained high activity on the resistant hybrid for a longer time period than on the susceptible hybrid (a hybrid by time period interaction was detected).

Overall, the existing community of aphidophagous insects in Texas appears to have responded rapidly to the sugarcane aphid's introduction. Natural enemy species recorded, with few exceptions, were observed on aphid-infested sorghum in both juvenile and adult life stages. Thus, these species are able to both locate sugarcane aphid outbreaks and exploit aphids across multiple generations. The continuity of species composition and population trends observed across both counties is evidence that the observed natural enemy response is not an isolated event. Natural enemy densities were similarly proportionate to aphid densities on both aphid-susceptible and aphid-resistant plants, suggesting that local natural enemies of the sugarcane aphid can complement host plant resistance.

2.2.   Introduction

The sugarcane aphid, *Melanaphis sacchari* (Zehntner), is an emergent sorghum pest in the southern United States. The aphid was first reported on sorghum in 2013 in southeast Texas (Bowling et al. 2015a). Within the same year, the aphid was later recorded in Louisiana, Oklahoma, and Mississippi. In 2014, the

sugarcane aphid was present in 12 states. As of fall 2015, the aphid had spread to a total of 17 states that together account for >95% of sorghum production in the US (USDA-NASS 2016). Its geographic range within the US extends south into Florida and Texas, north to Illinois and Kansas, and east-west from the lower east coast to the Great Plains. The aphid has also been reported in the Caribbean Islands and throughout the sorghum-growing regions of Mexico (Bowling et al. 2016).

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The sugarcane aphid is an established pest on sorghum and sugarcane (*Saccharum officinarum* (L.)) throughout much of Asia, Africa, Australia, and Central and South America (Singh et al. 2004; Zehntner 1897). The aphid was not recorded in North America until 1977, when it was found on Florida sugarcane (Mead 1978).

The current distribution of the aphid on sugarcane within the United States is limited to Hawaii, Florida, Texas, and Louisiana (Bowling et al. 2016; Singh et al. 2004). The sugarcane aphids that have spread throughout US sorghum since 2013 do not readily colonize sugarcane. Whether this host discontinuity is the result of a host switch originating within the US, or a separate invasion by a foreign biotype, is not yet clear (Bowling et al. 2016). However, genotyping with microsatellite markers has shown one aphid biotype to be predominant on sorghum in the US. This suggests that the outbreak has been caused largely by one asexual clone (Harris-Shultz et al. 2017).

Sugarcane aphid colonies are founded by alate adults (foundresses). Aphids initially colonize leaves of sorghum during vegetative growth. As aphids reproduce and new leaves emerge from the whorl, colony expansion typically develops from the lower to the upper part of the plant (Bowling et al. 2016; Villanueva et al. 2014). The majority of aphids are concentrated on the underside of leaves near the midrib; however, in very heavy infestations, sugarcane aphids have also been observed feeding on the stalk and grain sorghum head (Brewer et al. 2016). The aphids develop and reproduce most rapidly under warm, dry conditions (Brewer et al. 2016; Singh et al. 2004). Nymphs mature within 4-12 days (Chang et al. 1982), and each adult can produce 34-96 progeny over the course of its 2-5 week lifespan (Singh et al. 2004). A single heavily-infested plant can harbor 10,000-30,000 aphids (Bowling et al. 2016; Setokuchi 1977). Heavy aphid infestation causes early leaf chlorosis and necrosis, delayed or aborted panicle development, rapid decline in overall plant health, and reduction in quantity and nutritional quality of grain and forage yield

(Brewer et al. 2016; Villanueva et al. 2014; Singh et al. 2004). Sugarcane aphids also coat leaves with honeydew, which may interfere with grain harvest (Villanueva et al. 2014).

One means of preventing or reducing aphid damage is to cultivate sorghum varieties that have sugarcane aphid resistance (Sharma 1993). Since 1980, dozens of sugarcane-aphid-resistant sorghum varieties have been reported from various countries, particularly India (Mbulwe et al. 2016; Singh et al. 2004; Sharma 1993).

The sugarcane aphid has many natural enemies that may complement host plant resistance by acting as biological controls. Natural enemies that recruit to an aphid colony early in that colony's growth cycle can limit population growth and prevent aphids from causing economic damage (Colares et al. 2015a). A diverse assemblage of insects feed on the sugarcane aphid, including lady beetles (Coleoptera: Coccinellidae), hoverfly larvae (Diptera: Syrphidae), predatory gall midge larvae (Diptera: Cecidomyiidae), aphid fly larvae (Diptera: Chamaemyiidae), green lacewing larvae (Neuroptera: Chrysopidae), brown lacewing larvae (Neuroptera: Hemerobiidae), minute pirate bugs (Hemiptera: Anthocoridae), predatory seed bugs (Hemiptera: Lygaeidae), damsel bugs (Hemiptera: Nabidae), and parasitoid wasps (Hymenoptera: Aphelinidae, Braconidae) (Brewer et al. 2016; Colares et al. 2015a, Singh et al. 2004).

To develop effective and cost-efficient integrated pest management strategies for the sugarcane aphid, it is important to identify local aphid predators and parasitoids, and to evaluate whether and how they respond to this novel aphid on sorghum in the US. Natural enemies that are already native or naturalized within

sorghum-producing regions of North America can complement conventional control measures—such as aphid-resistant cultivars or appropriate insecticides—at little or no additional cost. Natural enemies that are able to attack sugarcane aphid colonies early in the season, while aphid densities are low, can suppress aphid populations by maintaining the aphid mortality rate above the aphid population growth rate (Obrycki et al. 2009; Rutledge and O'Neil 2005; Hindayana et al. 2001; Messina and Sorenson 2001; Obrycki and Kring 1998; Dean and Wilding 1973). In addition to causing aphid mortality through predation, natural enemies also disrupt aphid colonies indirectly through non-consumptive effects. A colony disturbed by predators or parasitoids will engage in defensive behaviors, which divert aphids' time and energy away from feeding and reproducing (Rutledge and O'Neil 2005). Surveying the natural enemies that recruit to aphid-infested sorghum over time may help to elucidate which species have the potential to be conservation biological controls.

To characterize this agroecosystem, this research was designed with the following objectives: A) to catalog the range of insect species that feed on the aphid in sorghum fields; B) to track relative abundance of natural enemies and aphids over the course of the growing season; and C) to determine whether natural enemies species composition and abundance differs between aphid-resistant and aphid-susceptible sorghum hybrids.

2.3. Methods

2.3.1. Field Locations

Research was conducted in 2015 and 2016 at two field locations: one in south Texas and one in central Texas. The south Texas research site was the Texas A&M Agrilife Research & Extension Center in Corpus Christi, Nueces County, TX (hereafter referred to as Nueces, or the Nueces County site). The Nueces County field plots for 2015 (GPS coordinates: 27.769908° N, -97.562039° W) were sampled from May through August. The 2016 Nueces County plots (27.774322° N, -97.560572° W) were sampled from June through August. The central Texas research site was the Texas A&M University Farm in Somerville, Burleson County, TX (hereafter referred to as Burleson, or the Burleson County site). In 2015, the Burleson County field plots (30.523613° N, -96.401023° W) were sampled from August through October. The 2016 Burleson County plots (30.539592° N, -96.421537° W) were sampled from June through September.

Burleson County
Neuces County
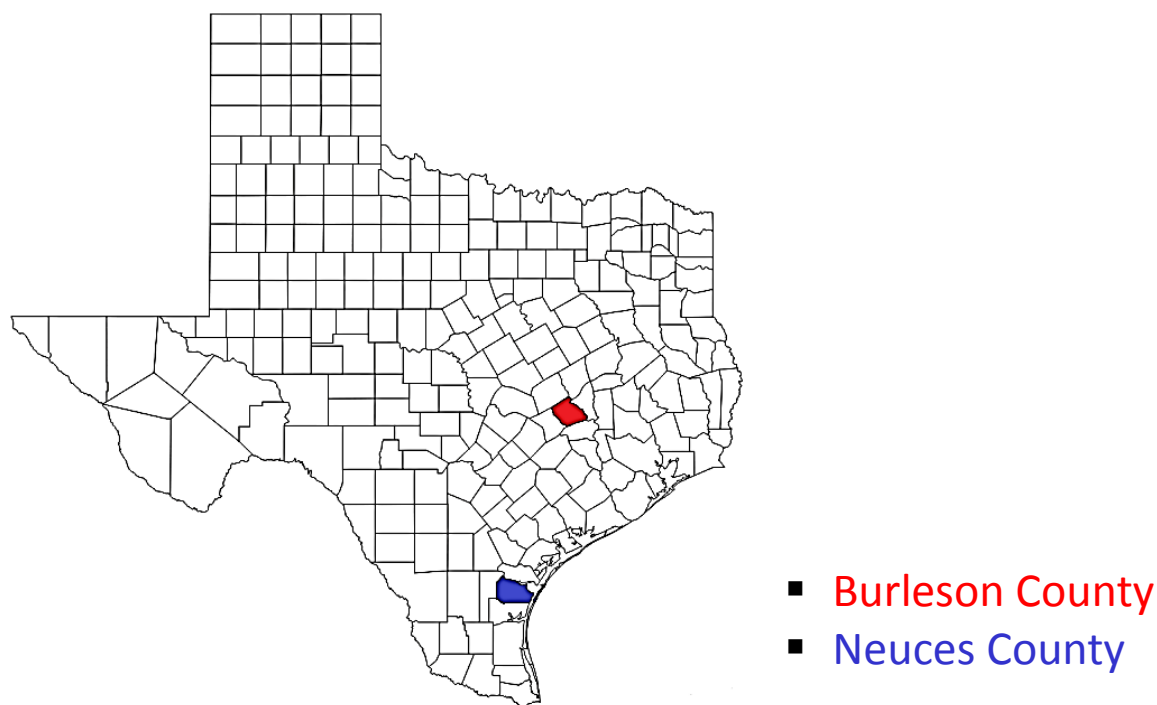
Fig. 2. Map of Texas counties highlighted to show the relative locations of the two field sites: the Texas A&M University Farm, Burleson County (red); and the Texas A&M AgriLife Research and Extension Center at Corpus Christi, Nueces County (blue).

2.3.2. Field Plot Design

In 2015 at both study sites, 8 sorghum plots were planted in a randomized complete block design of two hybrids replicated four times. Each plot contained 12 rows of sorghum, with between-row spacing of 96.52 cm and row length of 12.19 m. In Nueces County, the sorghum was planted in April 2015. In Burleson County, the sorghum was planted in July 2015. The plots consisted of the following sorghum hybrids: Dekalb DKS 53-67 (Monsanto, St. Louis, MO), an aphid-susceptible hybrid; and Dekalb DKS 37-07 (Monsanto, St. Louis, MO), a hybrid resistant to sugarcane aphid.

In April 2016 at the Nueces County site, 12 sorghum plots were planted in a randomized complete block design of three hybrids replicated four times. Each plot contained 6 rows of sorghum, with between-row spacing of 96.52 cm and row length of 9.14 m. The plots consisted of the following hybrids: ATx3408/RTx2783 (Resistant x Resistant cross); ATx3408/RTx436 (Resistant x Susceptible); and ATx631/RTx2783 (Susceptible x Resistant). In May 2016 at the Burleson County site, 20 sorghum plots were planted in a randomized complete block design of four hybrids replicated five times. Each plot contained 6 rows of sorghum, with between-row spacing of 96.52 cm and row length of 12.19 m. The plots consisted of the following sorghum hybrids: ATx3408/RTx2783 (Resistant x Resistant cross); ATx3408/RTx436 (Resistant x Susceptible); ATx631/RTx2783 (Susceptible x Resistant); and ATx631/RTx436 (Susceptible x Susceptible).

Across all field plots, no insecticide seed treatments or foliar insecticides were used for the duration of the experiment. Otherwise, agronomic practices standard for the growing regions were used.

2.3.3. Data Collection

Sampling was conducted once per week, beginning when vegetative sorghum had at least two leaves completely unfurled, and terminating when plants were senescent or scheduled for harvest. The majority growth stage of sorghum across all plots was recorded (e.g., vegetative, flag leaf, boot, flowering, soft dough, hard dough, senescent). In each plot, 10 plants were randomly selected. A relatively

healthy (visually >90% of the leaf surface was green) upper leaf and a lower leaf were selected from each plant, excluding the flag leaf.

Numbers of sugarcane aphids and natural enemies on the abaxial (downward-facing) surface of each leaf were recorded by visual observation. If a leaf contained more than 25 sugarcane aphids, the aphid population was estimated using number ranges modified from the Quick Aphid Checker (Bowling et al. 2015b). Populations of 26 to 49 aphids were rounded to 38. Aphid populations estimated to be between 50 and 100 aphids were rounded to the nearest multiple of 25 (i.e., 50, 75, or 100 aphids). Estimates between 101 and 800 aphids were rounded to the nearest multiple of 50 (i.e., 150, 200, 250, 300…). Estimated populations of 801 to 1,100 aphids were rounded to the nearest multiple of 100 (i.e., 1,000). Estimates greater than 1,100 aphids were rounded to the nearest multiple of 500. Natural enemies were identified and counted according to the following taxonomic categories: Aphelinidae- and Braconidae-type aphid mummies, Coccinellidae (lady beetles), Syrphidae (hoverflies), Chrysopidae (green lacewings), *Hemerobius* spp. (brown lacewings), and *Orius insidiosus* (minute pirate bugs). The age class of predators was recorded as either juvenile (nymphs, larvae, or pupae) or adult. Eggs were not counted. Due to the difficulty of identifying microhymenoptera in the field, adult wasps were not counted.

2.3.4. Laboratory Rearing of Natural Enemies

In 2015, representative samples of juvenile predators, as well as aphids mummified by braconid and aphelinid parasitoids, were collected from both field locations and reared to maturity in laboratory conditions. All juvenile predators were reared in petri dishes sorted by taxa and collection date of the field observations. Each dish was provisioned with live sugarcane aphids as food and sorghum leaf cuttings as a moisture source, both of which were restocked as needed. Predators and parasitoids that developed to maturity were identified to species using specimens at the Texas A&M University Insect Collection, published keys (Havelka et al. 2012; Gordon 1985), unpublished pictorial keys (J. Michaud, pers. comm.), and sending select specimens for identification to experts (see acknowledgements). Voucher specimens of all natural enemy species were deposited in the Texas A&M University Insect Collection (voucher #723).

2.3.5. Statistical Analysis

Field count data were analyzed separately for 2015 and 2016 and for the Nueces County and Burleson County locations. The decision to separate analyses was based on preliminary statistics that indicated substantial variability of aphid and natural enemy abundance across years and locations. Plot averages for each observation week were calculated to obtain an estimate of the number of aphids per lower and upper leaf and average aphids per leaf value. The same was done for

each natural enemy taxon and separately for adults, juveniles, and combined life stages (e.g., average adult coccinellids per leaf, average juvenile coccinellids per leaf, and average adult + juvenile coccinellids per leaf). Last, the mean aphids per leaf and natural enemies (by taxon) per leaf data were used to obtain the ratio of number of natural enemies per aphid per leaf. Preliminary analyses indicated no consistent difference in aphid, natural enemy, and adult and juvenile natural enemy counts between upper and lower leaves. Therefore, data from upper and lower leaves were combined and the upper/lower leaf distinction was excluded from statistical analysis. The estimated aphids per leaf, natural enemies per leaf (by taxon), and ratio of each natural enemy taxon per aphid per leaf were analyzed in a repeated measures ANOVA that conformed to the experimental design of a randomized complete block of the sorghum hybrid treatments and weekly observations used as the repeated measures.

2.3.6. Measuring Hyperparasitism of *Aphelinus* Mummies

During the experiments above, hyperparasitoids were detected when rearing the Aphelinid-type mummies. To determine the hyperparasitism rate of these mummies by the hyperparasitoid, *Syrphophagus aphidivorus*, unemerged *Aphelinus* mummies were collected from sorghum at the Burleson County field site in 2016 and reared in the lab. Mummies were collected from patches of sorghum with relatively high mummy density (24 per leaf or more). Collection began when high mummy densities were observed, and stopped once it became difficult to find leaves with 12

or more mummies. Because mummies were collected opportunistically based on density, we did not test for a relationship between hyperparasitism and plant hybrid type.

Using a fine brush or fine-point forceps, each mummy was transferred from the leaf to an individual rearing well in a tray of 96 wells. Each well was plugged with a neoprene stopper. The trays were then sealed in zipped plastic bags along with a damp paper towel to prevent desiccation. The bags were stored at room temperature. Mummies were kept for twenty days to allow adult emergence. Adult parasitoids were identified and counted. The following data were recorded for each collection date: total number of mummies collected, number of adult parasitoids emerged, number of emerged *Aphelinus* sp., and number of emerged *Syrphophagus aphidivorus*.

2.4. Results and Discussion

2.4.1. Natural Enemy Species Composition

A total of 22 natural enemy species and morphospecies were identified. Both Nueces and Burleson had nearly identical species composition. Almost all species (excluding *Leucopis argentata* and *Lysiphlebus testaceipes*) were observed on aphid-infested sorghum in both juvenile and adult stages, indicating that adults were able to locate sugarcane aphid populations and subsequently reproduce on aphid-infested plants. Additionally, specimens of all natural enemy species (except *L.*

argentata) were collected from the field as juveniles and subsequently reared on a strict sugarcane aphid diet in laboratory conditions. Juveniles of each laboratory-reared species survived and completed adult development. This suggests that these established natural enemies possess the necessary preadaptations to use sugarcane aphids as a food source (Colares et al. 2015b). Within each broad taxonomic group, the following species were observed.

Coleoptera: Coccinellidae (lady beetles) (Fig. 3): *Coccinella septempunctata* (Linnaeus) (Fig. 3D), *Coleomegilla maculata* (DeGeer) (Fig. 3E), *Cycloneda sanguinea* (Linnaeus) (Fig. 3A-B), *Harmonia axyridis* (Pallas) (Fig. 3F), *Hippodamia convergens* (Guérin-Méneville) (Fig. 3C), *Olla v-nigrum* (Mulsant) (Fig. 3G), and three dusky lady beetle (subfamily Scymninae) morphospecies (Fig. 3H-J)

Lady beetles have been intentionally introduced into agricultural systems to control aphid controls for more than a century (Obrycki et al. 2009; Obrycki and Kring 1998). Both larvae and adults are voracious predators of aphids and other soft-bodied insect crop pests. The time it takes for most lady beetles to develop from egg to adult is comparable to the longevity of a single aphid colony (Kindlmann and Dixon 1993). In order to provide their offspring with adequate food, adult female lady beetles preferentially oviposit in aphid colonies when or before the aphid population reaches its peak (Koch 2003). To further ensure that they have enough nutrition to complete development, coccinellid larvae engage in cannibalism, especially when aphid densities are low (Kindlmann and Dixon 1993).
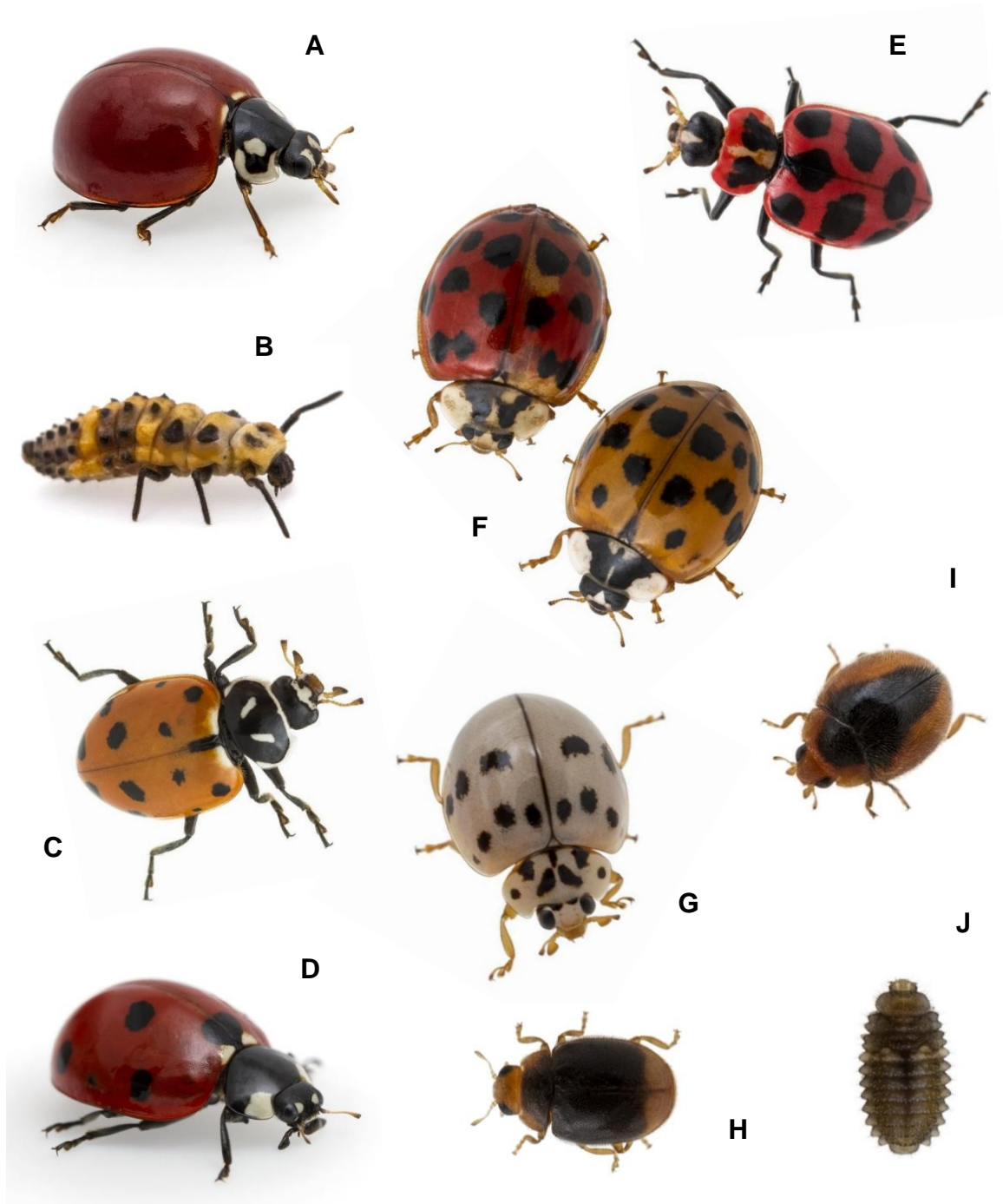
Fig. 3. Common lady beetles (Coleoptera: Coccinellidae) collected from sugarcane aphid colonies on sorghum. A: *Cycloneda sanguinea*; B: *C. sanguinea* larva; C: *Hippodamia convergens*; D: *Coccinella septempunctata*; E: *Coleomegilla maculata*; F: *Harmonia axyridis*, examples of color polymorphism; G: *Olla v-nigrum*, light morph; H: dusky lady beetle (Coccinellidae: Scymninae) morphospecies 1; I: dusky lady beetle morphospecies 2; J: dusky lady beetle larva.

