

THE EFFECTS OF PREDATION ON SUGARCANE APHIDS IN SORGHUM

A Thesis

by

JEREMY ALAN HEWLETT

Submitted to the Office of Graduate and Professional Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Chair of Committee,	Micky Eubanks
Committee Members,	David Kerns
	Ronnie Schnell
Head of Department,	David Ragsdale

August 2018

Major Subject: Entomology

Copyright 2018 Jeremy Alan Hewlett

ABSTRACT

The sugarcane aphid, *Melanaphis sacchari* Zehntner (Hemiptera: Aphididae), is an emerging pest in sorghum, *Sorghum bicolor* (L.), in the USA. Lady beetles and green lacewings are abundant in sugarcane aphid-infested sorghum fields, but little is known about the effects of these predators on the establishment and growth of sugarcane aphid populations in sorghum. I quantified the effect of two species of lady beetles (*Coccinella septempunctata* L. and *Harmonia axyridis* Pallas) (Coleoptera: Coccinellidae) and green lacewings (*Chrysoperla rufilabris* Burmeister) (Neuroptera: Chrysopidae) on aphid colony growth across several aphid densities. I incorporated these predator effects into a dynamic predator-prey model to predict the impact of predators on the short-term population dynamics of sugarcane aphids and their ability to suppress aphids below action thresholds in sorghum. I also compared the effects of aphid-resistant sorghum on predation by lacewing larvae.

All predators were able to significantly reduce the growth of sugarcane aphid populations at low to intermediate aphid densities (20, 40, and 80 aphids per colony). At the highest aphid density (160 aphids), *H. axyridis* larvae were much less effective and lacewing larvae did not suppress sugarcane aphid colony growth. The model found that all predators prevented aphid densities between 20-40 per leaf from reaching action thresholds and at densities of 80 aphids per colony, all predators suppressed aphids below threshold in two to four days. However, at the highest initial aphid density (160), *H. axyridis* larvae required seven days to suppress aphids below threshold and lacewing larvae never suppressed aphids below threshold.

Aphid-resistant cultivars sustained smaller populations of aphids compared to susceptible. However, predators were an overall more effective tool to reduce aphid densities

than use of only resistant varieties. As there is some evidence that predators may be more attracted to aphid-infested resistant varieties, predator effects may be enhanced on these varieties. My results suggest that common predators have the potential to reduce the number of insecticide applications needed to control sugarcane aphids and should be incorporated into Integrated Pest Management (IPM) programs. The simultaneous use of biological control and resistant varieties may yield synergistic suppression of sugarcane aphids in sorghum.

DEDICATION

To my family who put up with my nonsense while I went completely crazy.

I'm sure we'll all look back on it someday and laugh, right?

ACKNOWLEDGEMENTS

I would like to thank MacKenzie Kjeldgaard, Apuleyo Yanez, Andrew Graf and Constance Lin for all of their support and technical assistance. I certainly could not have done this without you. Too much work and too little time – thank you for offering your personal time. Thank you, Ada Szczepaniec, for providing much of the initial material like seeds and cages, and for your consistent interest in and comments on my work. I also thank my committee, Micky Eubanks, David Kerns, and Ronnie Schnell for their support and advice. I am most indebted to my advisor, Dr. Eubanks, for his patience, guidance, and passing of knowledge. I am honored to say I was a part of his lab in which I received a lifetime worth of valuable experience. Finally, thank you to the John A. Jackman Endowed Scholarship for that extra push to the finish line.

CONTRIBUTORS AND FUNDING SOURCES

This study was funded by the Department of Entomology at Texas A&M University, Texas A&M AgriLife Research, and the John A. Jackman Endowed Scholarship. This study was also supported by a committee consisting of Dr. Micky Eubanks and Dr. David Kerns of the Department of Entomology, and Dr. Ronnie Schnell of the Department of Soil and Crop Sciences. Insect collection and rearing for Chapters II and III were accomplished with assistance from Apuleyo Yanez and Andrew Graf. The olfactometry data were collected as part of an independent project by Apuleyo Yanez and directed by Hewlett. All other work contained within this document was completed by Hewlett.