Harmonia axyridis and *Coccinella septempunctata*, which were ubiquitous in both field locations, are non-native species that have become established throughout much of North America (Obrycki and Kring 1998). *H. axyridis* is a generalist predator of many crop pests including aphids, scale insects, spider mites, and insect larvae (Koch 2003). Among aphid-eating insects, this species is also one of the top intraguild predators: it frequently preys on conspecifics and other predators (e.g. syrphids, chrysopids, other coccinellids) when aphid density is low (Koch 2003). Although *H. axyridis* was the numerically predominant large (non-Scymnine) coccinellid in 2015, it was conspicuously scarce in both field sites in 2016.

Harmonia axyridis and *Hippodamia convergens* overwinter as adults and are consequently a potential source of early-season aphid predation (Obrycki et al. 2009; Obrycki and Kring 1998). However, in 2015, very few beetles of either species were observed on experimental sorghum until June. Few lady beetles were observed throughout 2016, though their low numbers in that year may have been influenced by the extreme scarcity of sugarcane aphids from spring through mid-summer.

The coccinellid *Olla v-nigrum* has two discrete color morphs: light grey with a variable number of black spots, and black with two red spots (Gordon 1985). Only the light morph was observed.

Four of the lady beetle species present at the Nueces and Burleson sites have been reported to colonize sorghum infested with sugarcane aphid in Kansas: *H. convergens*, *C. septempunctata*, *C. maculata*, and *H. axyridis*, with *H.*

convergens comprising more than 90% of the adult coccinellids (Colares et al. 2015a). *H. convergens* and *C. maculata* from Kansas have been successfully reared on a controlled diet of sugarcane aphids (Colares et al. 2015b). In the current study, larvae of all nine lady beetle species collected from Nueces and Burleson, including the four species known to feed on the aphid in Kansas, were successfully reared to maturity on a sugarcane aphid diet. This suggests that these lady beetle species are pre-adapted to exploit the sugarcane aphid as prey (Colares et al. 2015a).

Diptera: Chamaemyiidae (aphid flies): *Leucopis argentata*

Aphid flies, also known as silver flies (Diptera: Chamaemyiidae), are larval predators of aphids, scale insects, and mealybugs (Gaimari and Turner 1997). Although they have been found preying on aphids in agricultural systems (Kaiser et al. 2007), little research has been done on their efficacy as aphid biological controls, and much of their ecology is still unknown (Satar et al. 2015). The only evidence of aphid fly activity in this study consisted of two pupae collected from sorghum in Burleson County in 2015. One adult fly emerged; the other pupa yielded a parasitoid wasp.

The sole chamaemyiid specimen was identified as *Leucopis argentata* (Heeger), a specialist predator of the aphid *Hyalopteris pruni* on Common Reed (*Phragmites australis*) (Stephen Gaimari, pers. comm.). The 2015 Burleson County plots were planted in a riparian area, across the road from a pond. The specimen could have migrated from a reed to sorghum as a larva; however, aphid fly larvae normally feed and pupate on a single host plant (Krsteska 2015). Therefore, this

specimen provides only dubious evidence of aphid fly predation on sugarcane aphids.

Diptera: Syrphidae (hoverflies) (Fig. 4): *Allograpta obliqua* (Say) (Fig. 4A-B), *Eupeodes americanus* (Wiedemann) (Fig. 4C-D), and *Pseudodorus clavatus* (Fabricius) (Fig. 4E)

The larvae of aphidophagous hoverflies are voracious predators (Belliure and Michaud 2001; Hindayana et al. 2001). Each larva can consume approximately 50 aphids (Belliure and Michaud 2001). Adult syrphids are not predatory, but instead feed on plant products including nectar, pollen, and honeydew (Belliure and Michaud 2001). Adult female hoverflies require protein from pollen in order to produce viable eggs. Hoverflies can be highly effective aphid biological control agents if there are sufficient floral resources to support their reproduction (Chambers and Adams 1986). *Pseudodorus clavatus* is a particularly suitable candidate for aphid control due to its combination of high voracity and high fecundity (Belliure and Michaud 2001).

Allograpta obliqua comprised the majority of syrphid larvae and pupae on sorghum, and was particularly abundant in Nueces in 2015. *A. obliqua* has also been the predominant syrphid species recorded on sorghum infested with sugarcane aphids in Kansas (Colares et al. 2015a). Additionally, although adult syrphids rarely alighted on sorghum leaves and were not included in natural enemy counts, *A. obliqua* adults were frequently observed hovering throughout the sorghum fields. In contrast, adult *E. americanus* and *P. clavatus* were rarely encountered.
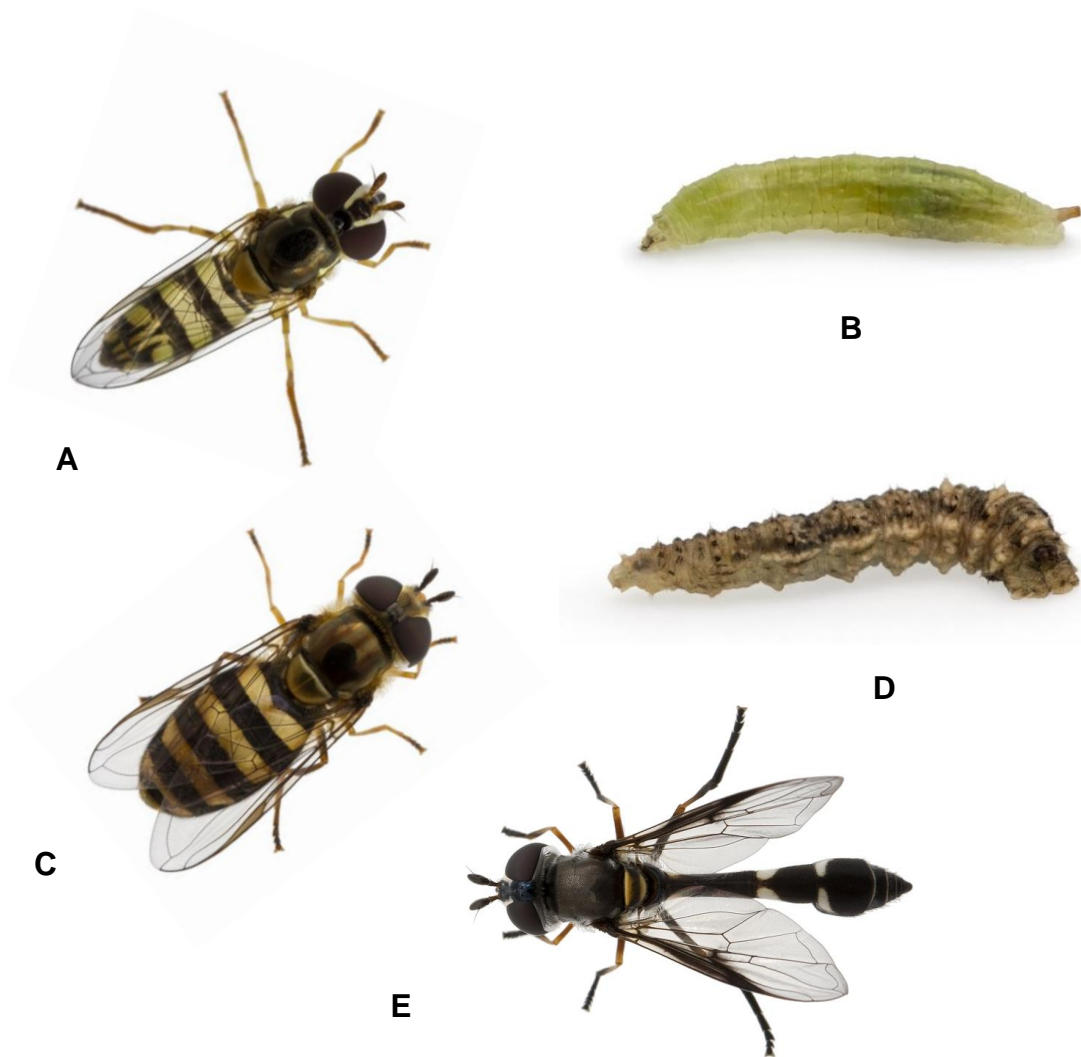
Fig. 4. Hoverflies (Diptera: Syrphidae) collected from sugarcane aphid colonies on sorghum. A: *Allograpta obliqua*; B: *A. obliqua* larva; C: *Eupeodes americanus*; D: *E. americanus* larva; E: *Pseudodorus clavatus*.

Laboratory-reared larvae of all three syrphid species were able to complete their development when fed only sugarcane aphids. Moreover, the syrphids killed and consumed aphids at such a rapid rate that, out of all the natural enemy families in

this study, lab-reared Syrphidae had to be resupplied with fresh aphids the most frequently (Maxson, pers. obs.).

At the Burleson Co. field site in 2016, the majority of adult syrphids observed in the spring and early summer were *Toxomerus* sp. However, local sugarcane aphid populations did not surpass 0.5 aphids/leaf until mid-August, by which time very few *Toxomerus* sp. were seen. Due to limited availability of sugarcane aphids throughout the period of peak *Toxomerus* activity, no *Toxomerus* larvae were reared. Larvae of some *Toxomerus* species are specialist pollen feeders rather than predators (Reemer and Rotheray 2008). This study therefore cannot verify whether the *Toxomerus* observed in 2016 were feeding on sugarcane aphids.

Hemiptera: Anthocoridae: *Orius insidiosus* (Say) (Fig. 5)

The minute pirate bug (*Orius insidiosus*) was present in trace numbers in both Texas field sites. *O. insidiosus* has also been detected feeding on sugarcane aphid in Kansas (Colares et al. 2015a). *O. insidiosus* is native to the United States (Rutledge and O'Neil 2005), and may be the most abundant anthocorid in North America (Dicke and Jarvis 1962) as well as one of the most ubiquitous insect predators in agroecosystems (Kiman and Yeargan 1985). The minute pirate bug is a polyphagous predator in all instars. It preys on a wide range of pests in agricultural systems, including aphids, thrips, spider mites, whiteflies, Lepidoptera, midges, and arthropod eggs (Rutledge and O'Neil 2005; Kiman and Yeargan 1985; Dicke and Jarvis 1962). Despite its diminutive size (<2.5mm), it sometimes attacks and feeds on arthropods larger than itself (Dicke and Jarvis 1962). Thrips and host plant pollen

27

are key determinants of *O. insidiosus* density, as pirate bug populations peak when these two food sources are readily available (Rutledge and O'Neil 2005).

    O. insidiosus nymphs have bright yellow-orange bodies that become dark brown in later instars. Because early-instar nymphs are similar in size, shape, and color to sugarcane aphids, they may have been under-reported in field counts. Adult pirate bugs are more conspicuous due to their black color and wings. However, adults were also difficult to accurately count due to their tendency to fly away when leaves were disturbed (E. Maxson, pers. obs.).
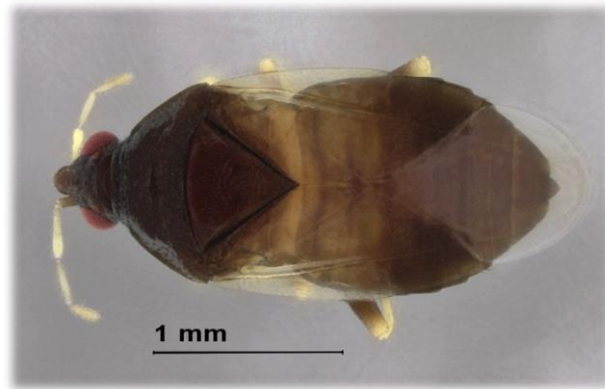
Fig. 5. Adult minute pirate bug (*Orius insidiosus*) collected from sugarcane aphid colony on sorghum.

Hymenoptera: *Aphelinus* sp*. varipes* species group (Fig. 6A-B), *Syrphophagus aphidivorus* (Fig. 6C-D), *Lysiphlebus testaceipes* (Fig. 6E-F)

    Three wasp species were observed to have trophic interactions with the sugarcane aphid. Two are parasitoids that preyed on the aphid directly:

Aphelinus sp. *varipes* group (Chalcidoidea: Aphelinidae) and *Lysiphlebus*

testaceipes (Ichneumonoidea: Braconidae). The third, *Syrphophagus aphidivorus*

(Chalcidoidea: Encyrtidae), was not a natural enemy of the aphid, but was a very

common hyperparasitoid of *Aphelinus*.

     Aphelinus varipes has a near-global distribution and has been used

successfully as a biocontrol of several agriculturally important aphid species,

including *Diuraphis noxia*, *Ropalosiphum padi*, and *Aphis glycines* (Hopper et al.

2005). There is strong evidence, however, that *Aphelinus varipes* is a complex of

cryptic species (Heraty et al. 2007). The *A. varipes* complex has a host range of at

least 40 aphid species across multiple genera, but the true host range of each

species is likely much smaller (Hopper et al. 2005). *Aphelinus* specimens reared

from *M. sacchari* appear to comprise a single species in the *A. varipes* complex.

Adult female *Aphelinus* cause aphid mortality both indirectly through parasitism, and

directly through host feeding (Hopper et al. 2005). *Aphelinus* larvae would fail to

develop in aphids that are killed by adult host feeding; consequently, *Aphelinus* host

feed on and oviposit into separate aphids (Boyle and Barrows 1978). A female

inserts one egg into each host aphid (Christiansen-Weniger 1994). The wasp's

progeny kills and mummifies its host during its last larval instar. During

mummification, the aphid's integument sclerotizes into a husk and its exocuticle

incorporates dark pigments that give the mummy a distinct blue-black color (Fig. 6-

B). The wasp completes pupation within the mummy and emerges as an adult.

A

© Jim Woolley

B

© Travis Ahrens

C

© Jim Woolley

D

E

1 mm

© Jewel Coffey

F

1 mm

© Jewel Coffey
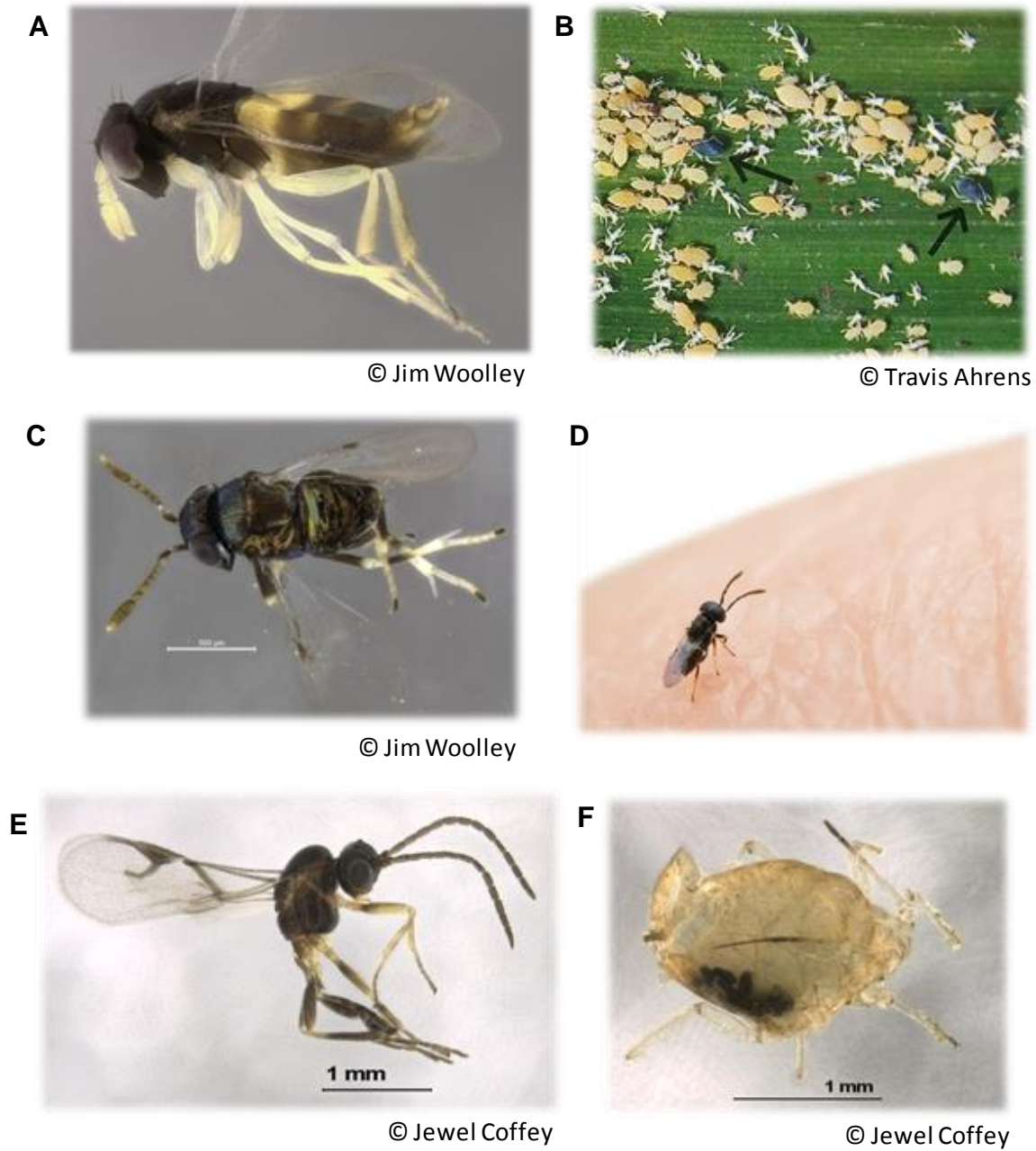
Fig. 6. Parasitoid and hyperparasitoid wasps (Hymenoptera) reared from sugarcane aphids. A: *Aphelinus* sp. *varipes* group; B: Sugarcane aphids mummified by *Aphelinus* sp. (arrows); C: *Syrphophagus aphidivorus*, a hyperparasitoid of *Aphelinus*; D: Live *S. aphidivorus* on author's finger; E: *Lysiphlebus testaceipes*; F: aphid mummified by *L. testaceipes*, showing characteristic tan color and swollen shape. Adult *L. testaceipes* had already emerged; meconium (larval frass) and exit hole are visible.

Lysiphlebus testaceipes (Hymenoptera: Braconidae) is a globally distributed,

oligophagous parasitoid that preys on more than 45 aphid species across 16 genera

(Starý et al. 1988). *L. testaceipes* acts as a biological control for many cereal aphids,

especially the greenbug, *Schizaphis graminum* (Rondani) (Elliott et al. 1999). Like

Aphelinus, it inserts one egg per aphid and mummifies its host (Starý et al. 1988);

unlike *Aphelinus*, *L. testaceipes* adults do not host feed (Brewer et al. 2008).

Lysiphlebus mummies can be distinguished from *Aphelinus* mummies by the light

brown, swollen appearance of the mummy (Fig. 6F) (Christiansen-Weniger 1994).

Across field locations and sorghum hybrids, *L. testaceipes* mummies were much

rarer than mummies of *Aphelinus*. The low number of *L. testaceipes* mummies may

be influenced by the presence of the bacterial endosymbiont, *Hamiltonella defensa*,

in the North American sugarcane aphid population (Colares et al. 2015a). The *H.

defensa* strain known to occur in *M. sacchari* appears to confer resistance to

parasitism by *L. testaceipes* but not *Aphelinus* (Colares et al. 2015b). Despite the

presence of *H. defensa*, some field-collected *L. testaceipes* mummies were

successfully reared to maturity. Some *L. testaceipes* may have been able to develop

on *M. sacchari* due to the fact that *H. defensa*'s ability to kill parasitoids is

temperature-dependent and compromised by heat stress (Guay et al. 2009). It is

also possible that the successful *L. testaceipes* developed on *M. sacchari* clones

that failed to inherit *H. defensa*.

Laboratory rearing revealed that many *Aphelinus* mummies were

hyperparasitized by *Syrphophagus aphidivorus* (Hymenoptera: Encyrtidae).

Syrphophagus aphidivorus is ubiquitous in many agroecosystems, both as a primary

parasitoid of Syrphidae (Japoshvili 2007; Liu et al. 2004; Belliure and Michaud 2001)

and as a secondary parasitoid of aphids that have already been parasitized by other

wasps (Buitenhuis et al. 2004; Müller et al. 1999). *Syrphophagus aphidivorus* is

relatively unique among hyperparasitoids in that it is not restricted to ovipositing into

hosts of a particular instar. The hyperparasitoid can successfully develop in

Aphelinus regardless of whether oviposition occurred while the *Aphelinus* host was a

larva in a living aphid or a pupa in an aphid mummy (Buitenhuis et al. 2004).

Syrphophagus aphidivorus comprised the overwhelming majority of the wasps that

emerged from field-collected, laboratory-reared *Aphelinus* mummies. Although *S.

aphidivorus*'s host range also includes *Lysiphlebus* spp. (Müller et al. 1999), none of

the *L. testaceipes* mummies yielded hyperparasitoids.

Neuroptera (Fig. 7)

Chrysopidae (green lacewings) (Fig. 7A-C): *Ceraeochrysa valida*, *Chrysopa

quadripunctata*, *Chrysoperla externa, Chrysoperla rufilabris*, and *Chrysoperla

plorabunda* species group

Hemerobiidae (brown lacewings) (Fig. 7D-E): *Hemerobius* sp.

Lacewings are well-known aphid biological control agents (New 1975).

Lacewing larvae are polyphagous predators that feed primarily on small arthropods

that are soft-bodied and slow-moving. Adult and larval Hemerobiidae both eat aphids

and have very similar diets overall despite the differences between their mouthparts

and feeding mechanisms. Some chrysopid species are predatory as adults, while

others are predators only as larvae. Although adult hemerobiids and chrysopids

were present on some aphid-infested sorghum leaves, only larvae were ever observed preying on aphids. Chrysopids of all life stages were more abundant on sorghum than were hemerobiids.