NOMENCLATURE

IPM	Integrated Pest Management
HPR	Host Plant Resistance
Action Threshold	Level of pest density where action should be taken to prevent economic injury

TABLE OF CONTENTS

	Page
ABSTRACT	ii
DEDICATION.....	iv
ACKNOWLEDGEMENTS	v
CONTRIBUTORS AND FUNDING SOURCES	vi
NOMENCLATURE	vii
TABLE OF CONTENTS.....	viii
LIST OF FIGURES	ix
LIST OF TABLES.....	xi
CHAPTER I INTRODUCTION	1
1.1 Introduction.....	1
1.2 Study System Introduction	2
CHAPTER II THE EFFECTS OF SUGARCANE APHID DENSITY IN SORGHUM ON PREDATION BY LADY BEETLES AND LACEWINGS	5
2.1 Introduction.....	5
2.2 Materials and Methods	7
2.3 Results	10
2.4 Discussion.....	16
CHAPTER III INTERACTIVE EFFECTS OF PREDATORS AND HOST PLANT RESISTANCE ON SUGARCANE APHIDS IN SORGHUM	19
3.1 Introduction.....	19
3.2 Materials and Methods	22
3.3 Results	27
3.4 Discussion.....	35
CHAPTER IV CONCLUSION	39
REFERENCES	41

LIST OF FIGURES

Page

- Fig. 1. Interactive effects of predators and initial sugarcane aphid density on sugarcane aphid suppression. Bars represent mean (\pm SEM) densities of aphids remaining on the plants after 48 h. Bars marked with different letters are significantly different ($P < 0.05$). Predators included adults and larvae of *C. septempunctata* (C7 Adults and C7 Larvae), and *H. axyridis* (Ha Adults and Ha Larvae), and larvae of *C. rufilabris* (Cr Larvae). 11
- Fig. 2. The effect of predators on final sugarcane aphid abundance averaged across aphid densities. Bars represent the mean (\pm SEM) of aphids remaining after 48 hours for each predator. Bars marked with different letters are significantly different ($P < 0.05$). Predators included adults and larvae of *C. septempunctata* (C7 Adults and C7 Larvae), and *H. axyridis* (Ha Adults and Ha Larvae), and larvae of *C. rufilabris* (Cr Larvae). 12
- Fig. 3. Predicted aphid densities after two or four days when exposed to predators. Initial aphid densities (40, 80, 160) indicated by solid line and action threshold indicated by dashed box. The effects of predators on initial aphid densities of 40 (A), 80 (C), and 160 (E) on day two are shown in column-1, densities of 40 (B), 80 (D), and 160 (F) on day four in column-2. Initial aphid densities of 20 are similar to 40 and thus omitted..... 14
- Fig. 4. The 14-day predator-prey model forecast for initial aphid densities. At low and intermediate densities (A, B), predators are able to continuously suppress aphid populations for as long as they are present. At high densities (C), all predators except lacewings can provide economic suppression. Predators included adults and larvae of *C. septempunctata* (C7 Adults and C7 Larvae), and *H. axyridis* (Ha Adults and Ha Larvae), and larvae of *C. rufilabris* (Cr Larvae). 15
- Fig. 5. Predator by variety interaction. Aphid colonies on resistant varieties, without a predator, show a 1.8-fold growth increase vs a 2.4-fold increase on the susceptible variety without a predator. However, predators suppress aphid populations to approximately the same final density on both. Cultivars are averaged across both densities (40, 120). Bars represent 95% CI..... 28
- Fig. 6. Variety by density interaction. At high initial densities (120), aphid populations on the susceptible variety have a greater final density than on the resistant variety. Predators have a larger suppressive effect at initial aphid densities of 120 rather than at 40. There was no difference between final aphid densities when initial aphid densities were low (40). Densities are averaged across both cultivars. Bars represent 95% CI. 29
- Fig. 7. The effects of predator on both cultivars at low (40) and high (120) aphid densities. Aphid populations are significantly higher on susceptible varieties when no predator

is present, however there was no significant difference between cultivars when predators were present. Bars represent \pm SEM.	30
Fig. 8. Volatile preference of lady beetles. On resistant cultivars with and without aphids, lady beetles chose with aphids significantly more often than without. This effect was not seen on susceptible varieties, where there was no difference in preference between the two options. No-choice is not shown.	32
Fig. 9. Predator-Prey model of aphid population growth on resistant and susceptible sorghum. Final aphid populations show little difference on either cultivar when predators are present. Aphid populations grew considerably faster on susceptible sorghum when predators were absent, suggesting predators could be more important on susceptible varieties.	34

LIST OF TABLES

	Page
Table 1. Analysis of variance for the effects of predator, variety, and density on aphid population growth.....	31

CHAPTER I

INTRODUCTION

1.1 Introduction

The sugarcane aphid, *Melanaphis sacchari* (Zehntner) (Hemiptera: Aphididae), is an emerging and remarkably damaging pest of sorghum, *Sorghum bicolor* (L.), in the United States (Kerns et al., 2015; Bowling et al., 2016; Brewer et al., 2016; Medina et al., 2017). Sugarcane aphids were first discovered infesting sorghum in southwest Louisiana in early 2013, and by the end of 2013, sorghum infestations were reported throughout Louisiana, Mississippi, southern Oklahoma, and along the Texas gulf coast (Kerns et al., 2015). By 2015, sugarcane aphid infestations of sorghum were confirmed in 17 states, covering 98% of the sorghum-producing region of the United States (Bowling et al., 2015; Bayoumy et al., 2016; Bowling et al., 2016). High aphid infestations are devastating and substantially reduce yield and overall grain quality by causing leaf chlorosis, necrosis, stunted growth, and plant death (Singh et al., 2004; Villanueva et al., 2014; Colares et al., 2015b; Bayoumy et al., 2016; Bowling et al., 2016). Moreover, fields not treated with insecticides may experience 100% yield loss when infested early in plant development (Catchot et al., 2015).

The economic losses associated with sugarcane aphids have been immense (Elliott et al., 2017). Production losses due to outbreaks in the Lower Rio Grande Valley in 2014-2015, for example, cost an estimated \$31.6 million (Villanueva et al., 2014; Zapata et al., 2016). Insecticides are currently the only effective means of suppressing sugarcane aphids in sorghum, but insecticide applications are costly (Elliott et al., 2017). With an average cost of \$20/acre, a single insecticide application nationwide (5.76M acres as of May 1, 2017) would exceed \$115

million (Zapata et al., 2016; USDA-NASS, 2017). In addition, insecticides registered for suppression of aphids in sorghum are limited to two Group 4 insecticides (sulfoxaflor and flupyradifurone), and their intense use is likely to result in rapid development of insecticide resistance (Colares et al., 2017). Thus, there is an urgent need to explore and integrate additional, non-chemical management tactics that will reduce management costs and limit insecticide resistance while still maintaining yield.

Two principal methods of reducing reliance on insecticides is through the use of natural enemies (predators) and use of resistant plant cultivars (Emden and Wearing 1965). However, the effects of predators on sugarcane aphid in sorghum, and the interactive effects of resistant sorghum cultivars has not yet been well studied in this system. This research is intended to identify predators that are effective at reducing sugarcane aphid outbreaks and provide the necessary groundwork to begin integrating these predators into current control programs.

1.2 Study System Introduction

1.2.1. Sorghum (Sorghum bicolor (L.) Moench)

Grown for food, fuel, fiber, and feed, sorghum is the fifth most important cereal grain in the world. In South Asia, Central America, and Africa, for example, sorghum is an important food staple crop, and in Australia, South America, and the United States, it is used primarily as fodder (Pinto et al., 2006; Paterson et al., 2009). Sorghum is also an important cash crop and its production can be seriously constrained through insect infestations (Sharma, 1993; Khan et al., 2010).

1.2.2. Sugarcane aphid (Melanaphis sacchari)

Sugarcane aphids can potentially infest sorghum plants from 2-3 weeks post-emergence through harvest (Fang, 1990; Singh et al., 2004). Life-history traits of aphids, including

parthenogenic, viviparous reproduction predispose the aphids to rapid population growth in warm, dry climates (Chang et al., 1982; Singh et al., 2004; Colares et al., 2015b). For example, an initial population of 50 aphids may grow to more than 500 aphids within one week, and, left unmanaged, an established population can reach densities of over 10,000 aphids per leaf (Bowling et al., 2016).