Lacewings feeding on sugarcane aphids in sorghum fields in Kansas have been reported to be in the *Chrysoperla carnea* (Stephens) species group (Colares et al. 2015a). Species in this group have also been reared on sugarcane aphid successfully (Colares et al. 2015b). However, no species in the *C. carnea* group were unambiguously detected at the Burleson or Nueces sites. Most female chrysopid specimens could not be identified past genus. Pending the development of female-based Chrysopidae keys, the possibility that some of these unidentified specimens are within the *C. carnea* species group cannot be ruled out. Despite these identification challenges, this study adds to the list of lacewing species known to recruit to and complete development on the sugarcane aphid.
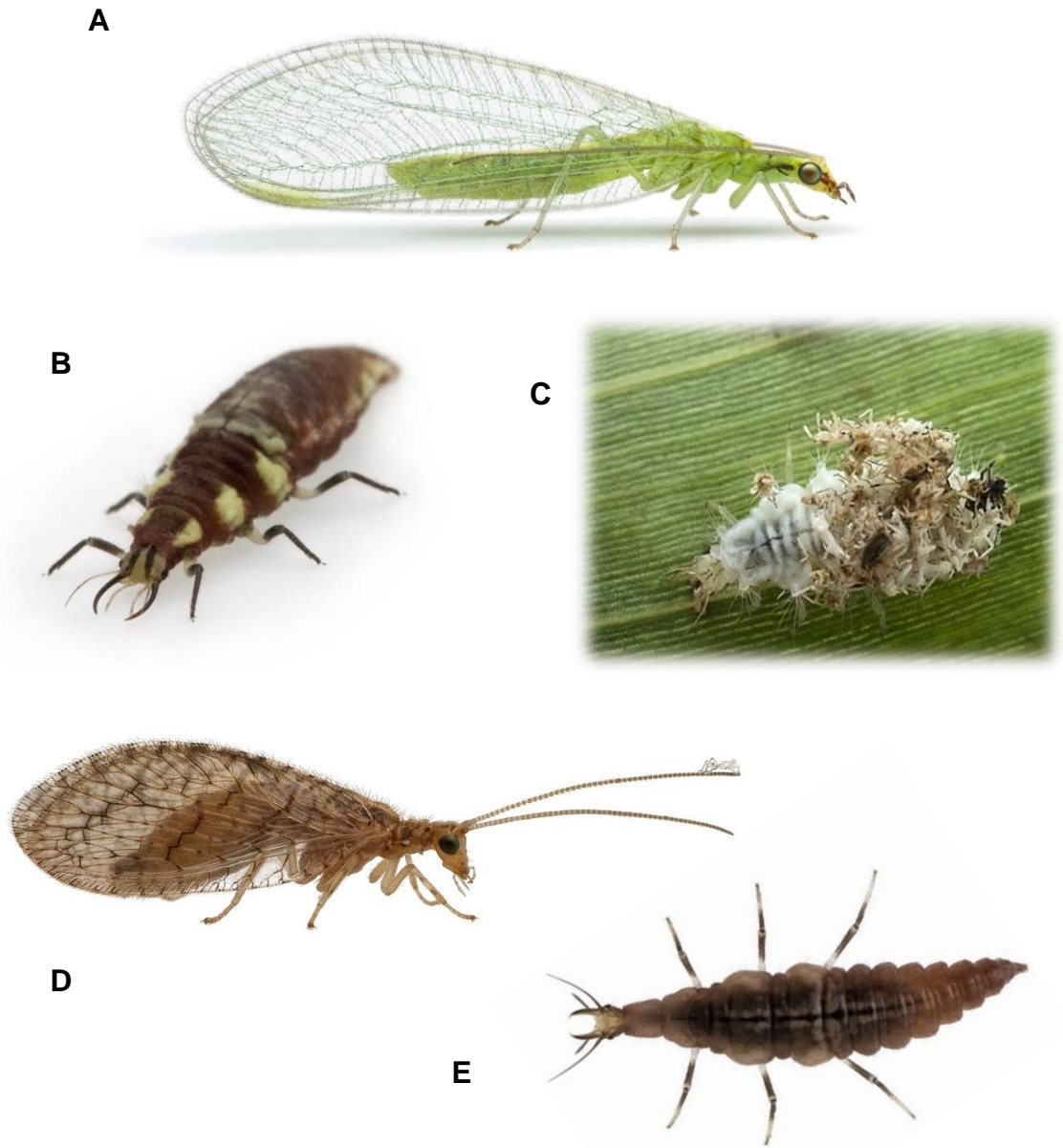
Fig. 7. Lacewings (Neuroptera) collected from sugarcane aphid colonies on sorghum. A: Green lacewing (Neuroptera: Chrysopidae) adult; B: Green lacewing larva; C: Green lacewing larva with a debris cloak of aphid remnants as worn by some species; D: Brown lacewing (Hemerobiidae: *Hemerobius* sp.) adult; E: Brown lacewing larva.

2.4.2. Other Ecological Observations

Melanaphis sacchari was the only aphid species on sorghum observed to undergo exponential population growth. Other aphids—such as corn leaf aphid (*Rhopalosiphum maidis* Fitch), greenbug (*Schizaphis graminum* Rondani), and yellow sugarcane aphid (*Sipha flava* Forbes)—were absent from the majority of leaves, and were never observed in colonies of more than 30-40 aphids per leaf.

The Red Imported Fire Ant (*Solenopsis invicta* Buren) was established throughout both field locations and was frequently observed foraging on sorghum. *S. invicta* is known to tend aphids, defending them from aphidophagous insects in order to gather their honeydew (Kaplan and Eubanks 2002). Fire ants that tend aphid colonies can deter or kill many aphid predators and parasitoids, thereby limiting natural enemies' efficacy as aphid control agents (Kaplan and Eubanks 2002; Vinson and Scarborough 1991). Despite the ubiquity of fire ants at both field locations, aphid-tending behavior was very rarely observed. Sugarcane aphid colonies at the height of their growth cycle excrete large volumes of honeydew, which accumulates on the lower leaves of the host plant. This honeydew is an attractive food source to fire ants and many other opportunistic foragers, such as flies, honey bees, and aculeate wasps. If the volume of honeydew on leaves was sufficient to meet the fire ant colony's resource needs, aphid tending may not have been necessary.

2.4.3. Seasonal Population Trends of Aphids and Natural Enemies on Sorghum

In 2015, peak abundance of sugarcane aphids and natural enemies was much higher on susceptible sorghum (DKS 53-67) than on resistant sorghum (DKS 37-07) in Nueces County during much of the growing season (Fig. 8, hybrid by date interaction: $F = 2.49$; d.f. = 11, 66 ; $P = 0.01$). This interaction reflects the differences in peak abundance of aphids and natural enemies between hybrids at mid-to-late season. No statistically significant interaction was detected for dates where aphid and natural enemy abundances are relatively low. In Burleson, where sorghum was planted in mid-summer and sampled into early fall, peak aphid populations were approximately six-fold higher than peak aphid populations in Nueces, where sorghum was planted in the spring and sampled during a normal grain sorghum production season. At Burleson, aphid populations did not significantly differ between hybrids (Fig. 9, hybrid by date interaction: $P > 0.05$).

Natural enemy composition was similar in both locations. In Nueces, *Aphelinus* and Coccinellidae had significantly higher density on the susceptible hybrid than the resistant hybrid during many observation dates (Fig. 8, *Aphelinus:* hybrid by date interaction: $F = 3.11$; d.f. = 11, 66; $P = 0.002$; Coccinellidae: hybrid by date interaction: $F = 3.29$; d.f. = 11, 66 ; $P = 0.001$). Aphelinid mummy counts weighted on a per aphid basis peaked earlier on the resistant hybrid (Fig. 8-9, hybrid by date interaction: $F = 3.01$; d.f. = 11, 66 ; $P = 0.003$), while weighted coccinellid counts were more consistent across dates on both hybrids (Fig. 8, $P > 0.05$). A few other significant differences were detected for syrphids and lacewings,
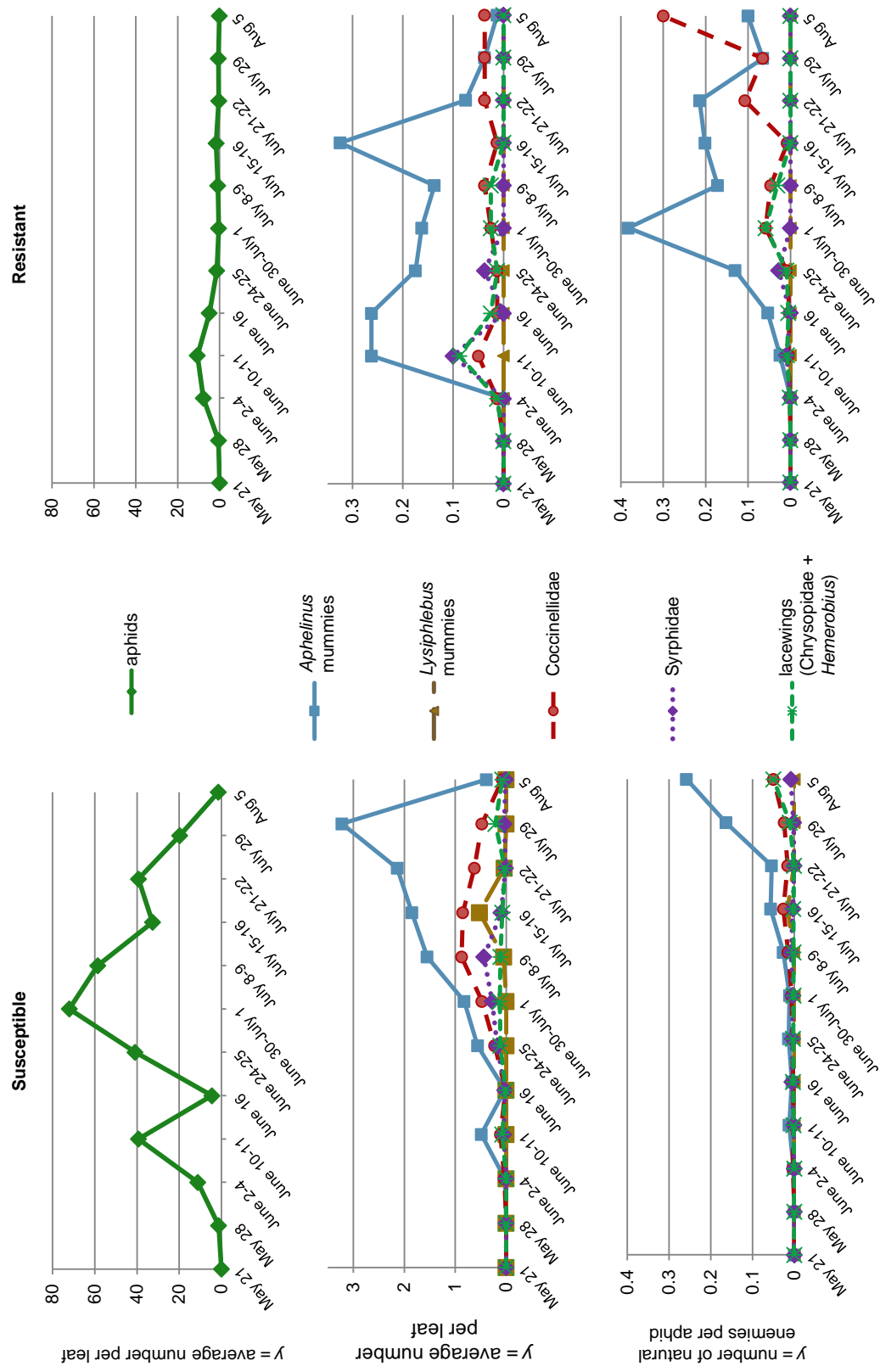
Fig. 8. Sugarcane aphid and natural enemy counts on aphid-susceptible sorghum (Dekalb DKS 53-67) (left column), and on aphid-resistant sorghum (Dekalb DKS 37-07) (right column), TAMU Agrilife Station, Corpus Christi, TX, 2015. Top row: average number of sugarcane aphids per leaf over time. Middle row: average numbers of aphid natural enemies per leaf (*note order-of-magnitude difference between y axes*). Bottom row: ratio of natural enemies to sugarcane aphids.

37
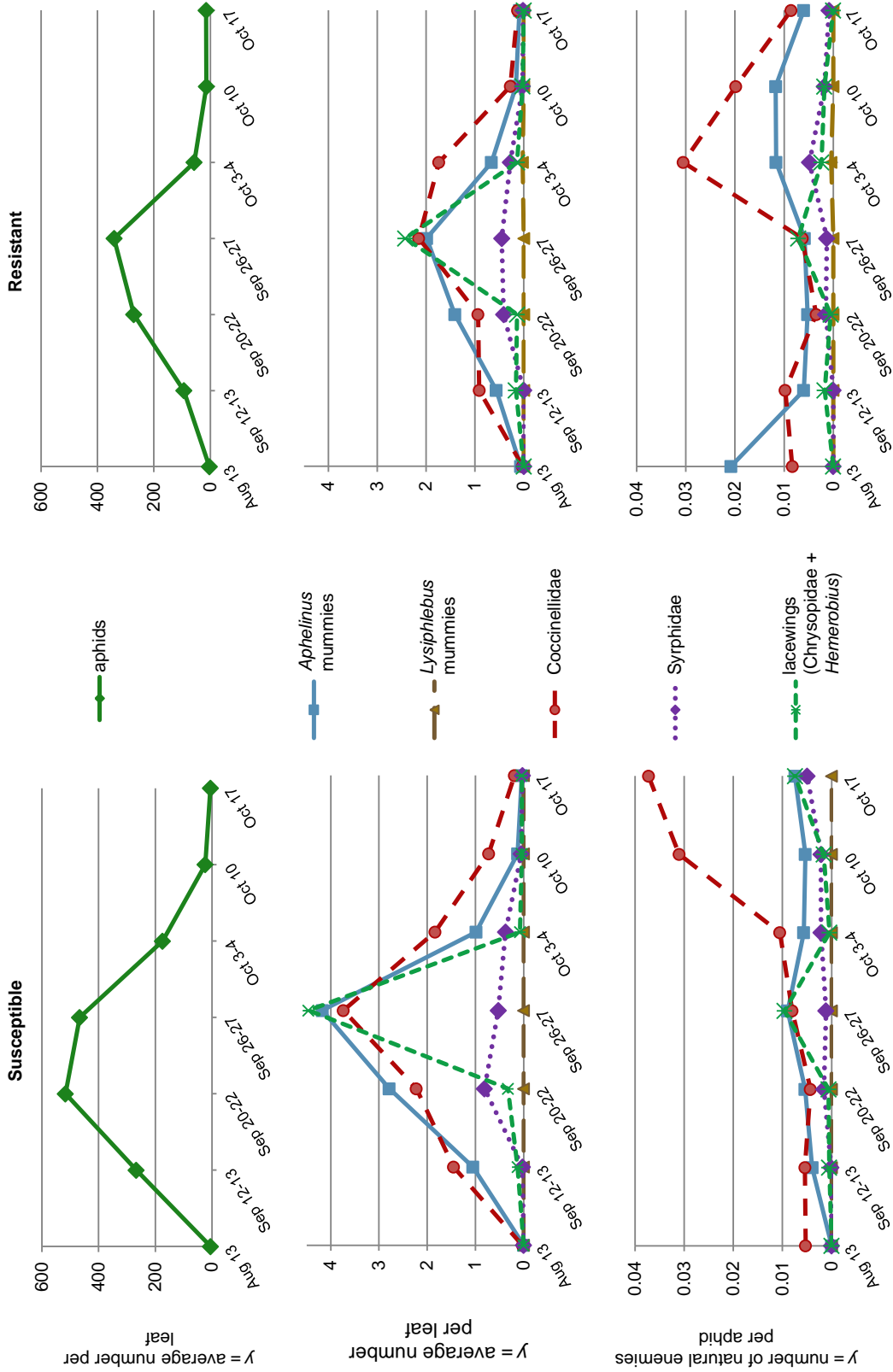
Fig. 9. Sugarcane aphid and natural enemy counts on aphid-susceptible sorghum (Dekalb DKS 53-67) (*left column*), and on aphid-resistant sorghum (Dekalb DKS 37-07) (*right column*), TAMU Farm, Somerville, TX, 2015. Top row: average number of sugarcane aphids per leaf over time. Middle row: average numbers of aphid natural enemies per leaf. Bottom row: ratio of natural enemies to sugarcane aphids.

38

but their population density never exceeded 0.5 per leaf on the susceptible hybrid and 0.1 per leaf on the resistant hybrid.

In Burleson in 2015, natural enemy density was numerically higher on the susceptible hybrid during most observation dates but was highly variable (Fig. 9). Weighted by aphid abundance, natural enemy abundance was an order of magnitude lower at this location than at Nueces. Significant differences in abundance between hybrids were detected only for *Aphelinus. Aphelinus* mummies were more abundant earlier on the susceptible hybrid (Fig. 9, hybrid by date interaction: $F = 2.92$; d.f. = 6, 36; $P = 0.02$), but differences were not detected when *Aphelinus* mummy abundance was weighted by aphid abundance (*P > 0.05*).

In 2016, at Nueces County, aphid populations peaked early (SxR hybrid: May 26th; SxR hybrid: May 26th; RxR hybrid: June 2nd) (Fig. 10). By June 9th, aphid populations had crashed across all hybrids and failed to recover. Aphid densities were relatively low compared to 2015, ranging from less than 10 to 30 aphids per leaf during the first two observation dates (Fig. 10). This trend was repeated across hybrids during each date ($P > 0.05$). The only significant differences in aphid density were detected across observation dates (date main effect: $F = 4.23$; d.f. = 5, 45; $P = 0.003$). From June 9th to July 8th, all hybrids averaged fewer than 0.5 aphids per leaf, with the exception of RxS hybrids on June 17th, where there were 1.5 aphids/leaf. Due to the prolonged and consistent paucity of sugarcane aphids, data collection at Nueces County ended on July 8th. Aphid density peaked at 27 aphids/leaf on June 2nd on the RxR plots (Fig. 10). In contrast, the other two hybrids had peak aphid populations on May 26th: 22.1 aphids/leaf on the SxR hybrid, and only 7.1
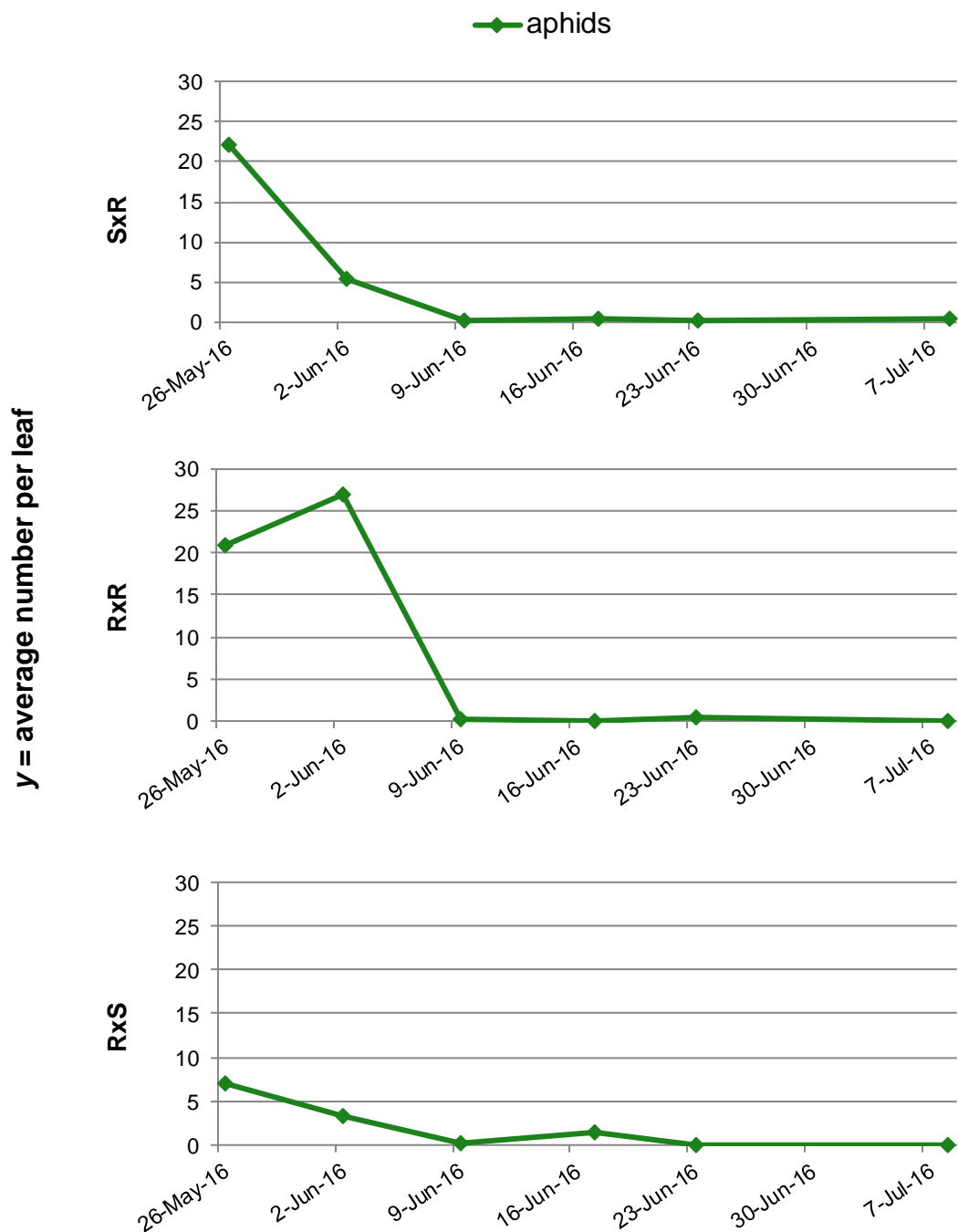
Fig. 10. Average number of sugarcane aphids per leaf over time on susceptible x resistant sorghum hybrid (ATx631/RTx2783) (*top*), resistant x resistant (ATx3408/RTx2783) (*center*), and resistant x susceptible (ATx3408/RTx436) (*bottom*), TAMU Agrilife Station, Corpus Christi, TX, 2016.

aphids/leaf on the RxS hybrid. It is unclear why sugarcane aphid populations were highest on the RxR cross. It is difficult to draw conclusions from the Nueces County 2016 data set, both because the SxS hybrid used in Burleson was unavailable in Nueces, and because aphids across all the Nueces plots failed to achieve the exponential population growth observed in other treatments.

Coccinellid densities in Nueces in 2016 were similar across hybrids on each sample date (Fig. 11; $P > 0.05$). Their densities differed across dates, generally following the same pattern as the aphids (date main effect: $F = 6.41$; d.f. = 5, 45; $P = 0.0001$). All other natural enemy taxa were much lower in abundance (Fig. 11). Differences were detected only across observation dates for syrphids, lacewings, and *Aphelinus* mummies (date main effect: $F < 2.75$; d.f. = 5, 45; $P < 0.04$). The number of natural enemies per aphid was highly variable (Fig. 12), in part due to the low numbers of aphids (Fig. 10). This ratio of natural enemies to aphids did not differ significantly between hybrids or across dates ($P > 0.05$).

At Burleson County in 2016, *Aphelinus* mummies and Coccinellidae followed the general population trends of the aphid (Figs. 13-14). In contrast to Nueces, the Burleson populations of aphids, *Aphelinus*, and Coccinellidae differed significantly in density across hybrids. Aphids reached peak abundance in late August (Fig. 13), when host plants were approaching senescence. Aphids, *Aphelinus* mummies, and coccinellids had greater peak abundance on the RxS and SxS hybrids than on the RxR and SxR hybrids (hybrid by date interactions were detected; aphid: $F = 1.76$; d.f. = 27, 144; $P = 0.02$; Fig. 13; *Aphelinus*: $F = 2.65$; d.f.
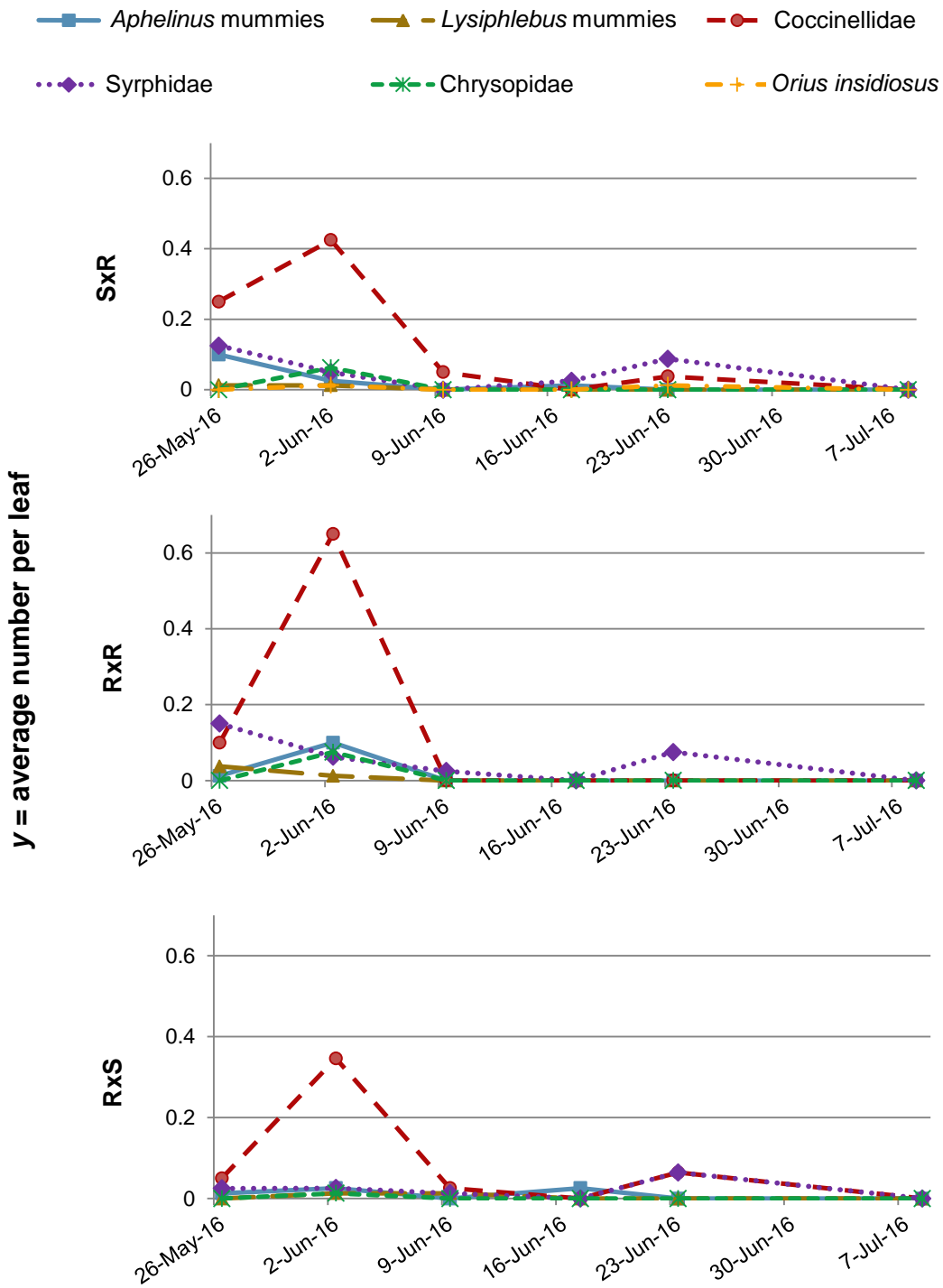
Fig. 11. Average number of aphid natural enemies per leaf over time on susceptible x resistant sorghum hybrid (ATx631/RTx2783) (*top*), resistant x resistant (ATx3408/RTx2783) (*center*), and resistant x susceptible (ATx3408/RTx436) (*bottom*), TAMU Agrilife Station, Corpus Christi, TX, 2016.

42
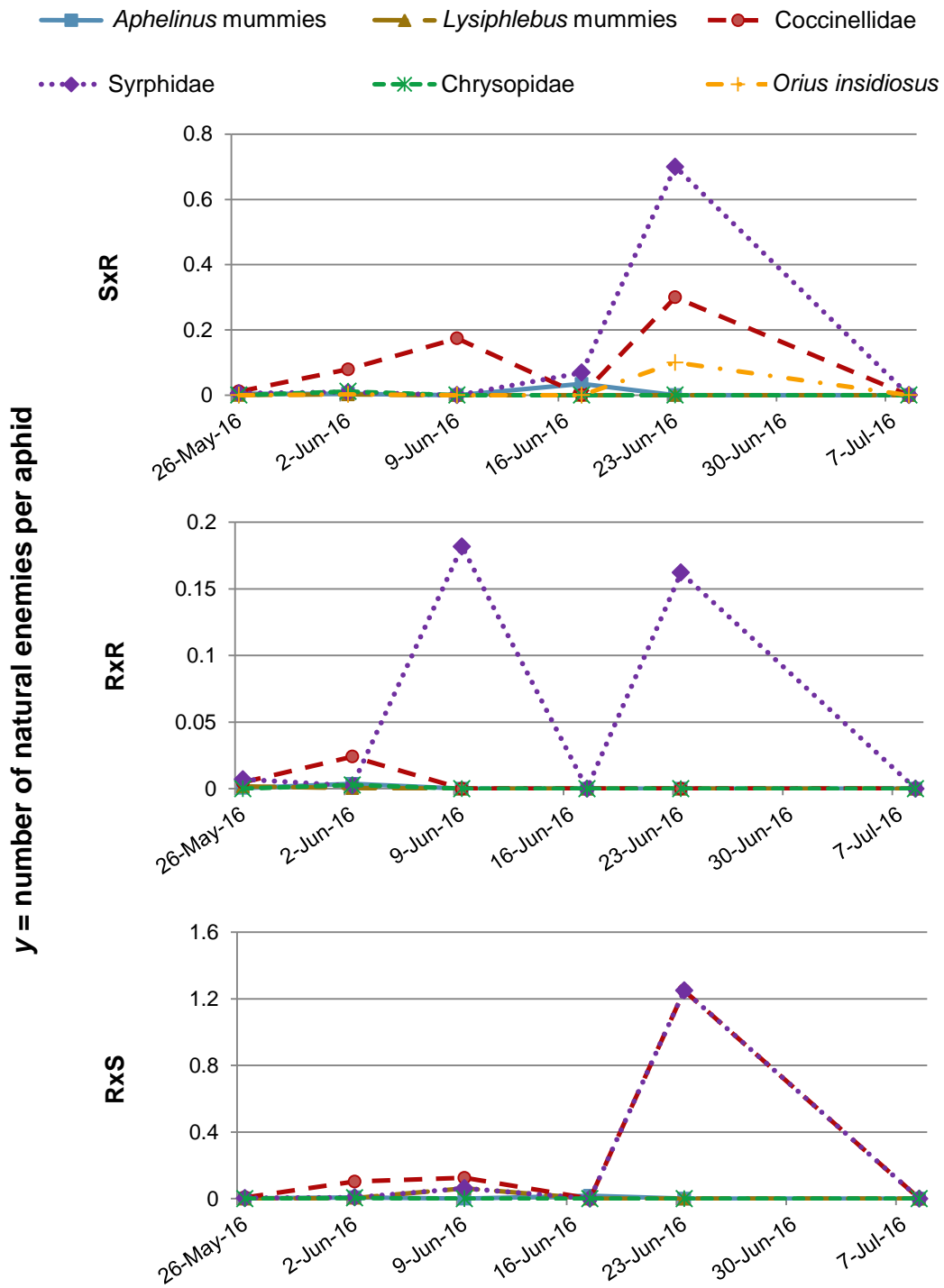
Fig. 12. Ratio of natural enemies to sugarcane aphids on susceptible x resistant sorghum hybrid (ATx631/RTx2783) (*top*), resistant x resistant (ATx3408/RTx2783) (*center*), and resistant x susceptible (ATx3408/RTx436) (*bottom*), TAMU Agrilife Station, Corpus Christi, TX, 2016. Note difference in scale of y axes.
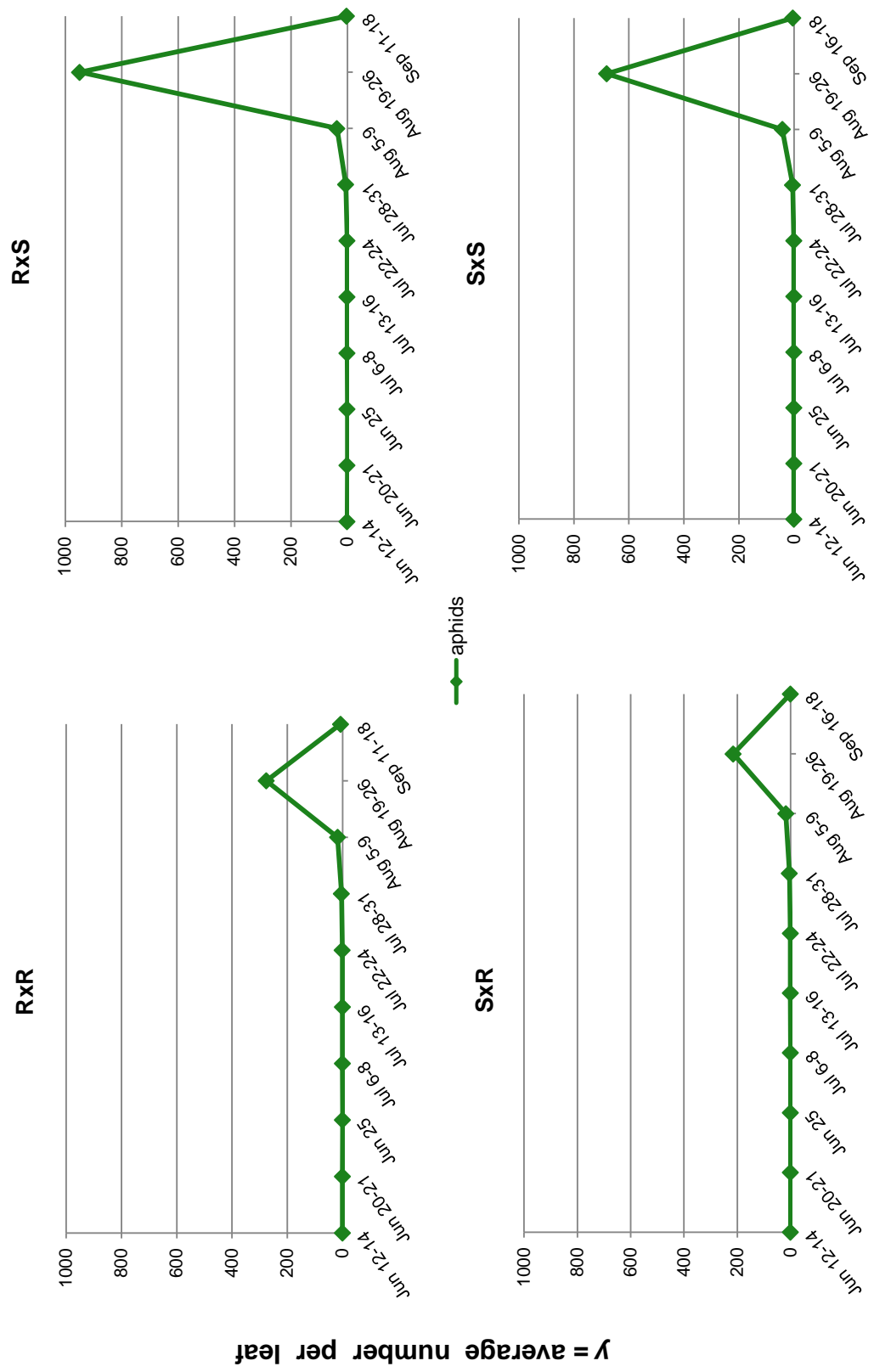
Fig. 13. Average number of sugarcane aphid per leaf over time on resistant x resistant sorghum hybrid (ATx3408/RTx2783) (*upper left*), resistant x susceptible hybrid (ATx3408/RTx436) (*upper right*), susceptible x resistant (ATx631/RTx2783) (*lower left*), and on susceptible x susceptible sorghum hybrid (ATx631/RTx436) (*lower right*), TAMU Farm, Somerville, TX, 2016.

44
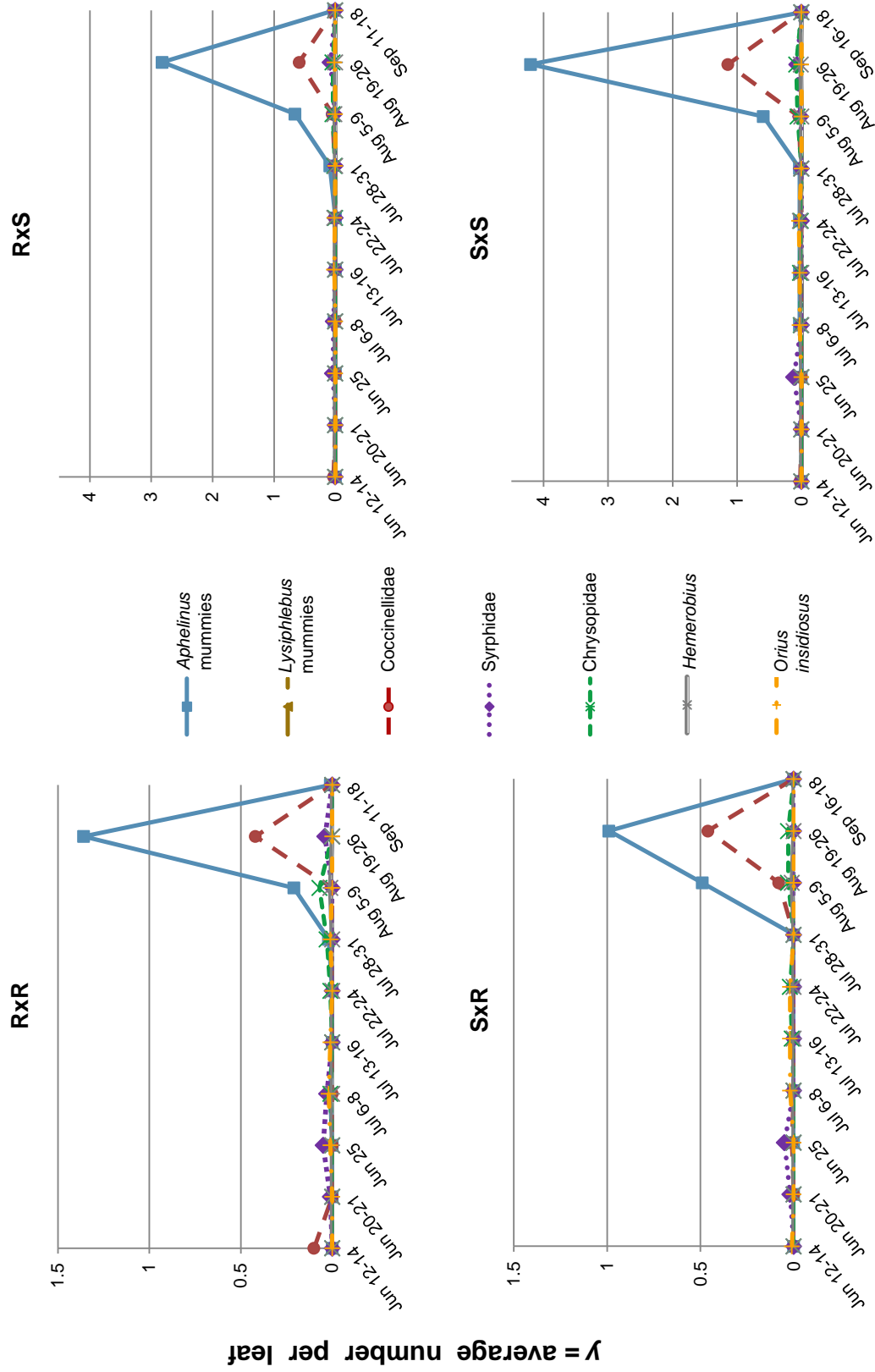
Fig. 14. Average number of aphid natural enemies per leaf over time on resistant x resistant sorghum hybrid (ATx3408/RTx2783) (*upper left*), resistant x susceptible hybrid (ATx3408/RTx436) (*upper right*), susceptible x resistant (ATx631/RTx2783) (*lower left*), and on susceptible x susceptible sorghum hybrid (ATx631/RTx436) (*lower right*), TAMU Farm, Somerville, TX, 2016. Note different *y* axis scale used for left versus right column.
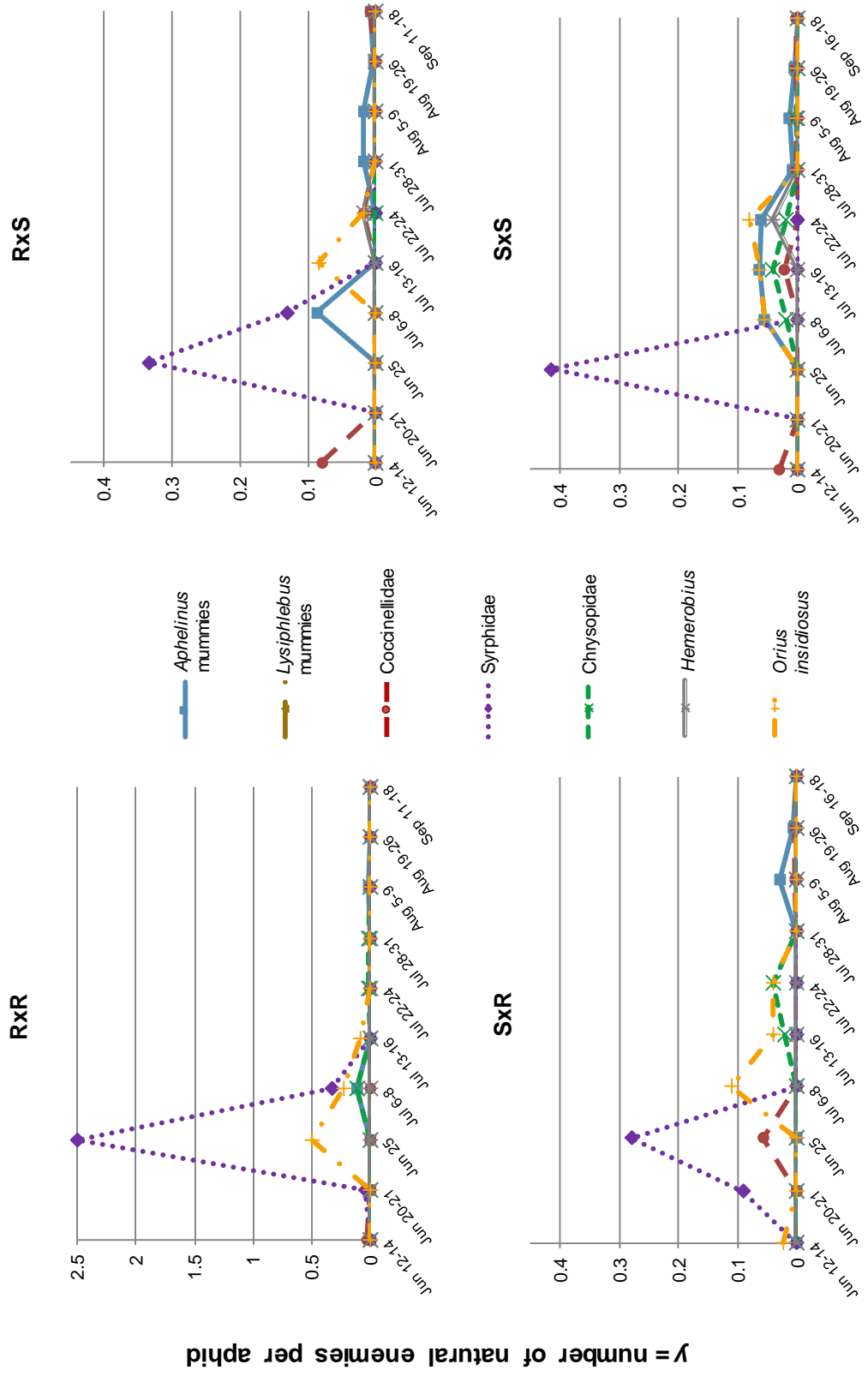
Fig. 15. Ratio of natural enemies to sugarcane aphids on resistant x resistant sorghum hybrid (ATx3408/RTx2783) (*upper left*), resistant x susceptible hybrid (ATx3408/RTx436) (*upper right*), susceptible x resistant (ATx631/RTx2783) (*lower left*), and on susceptible x susceptible sorghum hybrid (ATx631/RTx436) (*lower right*), TAMU Farm, Somerville, TX, 2016. Note different y axis scale used for upper left graph.