1.2.3. Lady beetle (*Coccinellidae*)

Lady beetles have a rich history of use in the biological control of many pests and are key natural enemies of aphids (Hodek, 1970; Obrycki and Kring, 1998; Dixon, 2000; P. Chowdhury et al., 2008). They are economically important in agricultural production because of their potential to successfully act as biocontrol agents in many crops, including sorghum (Hodek, 1970; Kring et al., 1985; P. Chowdhury et al., 2008; GenÇer et al., 2017). The two species used in this study, *Harmonia axyridis* Pallas, and *Coccinella septempunctata* L. are known to be present on aphid-infested sorghum in Texas and are recognized as voracious aphid predators in other systems (Hodek, 1973; Angalet et al., 1979; Olszak, 1988; Bowling et al., 2016). For example, Tsaganou et al. (2004) found that *H. axyridis* 4th instar larvae could consume an average of 59 cotton aphids (*Aphis gossypii* Glover) (Hemiptera: Aphididae) per day and adult females could consume an average of 281 aphids per day.

1.2.4. Green lacewing (*Chrysopidae*)

Green lacewings, *Chrysoperla rufilabris* Burmeister, are considered significant predators in agricultural crops (Hydorn and Whitcomb, 1979; Tauber and Tauber, 1983; Tauber et al., 2000). The larvae are predacious and have been used extensively as biological control agents against soft-bodied arthropods, including aphids (Ridgway and Kinzer, 1974; Hydorn and Whitcomb, 1979; Balasubramani et al., 1994; Legaspi et al., 1994; Arnett, 2000; Khan and Zaki,

2008). Some studies suggest they are capable of consuming between 100-600 aphids through larval development (Smith and Hagen, 1956; Garrett et al., 2005; McEwen et al., 2007).

CHAPTER II

THE EFFECTS OF SUGARCANE APHID DENSITY IN SORGHUM ON PREDATION BY LADY BEETLES AND LACEWINGS

2.1 Introduction

Nearly all aphid species attract natural enemies that can provide vital pest suppression (Losey and Vaughan 2006, Colares et al. 2015a). Incorporating natural enemies into Integrated Pest Management (IPM) programs has the potential to both reduce the cost of suppressing aphid populations and reduce insecticide use (Zhang and Swinton 2012). For example, the effects of predators of soybean aphids (*Aphis glycines* Matsumura) (Hemiptera: Aphididae) in soybean have been used to dynamically adjust the aphid action threshold (Zhang and Swinton 2009, Hallett et al. 2014). Hallett et al. (2014) demonstrated that implementing the dynamic action threshold resulted in delayed insecticide treatments and an overall reduction of insecticide applications by 67% while still maintaining soybean yield. Similar positive effects of adjusting pest management decisions based on natural enemy activity have been documented in other crops including cotton, sweet corn, and tomato (Conway et al. 2006, Musser et al. 2006, Walker et al. 2010).

Predators and parasitoids associated with sugarcane aphid-infested sorghum have been surveyed and described in extension bulletins (Brewer and Gordy 2016, Knutson et al. 2016) and peer-reviewed publications (Colares et al. 2015b, Bowling et al. 2016, Cortez-Mondaca et al. 2016). These reports indicate that predators are by far the most abundant and diverse natural enemies of sugarcane aphids in sorghum. Up to ten species of lady beetles (Coccinellidae) are associated with sugarcane aphids in sorghum as well as several species of lacewings

(Chrysopidae and Hemerobiidae), hover flies (Syrphidae), and some predaceous true bugs (Anthocoridae) (Colares et al. 2015b, Bowling et al. 2016, Cortez-Mondaca et al. 2016). In one notable example, the assemblage of predators and parasitoids recruited by sugarcane aphid-infested sorghum did not differ significantly from natural enemies recruited to sorghum infested with greenbugs (Colares et al. 2015b). This study supported the hypothesis that predators associated with an existing aphid pest of sorghum were readily recruited to the invasive aphid, and that they would consume sugarcane aphids (Colares et al. 2015b). Little research beyond these descriptive surveys has been conducted regarding the role of predators in the suppression of sugarcane aphids in sorghum.