= 27, 144; P = 0.0001; Fig. 14; and Coccinellidae: F = 1.74; d.f. = 27, 144; P = 0.02; Fig. 14). Consistent with the results from 2015, the hybrid by date interactions in Burleson in 2016 showed statistically significant differences in aphid and natural enemy abundance only at peak aphid densities. Other natural enemies differed only across dates. Some natural enemy taxa, particularly Syrphidae, had spurious peaks in abundance when weighted by aphid density (Fig. 15), but only during the first half of the sampling period, when aphid numbers were very low.

Sugarcane aphids were largely absent from Burleson from spring until August (Fig. 13). Across all four hybrid treatments, aphid populations peaked between August 19th and August 26th. From August 19th through 26th, the RxS hybrid had the highest average aphid density (948 aphids/leaf), followed by the SxS hybrid (680.2 aphids/leaf). Natural enemy populations in Burleson in 2016 were correspondingly low for the majority of the season (Figs. 14-15). Peak natural enemy abundance was observed from August 19th through August 26th, although *Aphelinus* and Coccinellidae were the only taxa present in greater than trace numbers (Fig. 14).

Frequent, heavy rains during the spring of 2016 may have contributed to the early scarcity of aphids. Rain can dislodge aphids from their host plant, thereby separating the aphids from their food source and exposing them to ground-foraging predators (Mann et al. 1995). Rain also raises relative humidity, which facilitates the infection of aphids by entomopathogenic fungi (Zygomycetes: Entomophthorales) (Feng et al. 1991; Dean and Wilding 1973). Fungal pathogens (e.g. *Entomophthora* spp., *Conidiobolus* spp.) are a major source of mortality in cereal aphids, and have been argued (Feng et al. 1991; Dean and Wilding 1973) to rival or exceed the

biological control effects of aphid predators and parasitoids under the right conditions.

Additionally, sorghum in the Burleson County site was infested with spider mites (Acari: Tetranychidae) for several weeks in July. The mites left webbing and minor stippling damage on the undersides of leaves near the midrib. On some plants, mite infestation extended to the flag leaf. Spider mite activity may have contributed to the relative scarcity of sugarcane aphids in the field prior to August. Spider mites were not observed at either location in 2015, nor were they observed in Nueces in 2016. Although the mites did not overwhelm infested plants and died off prior to August, the mite-infested sorghum may have been less attractive to aphids. A study of aphid-mite interactions on strawberry leaflets (Cédola et al. 2013) found that colonies of aphids and spider mites were spatially segregated, and that aphid foundresses were less likely to colonize leaflets if spider mites were present. Feeding damage from spider mites may render leaves unpalatable or unattractive to heterospecific herbivores (Xu et al. 2011), and mite webbing prevents some arthropods from accessing the leaf surface to feed (Sarmento et al. 2011). The morphological and chemical mechanisms underlying aphid and spider mite interactions have yet to be characterized, and may merit further study.

Natural enemy composition varied somewhat between 2015 and 2016 and between Nueces and Burleson, but there was a repeated trend of *Aphelinus* and Coccinellidae being the numerically dominant natural enemy taxa when natural enemies were at peak abundance (Figs. 8, 9, 11, 14). This trend did not appear to differ between sorghum hybrids. In Nueces in 2015, *Aphelinus* greatly outnumbered

48

other natural enemies on the majority of sample dates on both susceptible and resistant sorghum (Fig. 8). In Burleson in 2015, *Aphelinus* and Coccinellidae both had high relative abundance, which was slightly exceeded by lacewings only on September 26-27th (Fig. 9). In Nueces in 2016, when overall natural enemy density peaked on June 2nd, Coccinellidae outnumbered all other natural enemy taxa on all three sorghum hybrids (Fig. 11). In Burleson in 2016, on all four hybrids, *Aphelinus* far outnumbered other natural enemies on August 5th-9th and August 19th-26th, while Coccinellidae were the second most numerous taxon on August 19th-26th (Fig. 14). Based on these observed trends, Coccinellidae and *Aphelinus* may be expected to have the greatest impact on sugarcane aphid populations relative to other local natural enemies, and may therefore be the two most important sources of biological control against the sugarcane aphid.

Most peak natural enemy densities either coincided with peak aphid density or lagged behind aphid density by one week. Both aphids and natural enemies showed a trend of lower densities on aphid-resistant sorghum hybrids relative to aphid-susceptible hybrids (but see exceptions in Figs. 10, 13). However, the ratio of natural enemies to aphids within any given county and year was overall very similar on both susceptible and resistant sorghum hybrids (Figs. 8, 9, 12, 15). The fact that natural enemy populations remained roughly proportionate to aphid populations independent of whether the host plant was aphid-resistant indicates that the ability of natural enemies to recruit to and suppress aphid populations was not adversely affected by host plant resistance. This suggests that natural enemies and host plant resistance can play complementary roles in sugarcane aphid management.

Evidence for the compatibility of aphid natural enemies and some sources of host plant resistance has been reported in previous studies of tritrophic interactions between aphid pests, natural enemies, and cereal crops. In a study of the English Grain Aphid (*Sitobion avenae* F.) and the aphid parasitoid, *Aphidius* spp., on aphid-susceptible and aphid-resistant winter wheat, *Aphidius* mummy density was correlated with *S. avenae* density on all cultivars (Cai et al. 2009). On resistant wheat, peak densities of *S. avenae* and *Aphidius* were lower than on susceptible wheat, but percent parasitism of the aphid population was higher. These parasitoids were therefore able to cause proportionately greater reductions in aphid populations when aphids were on resistant host plants (Cai et al. 2009).

Farid et al. (1998) found that the Russian wheat aphid (*Diuraphis noxia* Mordvilko) had a lower population growth rate on aphid-resistant wheat (*Triticum aestivum* L.) than on susceptible wheat. *D. noxia* was parasitized by the wasp *Diaeretiella rapae* (M'Intosh). *D. rapae* performed equally well—as measured by percent aphid parasitism, percent mummy emergence, adult longevity, head width, and sex ratio—on susceptible and resistant wheat (Farid et al. 1998). Similarly, *D. rapae* and *Aphelinus albipodus* (Hayat and Fatima) have been shown to parasitize *D. noxia* on susceptible and resistant lines of barley (*Hordeum vulgare* L.) at equal rates in choice tests (Brewer et al. 1998). The peak population patterns that we observed in *Aphelinus* and *M. sacchari* on susceptible versus resistant sorghum are consistent with the results of these studies of other aphid-parasitoid-resistant host plant systems (Cai et al. 2009; Brewer et al. 1998; Farid et al. 1998).

2.4.4. Hyperparasitism of *Aphelinus* Mummies

The rate of hyperparasitism of *Aphelinus* mummies by *Syrphophagus aphidivorus*, as measured by adult emergence, was consistently higher than 80% across sample collection dates (Fig. 16). Summed across emerged adult *Aphelinus* and *S. aphidivorus*, net mummy survivorship was approximately 50 percent. Despite this high rate of hyperparasitism and low mummy survivorship, *Aphelinus* wasps nonetheless appeared to be major contributors to sugarcane aphid mortality in the field. *Aphelinus* mummies were one of the three most numerous natural enemy taxa across all field sites in 2015 (Figs. 8-9). *Aphelinus* mummies also accounted for most of the natural enemy activity observed in Burleson County in 2016 (Fig. 14). Though few studies have investigated the ecology of hyperparasitoids, available evidence suggests that aphid hyperparasitoids may have a smaller-than-expected effect on their primary parasitoid hosts because hyperparasitoids tend to have lower lifetime fecundity (Sullivan and Völkl 1999).
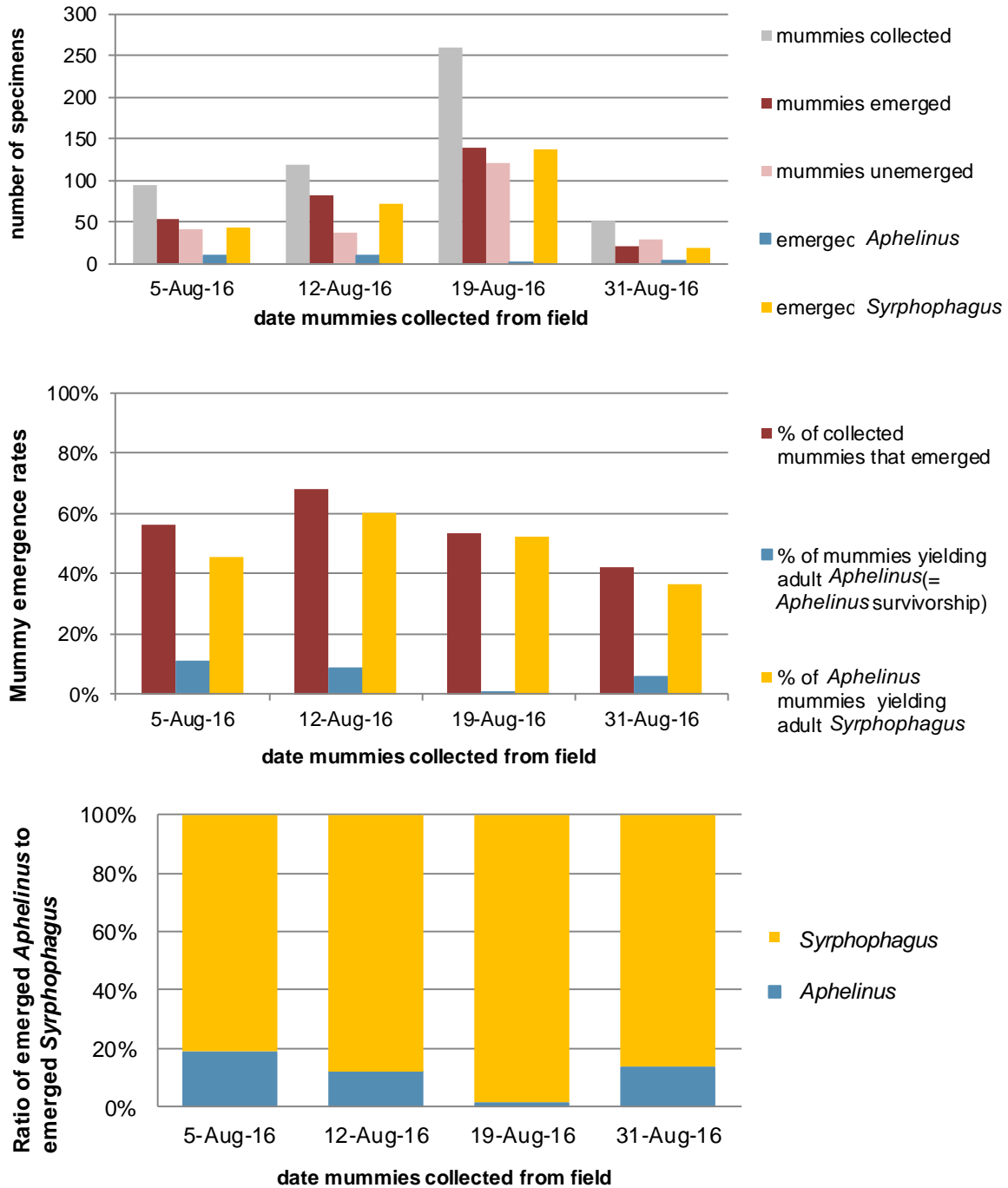
Fig. 16. Hyperparasitism of *Aphelinus* in *Melanaphis sacchari* mummies by *Syrphophagus aphidivorus*, TAMU Farm, Somerville, TX, 2016. All specimens were collected from the field as aphid mummies and individually lab-reared to maturity. Top panel: sample size of mummies collected on each date, and total numbers of wasps reared from corresponding sample. Middle panel: percentage of mummies from which wasps emerged. Bottom panel: percent of emerged wasps that were *Aphelinus* versus *S. aphidivorus*.

2.5. Conclusion

The existing community of aphid-feeding insects in Texas has responded rapidly to the introduction of the sugarcane aphid on sorghum. All natural enemies except Braconidae and Chamaemyiidae were observed on aphid-infested sorghum in both juvenile and adult life stages, and were successfully reared to maturity on a diet of sugarcane aphids. This suggests that these species are successful not only at locating sugarcane aphid outbreaks, but also at exploiting aphids across multiple generations. The continuity of species composition and population trends observed across two counties is evidence that this response is not an isolated event. Additionally, natural enemies were able to maintain activity on the resistant hybrid at levels proportionate to sugarcane aphid densities, suggesting that native and naturalized aphidophagous insects can complement host plant resistance as a component of integrated pest management.

Natural enemy densities increased following sugarcane aphid outbreaks. However, natural enemy activity was insufficient to prevent accelerated chlorosis and necrosis in plants that were already heavily infested (>1000 aphids/leaf). This observation is consistent with arguments from prior research that phenology is crucial to the efficacy of natural enemies; i.e., that natural enemies are most effective at controlling aphids early in the growing season when aphid populations are relatively low.

3. EVALUATION OF SUGARCANE APHID NATURAL ENEMIES USING EXCLUSION CAGES

3.1. Overview

The sugarcane aphid (*Melanaphis sacchari* (Zehntner)) is a new invasive sorghum pest in the United States. Aphid suppression by natural enemies of two size classes was evaluated by infesting sorghum with sugarcane aphids and enclosing the plants in partial and complete enemy exclusion cages. To test for interaction between natural enemy response and host plant resistance, two sorghum hybrids (aphid-resistant x susceptible, and susceptible x susceptible) were used. Natural enemy species with body width ≤2.5 mm were defined as small. Species >2.5 mm were defined as large. Standard mesh cages (aperture size: 2.5 mm) were used to measure small natural enemy response while excluding large species. Fine mesh cages (aperture size: 0.24 mm) screened out all natural enemies. Open-topped cages were used to measure the combined effects of large and small natural enemies. Caged aphids and natural enemies were sampled on three dates. Small lady beetles (Coccinellidae: Scymninae) were the only natural enemies to differ significantly in density between treatments. In the first sample, the numbers of Scymninae per leaf and per aphid were greatest in closed standard mesh cages. Scymninae per aphid was greater on the resistant x susceptible hybrid. On the second date, there were more Scymninae per leaf in standard mesh cages (open and closed) than in fine mesh. However, aphid density did not differ between cage or

hybrid treatments; therefore, aphid suppression by small and large species could not be measured. Additionally, results are inconclusive because cage integrity was compromised by several unanticipated problems. These problems and potential solutions are discussed.

3.2. Introduction

The sugarcane aphid, *Melanaphis sacchari* (Zehntner) (Hemiptera: Sternorrhyncha: Aphididae), is an emergent sorghum pest in the southern United States. The aphid is an established pest on both sorghum (*Sorghum bicolor* (L.) Moench) and sugarcane (*Saccharum officinarum* (L.)) throughout much of Asia, Africa, Australia, and Central and South America (Singh et al. 2004; Zehntner 1897). In the United States, *M. sacchari* became an established pest of sugarcane (*Saccharum officinarum* (L.)) in 1977 (Mead 1978), but the aphid was not observed damaging sorghum. In 2013, high populations on sorghum were detected along the Gulf Coast of Texas, and damage to sorghum was seen the same year (Bowling et al. 2015a). By fall 2015, the aphid had spread to 17 states that accounted for >95% of sorghum production in the US (USDA-NASS 2016). Its geographic range within the US extends south into Florida and Texas, north to Illinois and Kansas, and east-west from the lower east coast to the Great Plains. The aphid has also colonized the Caribbean Islands and the sorghum-growing regions of Mexico (Bowling et al. 2016).

The aphid is a major threat to sorghum production in Texas, which ranks second out of all states in total U.S. sorghum production (USDA-NASS 2016). For

example, sugarcane aphid in the Lower Rio Grande Valley caused an estimated economic loss of $31.60 million from 2013 through 2015, and an average loss of $49.76 per acre between 2014 and 2015 (Zapata et al. 2016). Available data suggest that yield losses of 10% to 50% are common throughout the aphid's US range (Villaneuva et al. 2014). Heavy aphid infestation causes leaf chlorosis and necrosis, delayed or aborted panicle development, rapid decline in overall plant health, and reduction in quantity and nutritional quality of grain and forage yield (Singh et al. 2004). Sugarcane aphids also coat leaves with a thick layer of sugary, sticky honeydew. Honeydew interferes with grain harvest by preventing automated grain separation and by clogging harvesting machinery (Villanueva et al. 2014).

Several aspects of the sugarcane aphid's ecology have facilitated its rapid spread through the sorghum-growing regions of North America. Sorghum-associated sugarcane aphids in the US are parthenogenetic, viviparous, anholocyclic females (Singh et al. 2004; Chang et al. 1982). Adults are polymorphic and are either alatae (winged) or apterae (wingless). Alatae can travel long distances via wind-aided dispersal (Irwin and Thresh 1988). At the peak of the colony growth cycle, a heavily infested plant may harbor 10,000-30,000 aphids (Bowling et al. 2016; Setokuchi 1977). Nymphs mature within 4-12 days (Chang et al. 1982), and each adult can produce 34-96 progeny over the course of its 2-5 week lifespan (Singh et al. 2004). Sugarcane aphids develop and reproduce most rapidly in hot, dry weather (Singh et al. 2004).

One means of preventing or reducing aphid damage is to cultivate sorghum that has sugarcane aphid resistance (Sharma 1993). Since 1980, dozens of

sugarcane-aphid-resistant sorghum varieties have been developed worldwide (Mbulwe et al. 2016; Singh et al. 2004; Sharma 1993). Morphological sources of sugarcane aphid resistance include reduction in size and number of leaves, increased wax production on abaxial leaf surfaces, and a taller stalk with greater distance between the bases of leaves (Armstrong et al. 2015). Chemical sources of resistance include herbivory-induced production of p-hydroxybenzaldehyde during the seedling stage. Changes in the nutrient profile of leaves—specifically, higher levels of phosphorus, polyphenols, and potassium—have antixenotic effects. Some cultivars show evidence of antibiosis: in no-choice tests, they are infested with 50%-80% fewer aphids compared to susceptible cultivars, and incur only moderate plant damage (Armstrong et al. 2015). Highly aphid-tolerant cultivars can withstand feeding by moderate numbers of aphids (e.g., 800 aphids/plant) without economic damage (Armstrong et al. 2015;). Resistance to other aphid species is not a reliable indicator of sugarcane aphid resistance. Some inbred lines that resist greenbug (*Schizaphis graminum* (Rondani)), a widespread grain pest, are highly susceptible to the sugarcane aphid (Armstrong et al. 2015), while others express resistance to both these aphids (Brewer, Rooney, pers. communication).

The sugarcane aphid has a diverse assemblage of natural enemies. Lady beetles (Coleoptera: Coccinellidae), lacewings (Neuroptera: Chrysopidae; Neuroptera: Hemerobiidae), hoverflies (Diptera: Syrphidae), and parasitoid wasps (Hymenoptera: Aphelinidae; Hymenoptera: Braconidae) are important natural enemies of cereal aphids including those on sorghum (Colares et al. 2015a, Singh et al. 2004, Brewer and Elliott 2004). Natural enemies that recruit to an aphid colony

57

early in that colony's growth cycle can suppress population growth, prevent the production of alates, minimize plant damage, and ultimately prevent yield loss (Colares et al. 2015a).

The goal of this study was to examine whether the natural enemies detected feeding on sugarcane aphid will complement host plant resistance. Natural enemies were separated by body size range using a partial and complete exclusion cage method. Exclusion cages have often been used to estimate the impact of natural enemies within a given size range on pest insect populations in agricultural systems (Perillo et al. 2015).

3.3. Methods and Materials

Partial and full exclusion cages were used to estimate the abundance and aphid suppression of small natural enemies (adult body width between 0.24 and 2.5 mm) and the combined effects of large (adult body width >2.5 mm) and small natural enemies. To determine whether natural enemies could complement host plant aphid resistance, the interaction of large and small natural enemies with sorghum-resistant status of the hybrid (aphid-susceptible x susceptible cross and aphid-resistant x susceptible cross) was considered experimentally. The field experiment was conducted during 2016 at the Texas A&M University Farm in Somerville, Burleson County, Texas (30.539592° N, -96.421537° W).

3.3.1. Natural Enemies

Aphid natural enemies were divided into two size classes, large and small, based on average adult body width. Species with an average adult body width greater than 2.5 mm were categorized as large, whereas species between 0.24 mm and 2.5 mm wide were categorized as small. Large natural enemies included the following taxa: hoverflies (Diptera: Syrphidae), green lacewings (Neuroptera: Chrysopidae), brown lacewings (Neuroptera: Hemerobiidae), and all non-scymnine lady beetles (Coleoptera: Coccinellidae). Small natural enemies included: aphelinid wasps (Hymenoptera: Chalcidoidea: Aphelinidae: *Aphelinus* sp.), braconid wasps (Hymenoptera: Braconidae: *Lysiphlebus testaceipes*), minute pirate bugs (Hemiptera: Anthocoridae: *Orius insidiosus*), and all scymnine lady beetles (Coleoptera: Coccinellidae: Scymninae).

3.3.2. Cage Design and Hybrid Treatments

Natural enemy exclusion cages were built with the following dimensions: 88.9 cm (length) x 38.1 cm (width) x 121.92 cm (height). Additionally, each cage had >15 cm of slack mesh at the bottom. The slack mesh was buried under soil to secure the bottom of the cage and to prevent natural enemies from crawling under the cage material.

Cage frames were constructed from PVC tubes (diameter approx. 1.9 cm) connected with PVC elbow pieces. Steel T-posts (1.83 m) were used to anchor the

cages in place. One post was driven into the ground on either side of each cage. Sturdy white rope was tied to rebar posts and threaded through loops at tops of cages, clothesline style, to suspend cages at a height of 121.92 cm. Loops at the tops of cages were made of white rope sewn onto the trim.

Each cage was constructed with one of two fabric types. The fabric used for fine mesh cages was white nylon organza mesh (aperture size <0.24 mm) selected to screen out both large and small natural enemies. The fabric used in standard mesh cages was white polyester mosquito netting mesh (aperture size: 2.5 mm) selected to permit movement of aphids and small natural enemies, while excluding large natural enemies. White twill fabric was used as a trim to reinforce the mesh seams.

Four cage treatments were used to fully, partially, and not exclude natural enemies from the cages:

1. Fine mesh, full-exclusion cage: a closed-top cage was constructed out of fine mesh to exclude all aphid natural enemies, regardless of body size. Aphids were unable to enter or exit the cage. This treatment was used to measure aphid abundance over time in the absence of aphid predators and parasitoids. A few natural enemies were found within the cage during sampling, these were removed during the inspections for aphids and natural enemies.

2. Standard mesh, partial exclusion cage: a closed-top cage was constructed out of mosquito netting mesh to allow small natural enemies (body width <2.5 mm) to enter and leave the cage, while screening out large natural enemies (body width >2.5 mm). This cage treatment was used to measure the abundance of small natural

enemies and aphids over time. A few large natural enemies were found within the cage during sampling, these were removed during the inspections for aphids and natural enemies.

3. Fine mesh, open cage: a control cage used to test for cage effect. Control cages had fine mesh sides but had no mesh on top. Slack mesh at the bottom was not buried. This allowed aphids and all natural enemies to freely enter or leave through the top or bottom of the cage.

4. Standard mesh, open cage: identical to treatment 3, except that the sides of the cage were constructed from mosquito netting.

Eight cages were built for each of the four cage treatments, for a total of 32 cages. The field was planted on April 2016, and was managed with standard agronomic methods except that insecticides were not used. Eight sorghum field plots were used, each consisting of 6 rows of sorghum with between-row spacing of 96.52 cm and row length of 12.19 m. Two sorghum hybrids differing in susceptibility to sugarcane aphid were used. Four of the eight plots were planted with a Susceptible x Susceptible hybrid (SxS, ATx631/RTx436), while the other four were planted with a Resistant x Susceptible hybrid (RxS, ATx3408/RTx436). Each cage and hybrid treatment combination had four replicates set out in a randomized complete block pattern. Cages were installed on June 21, 2016. In each plot, four cages (one of each treatment, in randomized order) were installed clothesline-style along a single sorghum row where plant growth was relatively even. Each cage contained approximately 10 plants. Tillers of a height similar to the main stalks were counted as individual plants, but each cage contained a minimum of three main stalks (i.e.,

plants from separate seeds). If a cage would contain more than 10 stalks, excess tillers were culled.

Due to a lack of naturally occurring sugarcane aphids at the field site, each cage was artificially infested with approximately 300-350 sugarcane aphids on June 28, 2016. The aphids had been laboratory-reared for several weeks to minimize the risk of introducing aphid parasitoids and predators into cages. Aphids were introduced onto three randomly-selected sorghum stalks per cage. To prevent stalks from growing through the top of the mesh, emerging sorghum heads in all cage treatments (including open-top cages) were bent 90° downward when they approached the cage height of 48".

3.3.3. Measurements and Data Collected

The cages were sampled for aphids and natural enemies three times: August 14-15, August 27-28, and September 8-10, 2016. For each sample, we selected two upper and two lower sorghum leaves from each cage. A relatively healthy (leaf surface at least 50% green) top leaf and bottom leaf were selected. The top leaf consisted of the uppermost fully unfurled leaf, excluding the flag leaf. The bottom leaf consisted of one of the lowermost three leaves on the plant, excluding dead leaves.

Aphid and natural enemy densities per leaf per cage were calculated visually by counting the insects of each taxon on the abaxial (downward-facing) surface of each selected leaf. Aphids were counted individually when there were 25 or fewer

aphids on the leaf. If a leaf contained more than 25 aphids, the aphid population was visually estimated using number ranges adapted from the Quick Aphid Checker (Bowling et al. 2015b). Populations of 26 to 49 aphids were rounded to 38. Aphid populations estimated to be between 50 and 100 aphids were rounded to the nearest multiple of 25 (i.e., 50, 75, or 100 aphids). Estimates between 101 and 800 aphids were rounded to the nearest multiple of 50. Estimated populations of 801 to 1,100 aphids were rounded to the nearest multiple of 100. Estimates greater than 1,100 aphids were rounded to the nearest multiple of 500, with 3,500 aphids as the upper maximum per leaf.

Natural enemies were identified and counted using the following taxonomic categories: Aphelinidae- and Braconidae-type aphid mummies, small coccinellids (Coccinellidae: Scymninae), large coccinellids (all non-scymnine lady beetle species), Syrphidae (hoverflies), Chrysopidae (green lacewings), *Hemerobius* spp. (brown lacewings), and *Orius insidiosus* (minute pirate bugs). Predator age class was recorded as either juvenile (nymphs, larvae, or pupae) or adult. Eggs were not counted, nor were emerged mummies (i.e. aphid mummies with visible exit holes). Due to the difficulty of identifying microhymenoptera in the field, adult aphelinids and braconids were not counted. Other signs of natural enemy activity—such as large volumes of predator frass, exuviae, eggs, or emerged mummies—were noted but were not quantified. Overall plant health in each cage was also noted.

3.3.4. Statistical Analysis

A two-way ANOVA was performed to examine the two experimental factors of plant hybrid and cage type. The residual was used as the error term for the hybrid x cage type interaction and the main factors, which were considered fixed effects. The interaction was not significant for all measures; therefore, measurement differences among the cage types were tested with Tukey's mean separation test ($\alpha = 0.05$). Because two hybrids were used, the results of the ANOVA were directly used to detect significant differences between the hybrids.

3.4. Results

In cages from which data were collected, mean sugarcane aphid density was highest on the first sampling date (August 14-15, 2016) and lowest on the final sampling date (September 8-10, 2016) (Figs 17, 20). Aphid density did not differ significantly between cage treatments or plant hybrids (hybrid x cage type interaction and main effects: $P > 0.10$). Aphid population growth on uncaged sorghum in the surrounding field appeared to lag behind caged aphid population growth by 1-2 weeks (compare average aphid densities across cage types with open field references in Table 1). One closed standard mesh cage containing RxS sorghum was not sampled due to heavy infestation by aggressive fire ants (*Solenopsis invicta*). Additionally, on the third sampling date one RxS standard mesh open cage could not be used because all the plants had died.

Fig. 17. Sugarcane aphid counts in fine mesh (full enemy exclusion) cages, TAMU Farm, Somerville, TX, 2016. Left column: susceptible x susceptible sorghum hybrid (ATx631/RTx436). Right column: resistant x susceptible sorghum hybrid (ATx3408/RTx436). Top row: experimental cages. Bottom row: open cages (control).
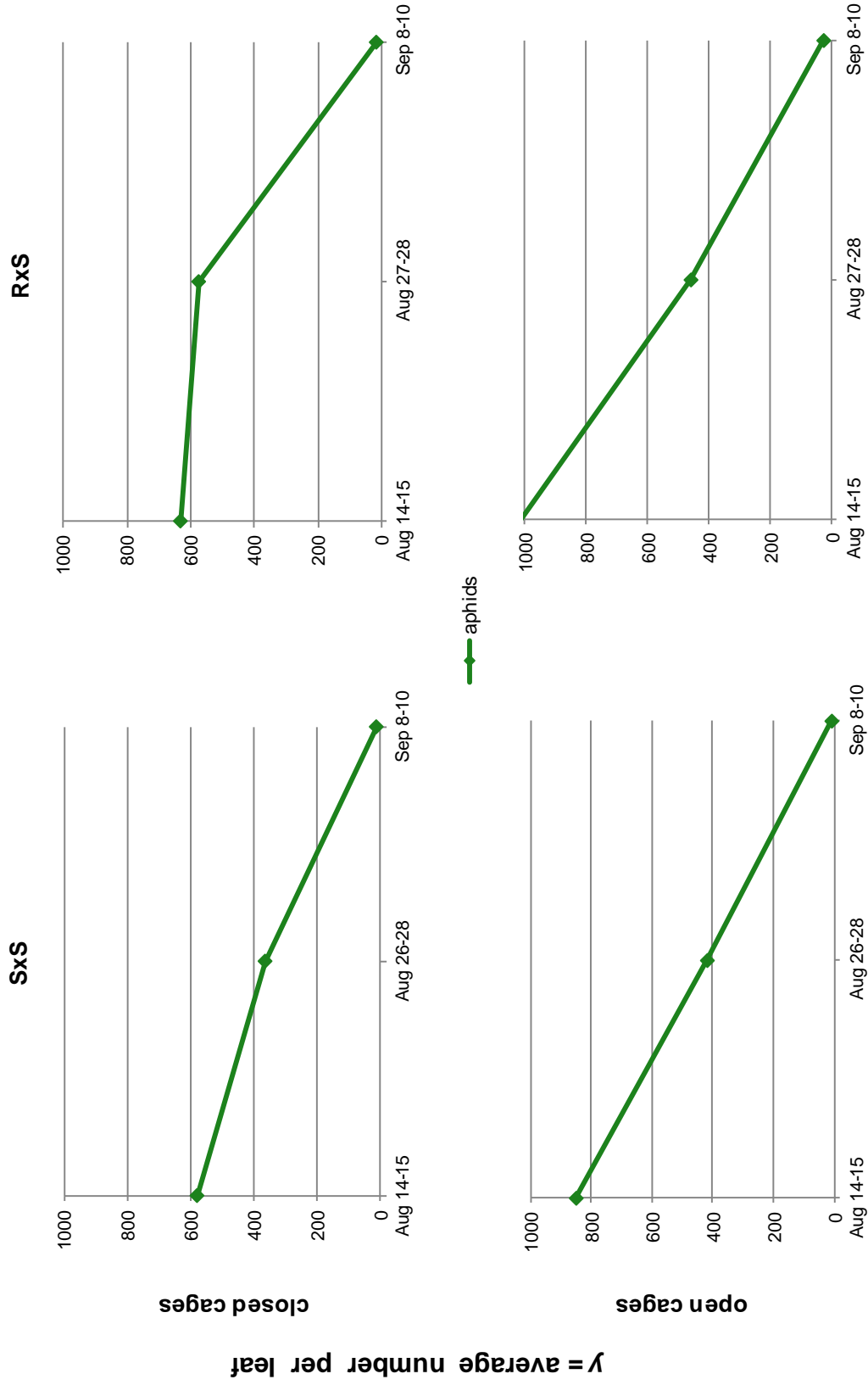
Fig. 18. Sugarcane aphid counts in standard mesh (partial enemy exclusion) cages, TAMU Farm, Somerville, TX, 2016. Left column: susceptible x susceptible sorghum hybrid (ATx3408/RTx436). Right column: resistant x susceptible sorghum hybrid (ATx631/RTx436). Top row: experimental cages. Bottom row: open cages (control).

Fig. 19. Natural enemy counts in fine mesh (full enemy exclusion) cages, TAMU Farm, Somerville, TX, 2016. Left column: susceptible x susceptible sorghum hybrid (ATx631/RTx436). Right column: resistant x susceptible sorghum hybrid (ATx3408/RTx436). Top row: experimental cages. Bottom row: open cages (control).

67

Fig. 20. Number of natural enemies per aphid in fine mesh (full enemy exclusion) cages, TAMU Farm, Somerville, TX, 2016. Left column: susceptible x susceptible sorghum hybrid (ATx631/RTx436). Right column: resistant x susceptible sorghum hybrid (ATx3408/RTx436). Top row: experimental cages. Bottom row: open cages (control). Note different scale of y axis on experimental resistant x susceptible sorghum (upper right).

68
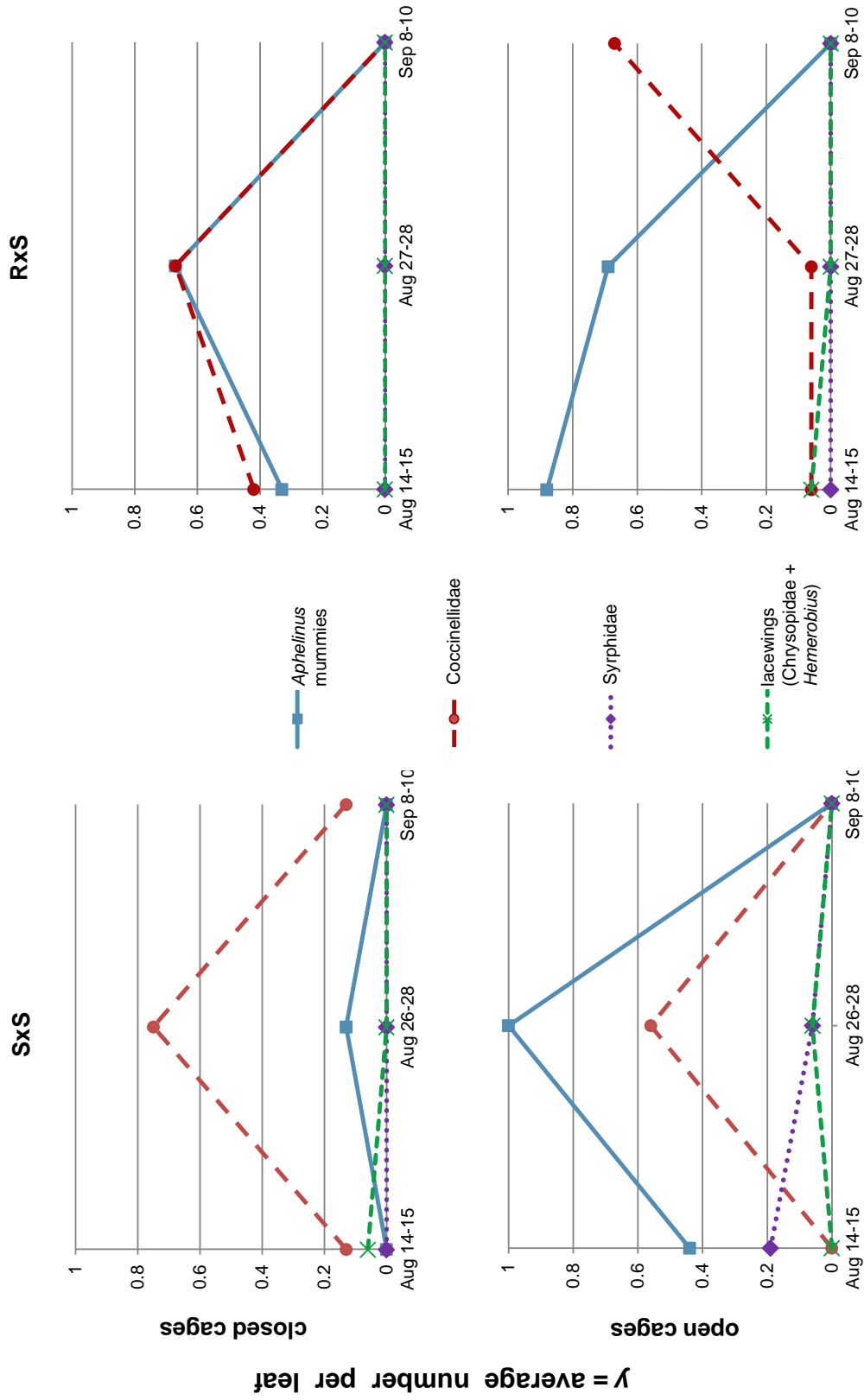
Fig. 21. Natural enemy counts in standard mesh (partial enemy exclusion) cages, TAMU Farm, Somerville, TX, 2016. Left column: susceptible x susceptible sorghum hybrid (ATx631/RTx436). Right column: resistant x susceptible sorghum hybrid (ATx3408/RTx436). Top row: experimental cages. Bottom row: open cages (control).
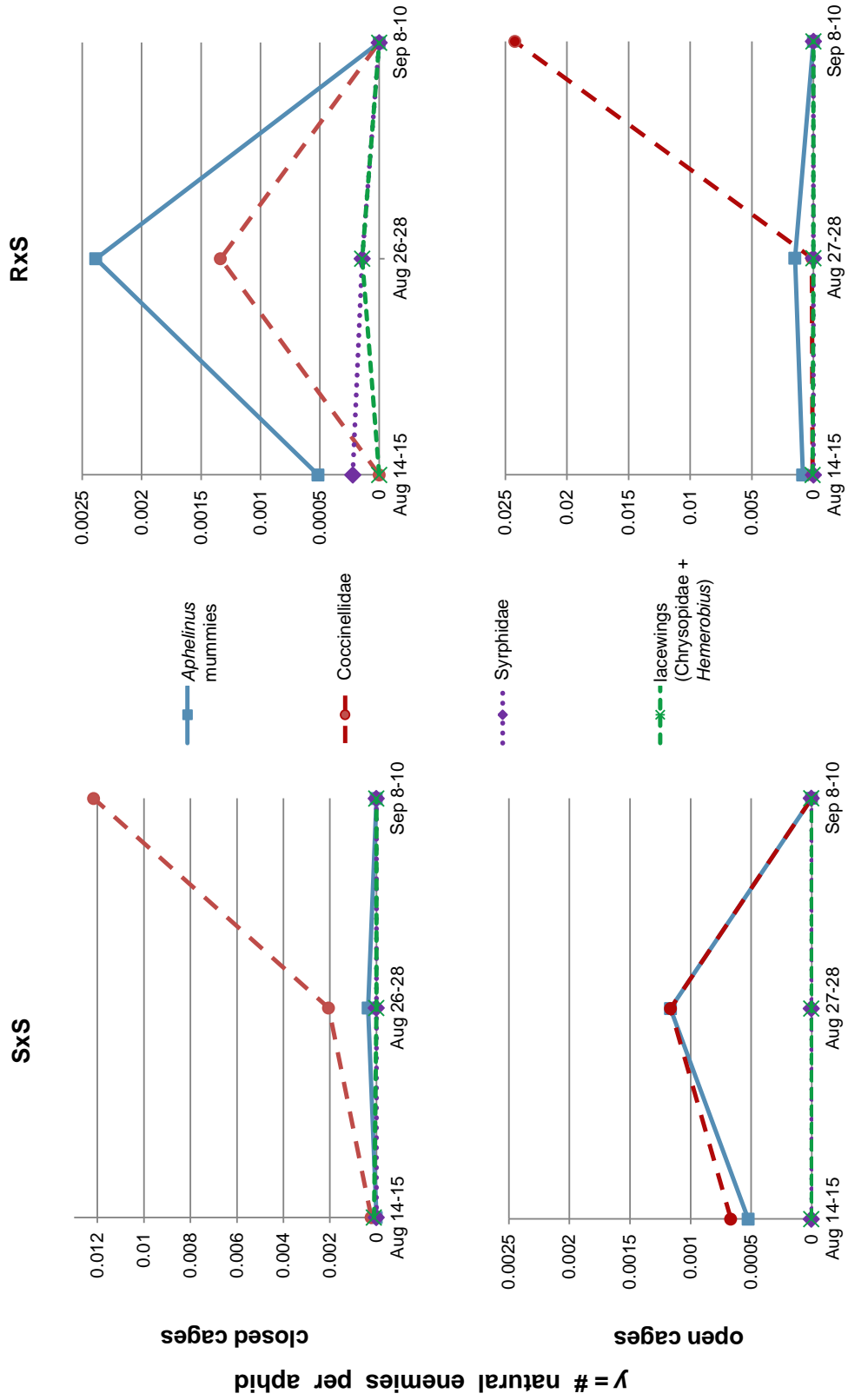
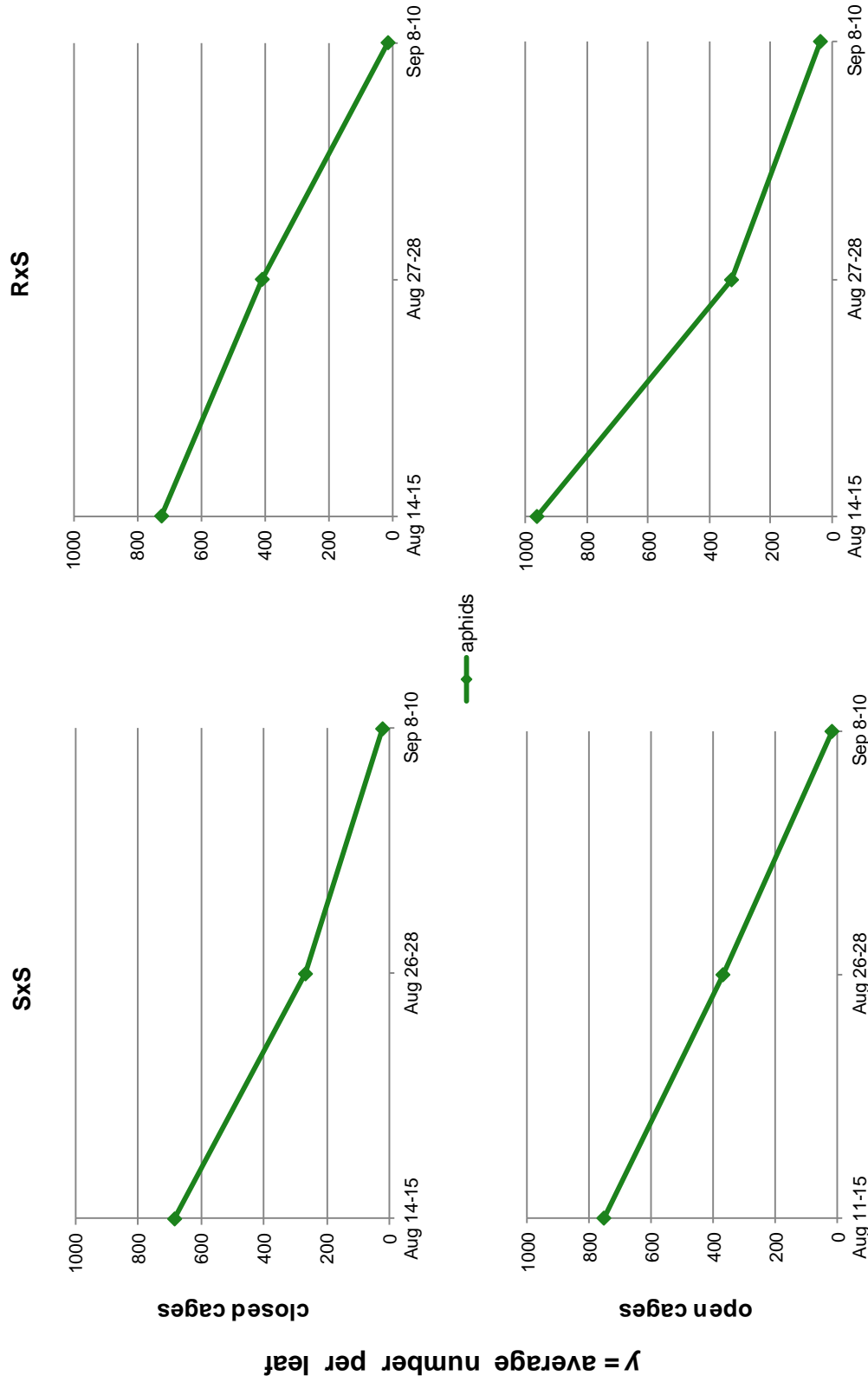**Fig. 22.** Number of natural enemies per aphid in standard mesh (large enemy exclusion) cages, TAMU Farm, Somerville, TX, 2016. Left column: susceptible x susceptible sorghum hybrid (ATx631/RTx436). Right column: resistant x susceptible sorghum hybrid (ATx3408/RTx436). To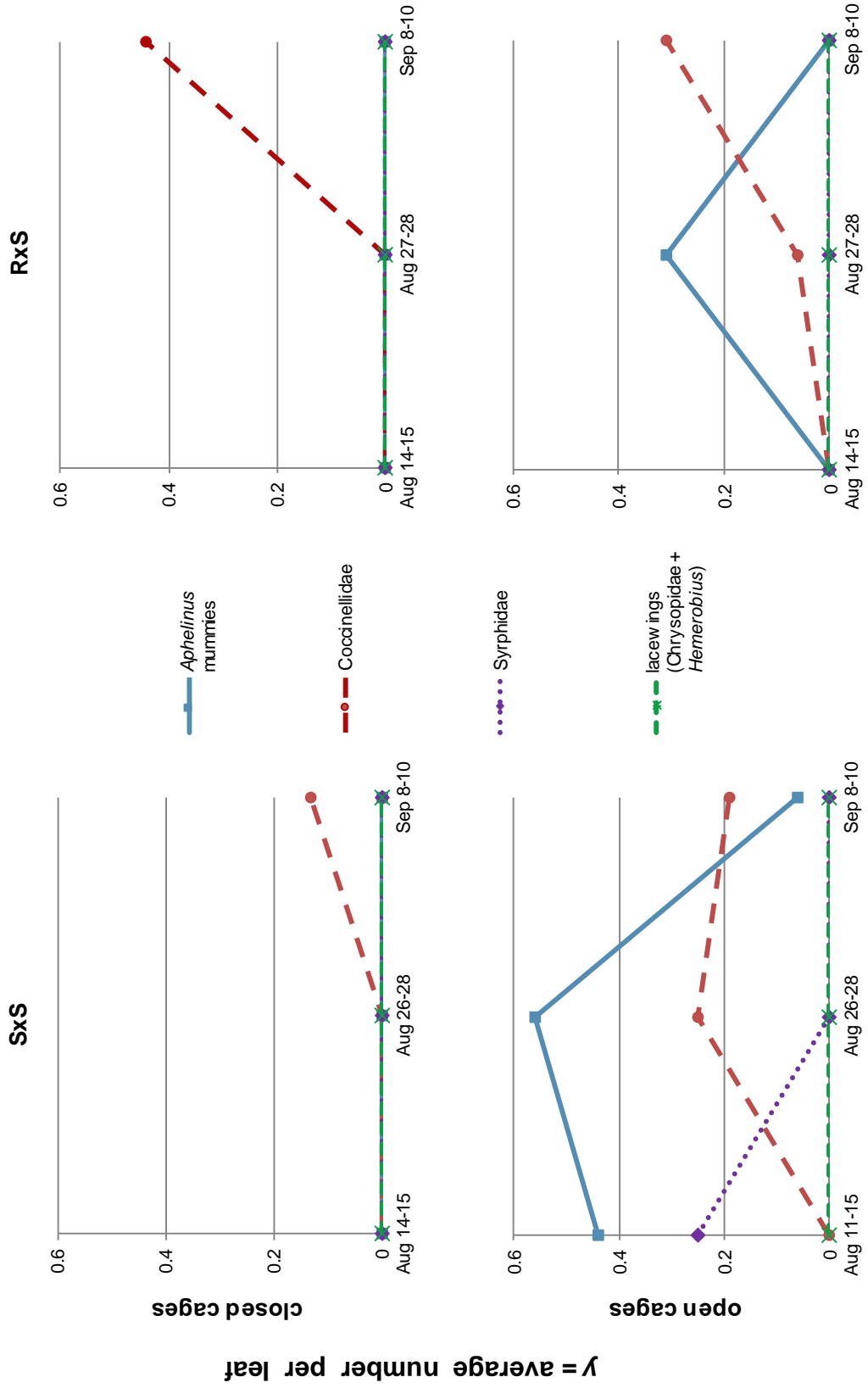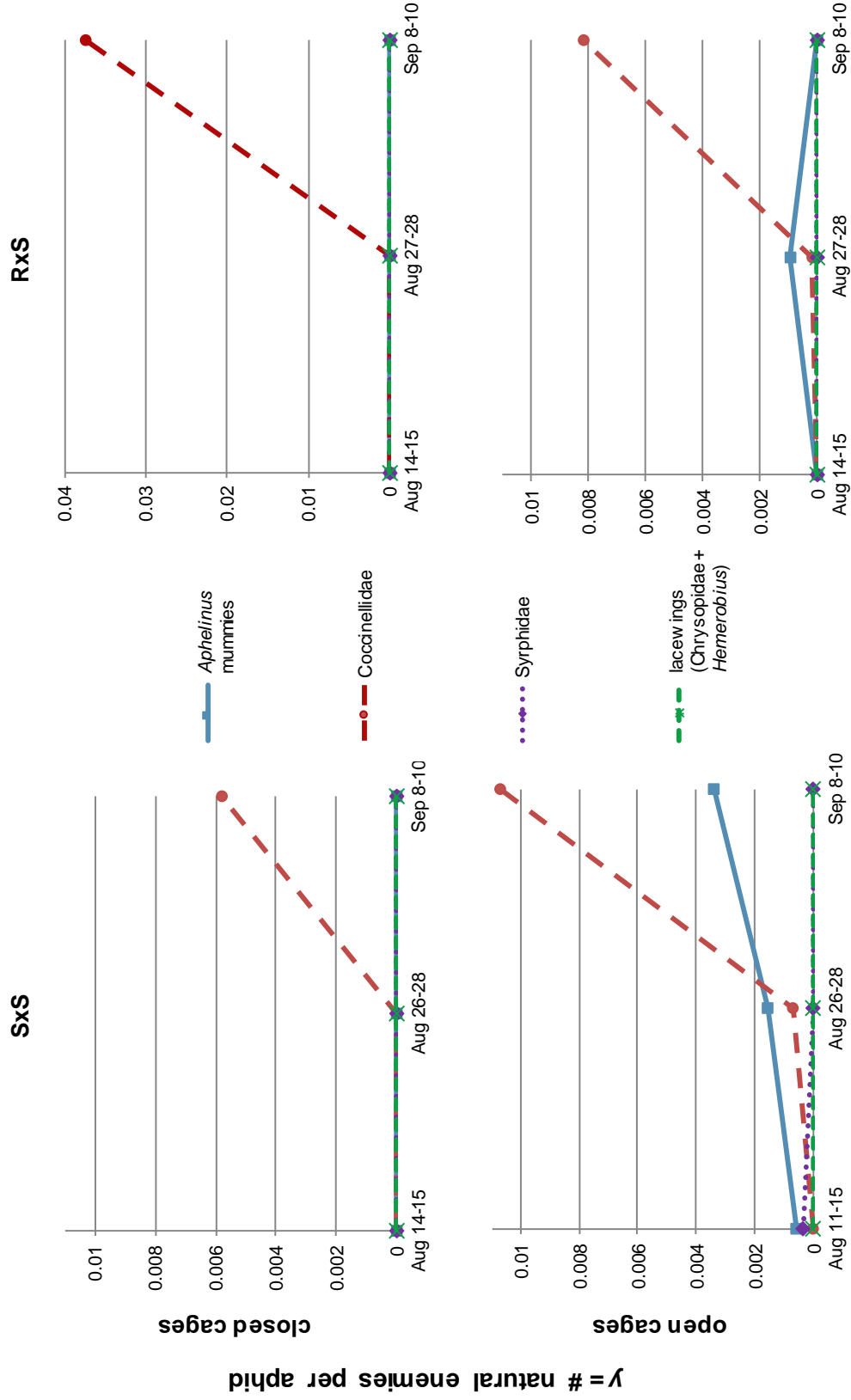p row: experimental cages. Bottom row: open cages (control). Note different scale of y axis on experimental susceptible x susceptible sorghum (upper left).