Despite a diverse community of predators, natural enemies have not been incorporated into dynamic action thresholds for sugarcane aphids in sorghum. A key challenge to developing a dynamic threshold that incorporates natural enemies is quantifying the impact of predators on aphids at different aphid densities (Zhang and Swinton 2012, Hallett et al. 2014, Giles et al. 2017). To address this, I performed a greenhouse experiment to quantify the effects of several common predators on the suppression of sugarcane aphids. I then incorporated these predator effects into a dynamic predator-prey model to predict the impact of predators on the short-term population dynamics of sugarcane aphids in sorghum and the ability of these predators to suppress aphids below action threshold. My research provides foundational steps necessary to improve the sustainability and profitability of sorghum production in the U.S. and advance the goals of the National IPM Roadmap of decreasing exposure of non-target organisms to insecticides and promoting tactics that improve economic benefits of crop production.

2.2 Materials and Methods

Sugarcane aphid-susceptible grain sorghum (*Sorghum bicolor* (L.) Moench) hybrid DKS38-08 (Monsanto, St. Louis, Missouri) was used in all experiments. One seed was planted per pot (15.24 cm diameter x 13.97 cm depth) (Dillen, Middlefield, OH) in Sunshine Mix #1 (Sungro, Agawam, MA) growing medium. Sorghum was cultivated in a greenhouse at 28°C - 32°C and supplemented with artificial light on a L:D (light:dark) cycle of 12:12. Sorghum was grown in 60 x 60 x 60 cm insect-proof cages (MegaView Science Co., Ltd., Taichung, Taiwan) for two weeks to an approximate size of 20 cm (~six true leaves). All sorghum was top-watered *ad libitum* when the first ~2.5 cm of soil was dry.

2.2.1. Aphid rearing

A sugarcane aphid colony was established using aphids originally collected from sorghum in Bushland, TX in 2016. The colony was maintained on sorghum in insect-proof enclosures for three months prior to the start of experiments. Sorghum plants used to support the colony were grown under the previously described conditions (section 3.1), but with 8-10 seeds per pot. Subsets of aphid colonies were moved by donor leaves to new plants approximately every two weeks to prevent overcrowding in the colonies.

2.2.2. Aphid predators

All predators were collected from sorghum fields in College Station and Thrall, TX. Two environmental chambers were used: a rearing chamber set to 27°C, 75% RH, and 12:12 (L:D) photoperiod, and a storage chamber set to 15.4°C, 75% RH, and 12:12 (L:D) photoperiod (see below). All predators were fed sugarcane aphids on sorghum clippings 2-3 times each week in the rearing chamber, once each week in the storage chamber, and provided a moistened cotton ball for water.

Adult lady beetles (*H. axyridis*, and *C. septempunctata*) were collected from sorghum fields and allowed to breed and oviposit on sorghum for approximately one week. Thereafter, adults were maintained in the storage chamber until sufficient numbers of larvae were available for the experiments. The majority (80%) of larvae were reared from eggs. Larvae were separated from the clutch using a fine-tip paintbrush and placed individually into 9 cm petri dishes at 2nd instar. To maximize aphid consumption potential, larvae were reared to the last instar (4th) for this study. Only immature green lacewings (*C. rufilabris*) were used in this study because adults are not predacious. Green lacewing larvae were reared from adult lacewings collected from sorghum fields. Gravid adults were allowed to oviposit, and the larvae were removed from the clutch at 2nd instar with a fine-tip paintbrush and placed into individual 9 cm petri dishes. Again, all larvae were reared to the last instar (3rd) for this study.

2.2.3. Plant enclosures

Each cage was built from 3.8 L, 25 cm tall wide-mouth plastic jars (Uline, Pleasant Prairie, WI) with the sides and bottom cut out and replaced with a fine mesh to permit airflow. Jars were turned upside down over the plants and pressed into the soil. Each experimental unit was then placed on a small stand inside a 59.7 cm x 42.9 cm x 14.9 cm plastic bin (Sterilite, Townsend, MA) and moated (partly filled with water) to prevent insects outside of the experiment (e.g., ants) from reaching the arena.