Aphelinus mummies and small (scymnine) lady beetles comprised the majority of natural enemies in cages (Figs 18-19, 21-22). Very few hoverflies, lacewings, and large (non-scymnine) lady beetles were observed. No minute pirate bugs or *Lysiphlebus* mummies were observed in any cages.

Two natural enemy variables showed significant statistical differences among hybrid and cage type main effects: small lady beetles (Coccinellidae: Scymninae) per leaf, and ratio of Scymninae to aphids. On the first sampling date (August 14-15, 2016), Scymninae per leaf differed among cage types (F = 6.50: d.f. = 3,20; P < 0.003) and ratio of Scymninae to aphids differed among cage types (F = 4.72: d.f. = 3,20; P < 0.012) . There were significantly more Scymninae per leaf and more Scymninae per aphid in closed standard mesh cages than in all other cage treatments (Table 1). Differences across the two hybrid types were detected for the Scymninae per aphid measure (F = 4.60: d.f. = 1,20; P < 0.044). In cages with relatively high aphid density, honeydew covered both the lower leaves and the cage mesh. Some infested leaves were starting to turn yellow or were developing patches of necrosis.

On the second sampling date (August 27-28, 2016), Scymninae per leaf differed among cage types (F = 3.09: d.f. = 3,20; P = 0.05). There were significantly more Scymninae per leaf on standard mesh cages (both closed and open treatments) than on fine mesh cages. No other significant differences were detected. During this sampling period, most caged plants showed visible decline. More than half of all leaves were yellowed or necrotic. Some plant stalks had withered. Leaves beneath large aphid colonies were covered with sooty mold.

Table 1. Comparison of mean sugarcane aphids per leaf, Scymninae (small lady beetles) per leaf, and number of Scymninae per aphid across cage treatments and sorghum hybrids over time.

	aphids/leaf (+/- SEM)			small (scymnine) lady beetles/leaf (+/- SEM)			small (scymnine) lady beetles/aphid (+/- SEM)		
	SxS hybrid (ATx631/RTx436)	RxS hybrid (ATx3408/RTx436)	across hybrids	SxS hybrid (ATx631/RTx436)	RxS hybrid (ATx3408/RTx436)	across hybrids	SxS hybrid (ATx631/RTx436)	RxS hybrid (ATx3408/RTx436)	across hybrids
Aug 8-9 2016									
Open field reference	47.49	39.64		0.05	0.0625		0.0011	0.0016	
Aug 14-15 2016									
Fine mesh cage	683.25 (529.72)	722.31 (234.15)	703 (268) a	0 (0)	0 (0)	0 (0) b	0 (0)	0 (0)	0 (0) b
Fine mesh open cage (control)	754.44 (444.63)	963 (429.48)	856 (289) a	0 (0)	0 (0)	0 (0) b	0 (0)	0 (0)	0 (0) b
Standard mesh cage	580.44 (380.75)	629 (332.14)	601 (239) a	0.125 (0.072)	0.417 (0.220)	0.25 (0.11) a	0.0007 (0.0005)	0.0062 (0.0034)	**0.0030 (0.0017) a**
Standard mesh open cage (control)	853.19 (365.47)	1015.69 (312.19)	934 (225) a	0 (0)	0.0625 (0.0605)	0.031 (0.031) b	0 (0)	0.0019 (0.0002)	0.0001 (0.0001) b
across cage types	718 (196) a	846 (155) a		0.031 (0.021) a	**0.100 (0.059) b**		0.0002 (0.0001) a	0.0013 (0.0009) a	
Aug 22-26 2016									
Open field reference	1620.7	1114.86		1.225	0.625		0.0008	0.0006	
Aug 27-28 2016									
Fine mesh cage	267.94 (107.24)	407.06 (114.25)	338 (77.2) a	0 (0)	0 (0)	0 (0) b	0 (0)	0 (0)	0 (0) a
Fine mesh open cage (control)	368.75 (169.06)	328.13 (150.18)	348 (105) a	0.25 (0.25)	0.0625 (0.0625)	0.156 (0.124) b	0.0073 (0.0073)	0.0019 (0.0019)	0.0046 (0.0036) a
Standard mesh cage	365.063 (120.46)	575 (101.04)	455 (86.0) a	0.812 (0.504)	0.583 (0.167)	**0.714 (0.280) a**	0.0127 (0.0068)	0.0047 (0.0019)	0.0093 (0.0041) a
Standard mesh open cage (control)	419.19 (159.78)	462.5 (87.05)	441 (84.6) a	0.562 (0.277)	0.0625 (0.0625)	**0.313 (0.162) a**	0.0169 (0.0138)	0.0006 (0.0006)	0.0087 (0.0071) a
across cage types	355 (64.9) a	434 (57.3) a		0.406 (0.161) a	0.150 (0.068) a		0.00921 (0.0042) a	0.0016 (0.0007) a	
Sep 8-10 2016									
Fine mesh cage	22.31 (11.49)	11.75 (11.25)	17.03 (7.71) a	0.125 (0.072)	0.375 (0.298)	0.250 (0.149) a	0.0633 (0.0575)	0.361 (0.325)	0.212 (0.163) a
Fine mesh open cage (control)	17.75 (10.25)	37.75 (21.37)	27.75 (11.60) a	0.189 (0.120)	0.3125 (0.237)	0.250 (0.125) a	0.0424 (0.0438)	0.112 (0.0967)	0.0887 (0.0630) a
Standard mesh cage	10.69 (4.75)	14.75 (8.98)	12.43 (4.32) a	0.125 (0.125)	0 (0)	0.0714 (0.0714) a	0.116 (0.116)	0 (0)	0.0560 (0.0560) a
Standard mesh open cage (control)	10.5 (9.17)	27.67 (22.5)	17.86 (10.41) a	0 (0)	0.667 (0.363)	0.286 (0.192) a	0 (0)	0.174 (0.105)	0.0580 (0.0457) a
across cage types	15.31 (4.34) a	23.23 (8.18) a		0.109 (0.045) a	0.339 (0.133) a		0.0527 (0.0304) a	0.172 (0.103) a	
Sep 16-18 2016									
Open field reference	5.1	2.975		0	0.0125		0	0.0042	

Footnote: Means +/- SEMs are presented. The hybrid x cage interaction was not significant in all cases. Therefore, Tukey's Means Separation test (alpha<0.05) was conducted on cage means averaged across hybrids. Because there were two hybrids, the F test directly from the ANOVA was used to test for significant differences (p <0.05) on hybrid means averaged across cage types. Same letters (a or b) across hybrid and cage means indicate a lack of significant difference. Statistically significant differences are **bolded**. Open field aphid per leaf reference counts are shown for comparison but were not included in data analysis.

No significant results were found for the third sample date (September 8-10 2016). The majority of caged plants were dead or dying. By this time, aphid and natural enemy densities in cages were very low overall, so the lack of significant results was not surprising.

Small natural enemies consistently outnumbered large natural enemies across all cage types, hybrids, and sample dates (Figs 23-26). This trend is consistent with our expectations for the closed standard mesh cages, which were designed to admit small enemies while excluding large enemies. However, small natural enemies also outnumbered large natural enemies in open cage treatments where large enemies could move through the tops and bottoms of cages.

The significantly higher number of Scymninae per leaf in standard mesh cages versus fine mesh cages on the first two sampling dates was expected. Scymninae are small enough to fit through the holes in the standard mesh, but are too large for the holes in the fine mesh. The closed fine mesh cages were designed to exclude all natural enemies; therefore, we expected to find no Scymninae in this treatment. Nonetheless, Scymninae and some other natural enemies were recorded in closed fine mesh cages. Scymninae populations were proportionate with aphid populations on both plant hybrids. In the significant hybrid difference detected, the ratio of Scymninae to aphids was higher on RxS sorghum than on SxS. This result suggests the possibility of a synergistic or additive aphid suppression effect resulting from interaction between host plant resistance and predation by Scymninae. Further research would be needed to confirm or characterize this potential interaction.
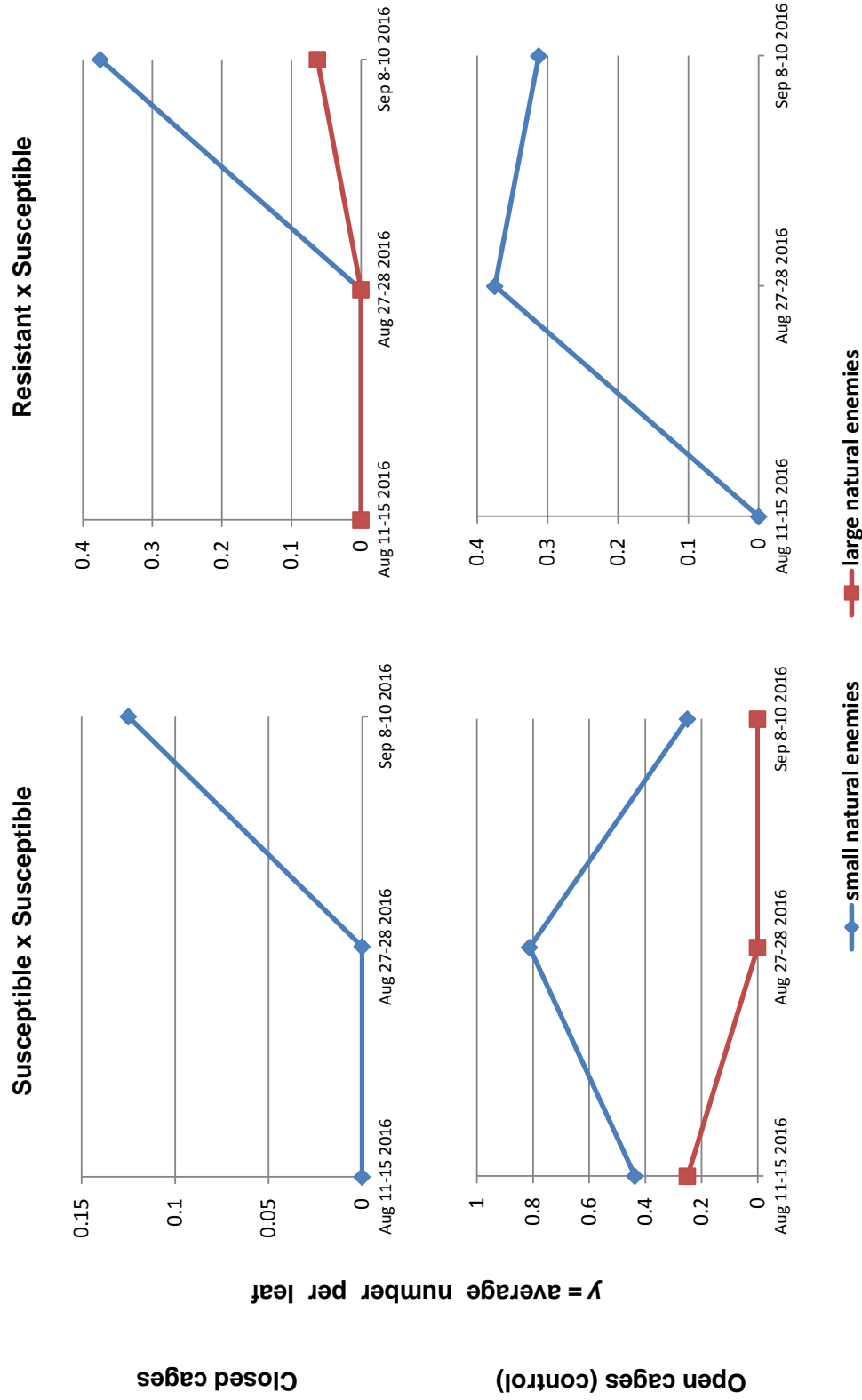
Fig. 23. Per-leaf density of small (body width < 2.5 mm) and large (body width ≥ 2.5 mm) aphid natural enemies in fine mesh (full enemy exclusion) cages, TAMU Farm, Somerville, TX, 2016. Left column: susceptible x susceptible sorghum hybrid (ATx3408/RTx436). Right column: resistant x susceptible sorghum hybrid (ATx631/RTx436). Top row: experimental cages. Bottom row: open cages (control). Note differences in scale of *y* axes. No large natural enemies were observed in the closed susceptible x susceptible and open resistant x susceptible treatments.
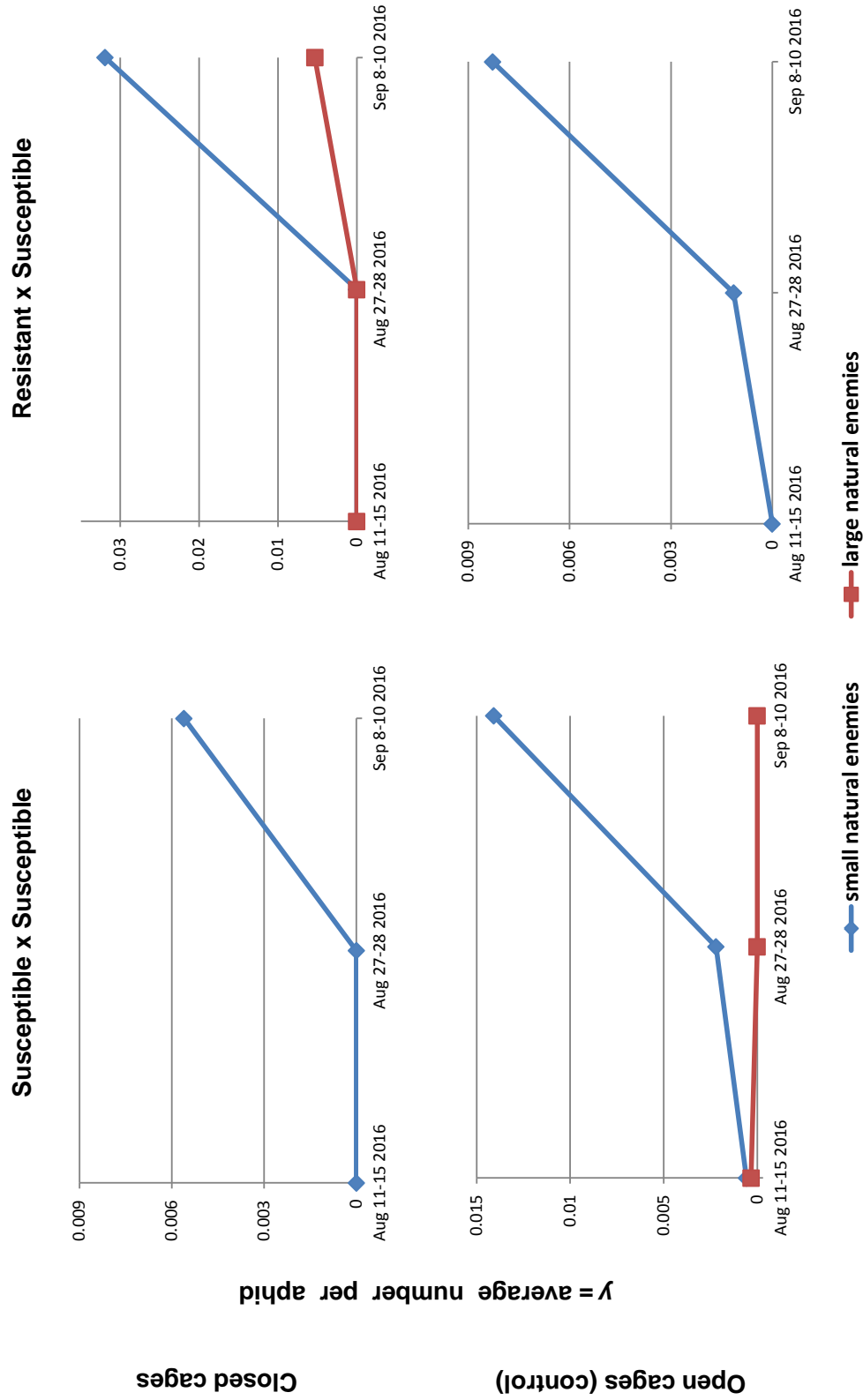
Fig. 24. Number of small (body width < 2.5 mm) and large (body width ≥ 2.5 mm) aphid natural enemies per sugarcane aphid in fine mesh (full enemy exclusion) cages, TAMU Farm, Somerville, TX, 2016. Left column: susceptible x susceptible sorghum hybrid (ATx3408/RTx436). Right column: resistant x susceptible sorghum hybrid (ATx631/RTx436). Top row: open cages (control). Bottom row: experimental cages. Note differences in scale of y axes. No large natural enemies were observed in the closed susceptible x susceptible and open resistant x susceptible treatments.
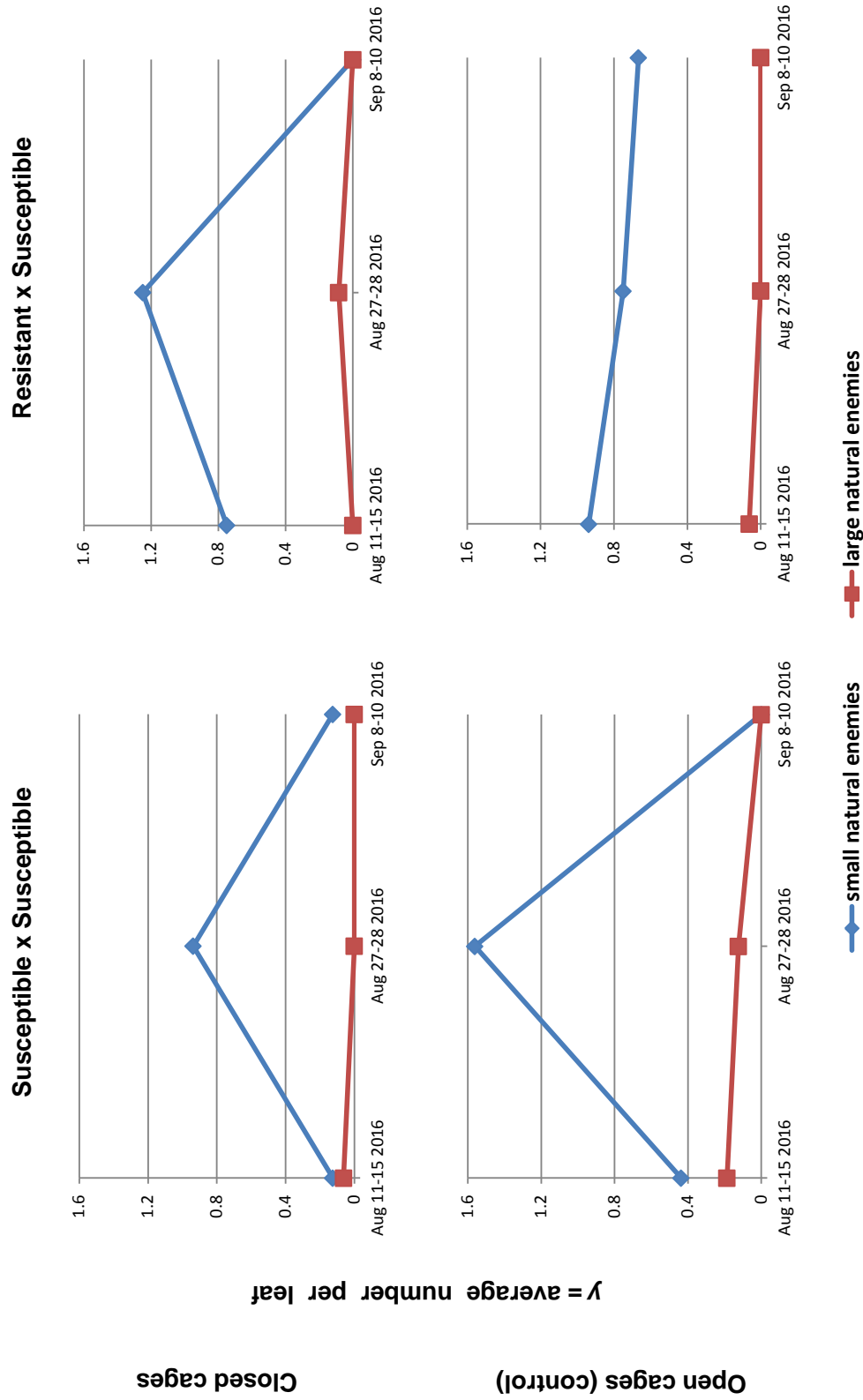
Fig. 25. Per-leaf density of small (body width < 2.5 mm) and large (body width ≥ 2.5 mm) aphid natural enemies in standard mesh (large enemy exclusion) cages, TAMU Farm, Somerville, TX, 2016. Left column: susceptible x susceptible sorghum hybrid (ATx3408/RTx436). Right column: resistant x susceptible sorghum hybrid (ATx631/RTx436). Top row: experimental cages. Bottom row: open cages (control).
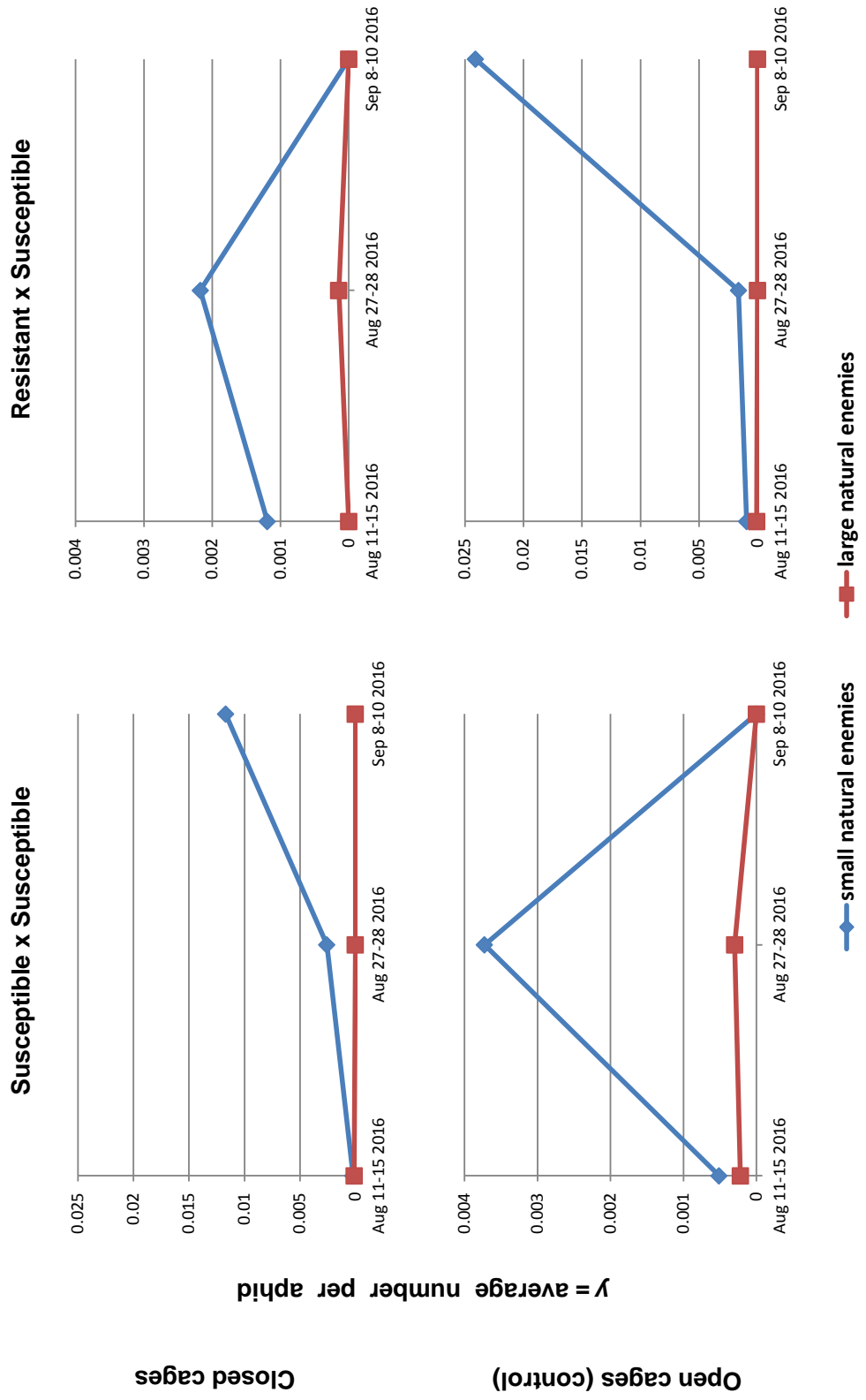
Fig. 26. Number of small (body width < 2.5 mm) and large (body width ≥ 2.5 mm) aphid natural enemies per sugarcane aphid in standard mesh (large enemy exclusion) cages, TAMU Farm, Somerville, TX, 2016. Left column: susceptible x susceptible sorghum hybrid (ATx631/RTx436). Right column: resistant x susceptible sorghum hybrid (ATx3408/RTx436). Top row: experimental cages. Bottom row: open cages (control). Note differences in scale of y axes.

In a similar experiment by Costamagna et al. (2008), partial and full exclusion cages, as well as open sham cages, were used to evaluate separately the impact of small (<2 mm) and large (>2 mm) natural enemies on the soybean aphid (*Aphis glycines* Matsumura) on soybean, *Glycine max* (L.) Merr. Large predators—of which the majority were Coccinellidae—were found to have a much larger suppression effect on aphid populations than small predators did. Moreover, only large enemies were able to prevent aphid populations from reaching economic injury thresholds. These conclusions were based on the finding that yield loss and peak aphid density were lowest in the open cage and uncaged treatments, which were the only treatments where large natural enemies were not excluded.

Taken together, the findings of Costamagna et al. (2008) are evidence that large natural enemies are more efficient aphid biological control agents than small natural enemies and are therefore of greater importance to integrated pest management. However, this may not necessarily be the case for natural enemies of the sugarcane aphid on sorghum. There were large differences in natural enemy community composition between Costamagna et al. (2008) and our cage study. In Costamagna et al. (2008), although Coccinellidae comprised the vast majority (73%) of the large natural enemies and were the most influential natural enemy taxon overall, no scymnine coccinellid species were observed. In contrast, while coccinellids were overall the most numerous natural enemies that we observed in cages (Figs. 19-22), nearly all of the coccinellids were Scymninae. Additionally, Costamagna et al. (2008) found *O. insidiosus* to be the most common small natural enemy. In our study, *O. insidiosus* was not detected in any cages. Finally,

78

Costamagna et al. (2008) detected very few parasitoid wasp mummies (percent aphid parasitism <1%), as opposed to our study where *Aphelinus* was the predominant natural enemy taxon in some treatments on some sampling dates (Figs. 19-22). Given these differences in the composition of natural enemies in both size classes, the finding that large natural enemies contribute more to aphid control on soybean (Costamagna et al. 2008) should not be assumed to be generalizable to the relative impact of large and small natural enemies of the sugarcane aphid on sorghum. In our study, due to the lack of statistically significant differences in aphid density between treatments, the relative aphid suppression caused by small and large natural enemies could not be measured. However, future exclusion cage studies have the potential to reveal which natural enemy size class offers the most efficient biological control of sugarcane aphids in sorghum agroecosystems.

3.4.1. Methodological Challenges and Suggested Revisions

This study's methods were compromised by several confounding variables that had not been anticipated. There are at least two explanations for the presence of natural enemies in cages intended to exclude them. First, cage integrity was compromised by the activity of corn earworm (*Helicoverpa zea* Boddie) and fall armyworm (*Spodoptera frugiperda* Smith). Larvae infested the sorghum heads and chewed holes through the mesh. The holes were in many cases wide enough to permit the movement of all large aphid predators. Holes were patched with duct

tape; however, aphids and natural enemies could have moved through the holes in the time before they were sealed.

Cage effects may have differed between fine mesh and standard mesh and may have changed over the course of the sampling period. Both mesh fabrics were white, but the mosquito mesh was coarse and matte, whereas the fine mesh (organza) was smooth and translucent with a pearlescent sheen. Additionally, cages accumulated debris including mud, pollen, honeydew, aphid exuviae, and sooty mold. Differences in the appearance, texture, and chemical profile of the two fabrics over time may have affected cage microclimate and cage attractiveness to natural enemies.

If this experiment is to be repeated, the following methodological changes may help avoid or control for such confounding factors:

1. Cage mesh should be durable enough to withstand chewing by caterpillars and other arthropods. All mesh should be the same color, have the same light reflectance, be made from the same material, and have the same surface texture, to the extent feasible.

3. Natural enemies and pests should be eliminated prior to cage installation. Plants selected for caging should be thoroughly inspected in order to remove as many natural enemies and potentially disruptive organisms (e.g., corn earworm) as possible.

4. Cages should be infested with aphids early in the growing season, while sorghum is at the flag leaf or boot stage. This will allow for a longer sampling period before the plants senesce. To benefit from the prolonged experimental duration,

cages should be sampled on five or more dates. This will provide a more detailed and accurate estimation of changes in aphid and natural enemy densities over time.

5. To compensate for the very low average number of natural enemies per leaf, the number of leaves sampled per cage should be increased.

6. To provide reliable open field reference data, the uncaged plants in each plot should be sampled on the same dates as caged plants.

7. A third mesh grade would be useful to more accurately compare the aphid suppression effects of small and large natural enemies while controlling for cage effect. This mesh must be wide enough to allow the movement of large aphid natural enemies. A mesh aperture diameter of approximately 7 mm should be sufficient for the largest major aphid predators. In the current experiment, large natural enemies were able to enter only the open cages, and only through the mesh-free zones at the top and bottom. Wide mesh cages would allow large enemies to enter cages from all sides, so that large enemies would be able to access cages through all—rather than just two—of the same routes that small enemies can use. Wide mesh cage treatments will measure the combined aphid suppression effects of large and small natural enemies.

3.5. Conclusion

On the first sample date, there were significantly more small lady beetles (Coccinellidae: Scymninae) per leaf and per aphid in closed standard mesh cages than in other cage treatments. On the second sampling date, there were significantly more Scymninae per leaf on open and closed standard mesh cages than on fine mesh cages. Scymnine lady beetles are small enough to crawl through the apertures in the standard mesh, but are too large to pass through the fine mesh; therefore, these results were expected. The ratio of Scymninae to aphids was also significantly higher on the RxS sorghum hybrid than on the SxS hybrid on the first sample date. There were no Scymninae x hybrid interactions on this and other sampling dates. These results suggest that the combination of aphid-resistant sorghum and Scymninae lady beetles may have an additive aphid suppression effect.

Sugarcane aphid density did not differ significantly between no-enemy, small-enemy, and all-enemy cage treatments. Consequently, aphid suppression by natural enemies of either size class could not be quantified. The data show an expected trend of smaller enemies outnumbering large enemies in closed cage treatments; however, smaller enemies were also more numerous than large enemies in open cage treatments. Our results may have been compromised by confounding factors that were not detected until the experiment was underway. Conclusions drawn from our results should be verified in further experimentation using revised methodology. The potential for an interaction between natural enemies and aphid-resistant

sorghum resulting in additive sugarcane aphid suppression is an especially

promising subject for further study.

4. CONCLUSIONS

This study found ample evidence that many of the aphid natural enemy species established in the United States have been successfully exploiting the sugarcane aphid in sorghum agroecosystems, despite the aphid's status as a novel prey species. 22 morphospecies of natural enemies were identified. The species community included two parasitoid wasps (*Aphelinus* sp. and *Lysiphlebus testaceipes*), ten lady beetles (Coccinellidae), three hoverflies (Syrphidae), five green lacewings (Chrysopidae), brown lacewings (Hemerobiidae), and minute pirate bugs (*Orius insidiosus*). The numerically dominant natural enemy groups were Coccinellidae and *Aphelinus*, despite the fact that *Aphelinus* mummies were observed to be hyperparasitized by *Syrphophagus aphidivorus* at a rate of approximately 90%. *Aphelinus* and Coccinellidae were the only natural enemies for which a hybrid by time interaction was detected: both taxa maintained high activity on resistant sorghum for a longer period than on susceptible sorghum, although the cause of this difference is unclear. Chrysopidae and Syrphidae were the most abundant natural enemy groups after Coccinellidae and *Aphelinus. Lysiphlebus testaceipes* and *Orius insidiosus* were present only in trace numbers.

Species composition showed strong continuity across the central and south Texas field sites. Nearly all of these species were observed on aphid-infested sorghum in both juvenile and adult life stages. The juveniles were able to survive and complete development when reared on a diet of sugarcane aphids alone. This indicates that these natural enemies are not only colonizing aphid-infested plants as

adults, but are also successfully reproducing and exploiting the aphid across multiple generations over the course of the growing season.

Both aphids and natural enemies showed a trend of higher peak abundance on sorghum cultivars with relatively high susceptibility to the aphid. Aphid populations showed a trend of one dramatic boom-and-bust cycle per season, consistent with the lifecycle of a sugarcane aphid colony. Peak abundance of natural enemies tended to lag behind peak abundance of aphids by one to two weeks, likely representing the time required for natural enemies to locate the aphids and subsequently reproduce.

Average sugarcane aphid density was lower on most resistant hybrids. While both aphid and natural enemy populations were lower on resistant plants, natural enemy populations remained roughly proportionate to aphid populations across hybrids. The similar ratio of natural enemies per aphid on susceptible and resistant sorghum suggests that the plants' aphid resistance traits did not negatively affect the ability of natural enemies to suppress aphid populations. This suggests that natural enemies and host plant resistance are able to play complementary roles in aphid control.

Yield was not measured in this study. An aphid-resistant cultivar is unlikely to be widely adopted unless the cultivar's maximum yield is comparable to that of high-yielding, susceptible cultivars even when the target aphid is absent. To provide more information to growers concerning the performance of resistant versus susceptible sorghum, future studies of interactions between aphids, natural enemies and

resistant host plants could culminate with harvesting and weighing the grain yield of each hybrid separately.

The natural enemy exclusion cage trials did not run as intended. Several methodological problems and oversights, such as damage to cage mesh by non-target species, were not detected until the experiment was already underway. These confounding factors raise questions about the validity of the cage data; therefore, any conclusions suggested by the results should be treated as tentative. Small lady beetles (Coccinellidae: Scymninae) alone were found to differ significantly in per-leaf and per-aphid density across treatments on some sample dates. Other natural enemies showed no significant differences in per-leaf or per-aphid density. Sugarcane aphid density did not differ between cage or hybrid treatments; therefore, aphid suppression by small and large species could not be measured. However, with appropriate methodological revisions, exclusion cages may be an informative tool in future research on interactions between the sugarcane aphid, natural enemies, and sorghum cultivars.

This study found strong continuity in natural enemy species composition and population trends across both counties and years, indicating that the natural enemy responses observed are not isolated events. Continued sampling of natural enemies throughout the sugarcane aphid's US range is recommended in order to aid and inform pest management decisions. Sampling across different growing regions can reveal continuity or discontinuity of natural enemy species distribution or species richness. Such data could also reveal regions that are natural enemy hot spots, or areas where the existing natural enemy community is depauperate.

In order to track stability or change in natural enemy composition or efficacy over time, continued surveying of sugarcane aphids and natural enemy communities is recommended. For example, repeated field studies showing a steady increase in natural enemy density and a steady decrease in frequency and severity of sugarcane aphid outbreaks could be evidence that established natural enemy species are adapting to exploit this introduced pest with greater efficiency.

Due consideration should be given to IPM strategies that facilitate aphid suppression by established natural enemies, such as intercropping sorghum with strips of native vegetation or refuge crops able to sustain high natural enemy populations. Likewise, care should be taken with the timing and frequency of pesticide applications such that the aphid suppression contributions of local natural enemies are minimally affected. Natural enemies may be unable to regulate the aphid population growth rate, and thus fail to prevent yield loss, when there are already hundreds or thousands of aphids per leaf. However, natural enemies that colonize sorghum fields early in the growing season and in sufficient numbers can suppress aphids effectively by targeting aphid colonies are at their earliest and most vulnerable growth stages.

Overall, the established natural enemies identified in this study show strong potential as a source of conservation biological control of the invasive sugarcane aphid. The ability of local species to adapt to and suppress this novel pest should not be underestimated.

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