2.2.4. Experimental design and analysis

I conducted a 4 x 6 randomized factorial complete block greenhouse experiment to quantify the effects of sugarcane aphid density (20, 40, 80, or 160 per plant) and predator identity (adults and larvae of *C. septempunctata*, adults and larvae of *H. axyridis*, larvae of *C. rufilabris*, or no predator) on aphid abundance after 48 hours of exposure to predators. Each experimental

unit consisted of one caged sorghum plant, which was randomly assigned to one of the 24 treatment combinations. Aphids were introduced to each cage by transferring infested leaves from lab-reared aphid colonies and were allowed to acclimate for 24-48 hours. During the acclimation period, predators were maintained at 27°C in a growth chamber without any prey. A final visual inspection was made to ensure aphids had established at the assigned densities, and then a single predator was added to each cage with the exception of cages assigned to the no predator treatment. The number of aphids were counted 3 times per day for 48 hours, at 09:00h, 13:00h, and 17:00h respectively. The greenhouse was maintained at 28°C-33°C during the experiment. The experiment was replicated four times over the span of two months.

2.2.5. Statistical analysis

Data were tested for normality (Shapiro–Wilks test) and homogeneity of variance (Levene’s test) to verify ANOVA assumptions. The effects of sugarcane aphid density, predators, and their interaction on the number of aphids were analyzed using repeated measures ANOVA with initial aphid density and predator identity as fixed independent variables. Analyses were conducted using R v3.4.2 (R Core Team 2017).

2.2.6. Predator-Prey model

I used the predator effects from our experiment to parameterize a simple predator-prey model that estimates aphid population growth with and without predators. The estimated rate of increase (λ) of aphid population per time step (two days) was estimated using Eq. (1), where N is aphid population density and t is time. I used two-day time intervals because our predation experiment lasted 48 hours.

$$\lambda_{\text{experiment}} = \frac{N_{t+1}}{N_t} \quad (1)$$

I then used a dynamic version of our model to simulate aphid colony growth with or without predator effects over 14 days, Eq. (2). I was particularly interested in the ability of predators to maintain or suppress aphids below sugarcane aphid action thresholds in sorghum. I used an action threshold of 50-75 aphids per leaf using data from Gordy et al. (unpublished), which was based on previous work by Brewer and Bowling (2014).

$$\frac{dN}{dt} = \lambda_{\text{experiment}} \times N_{t-1} \quad (2)$$

2.3 Results

2.3.1. Predation study

There was a significant interaction between predator identity and initial aphid density on the final density of sugarcane aphids in our experiment ($F_{15,491} = 11.65, P < 0.001$; Fig. 1). At low initial aphid densities (20 and 40 aphids per plant), all predator species and life stages significantly suppressed aphids (Fig. 1). This suppression was dramatic. For example, when initial aphid densities were 20 and 40 per plant, predators suppressed the number of aphids by 81.8% to 94.5% and 60.5% to 94.4%, respectively. At higher densities (80 and 160 aphids), the impact of predators varied (Fig. 1). *Coccinella septempunctata* larvae maintained a high degree of suppression at all aphid densities, but the effect of *H. axyridis* larvae on aphid colony growth dropped from 90.9% to 53.9% as aphid density increased from 20 to 160. Green lacewing larvae were effective predators at relatively low aphid densities, but did not significantly suppress colony growth when there were 160 aphids per plant (Fig. 1). The effects of *C. septempunctata* adults and larvae and *H. axyridis* adults varied among aphid densities, but all three significantly, and in most cases dramatically, suppressed aphid colony growth at all densities (Fig. 1).

There was also a main effect of predator identity, in which aphid population abundance varied with predator species and life stages ($F_{5,491} = 87.53, P < 0.001$). Overall, *C. septempunctata* were more effective at suppressing aphid abundance than *H. axyridis* or *C. rufilabris* (Fig. 2). For example, *C. septempunctata* adults reduced aphid densities by 76% and larvae reduced aphid densities by 89%. Adults and larvae of *H. axyridis* reduced densities by 69% and 64%, respectively. The larvae of *C. rufilabris* were the least effective predator, reducing average aphid numbers by only 41.5%.

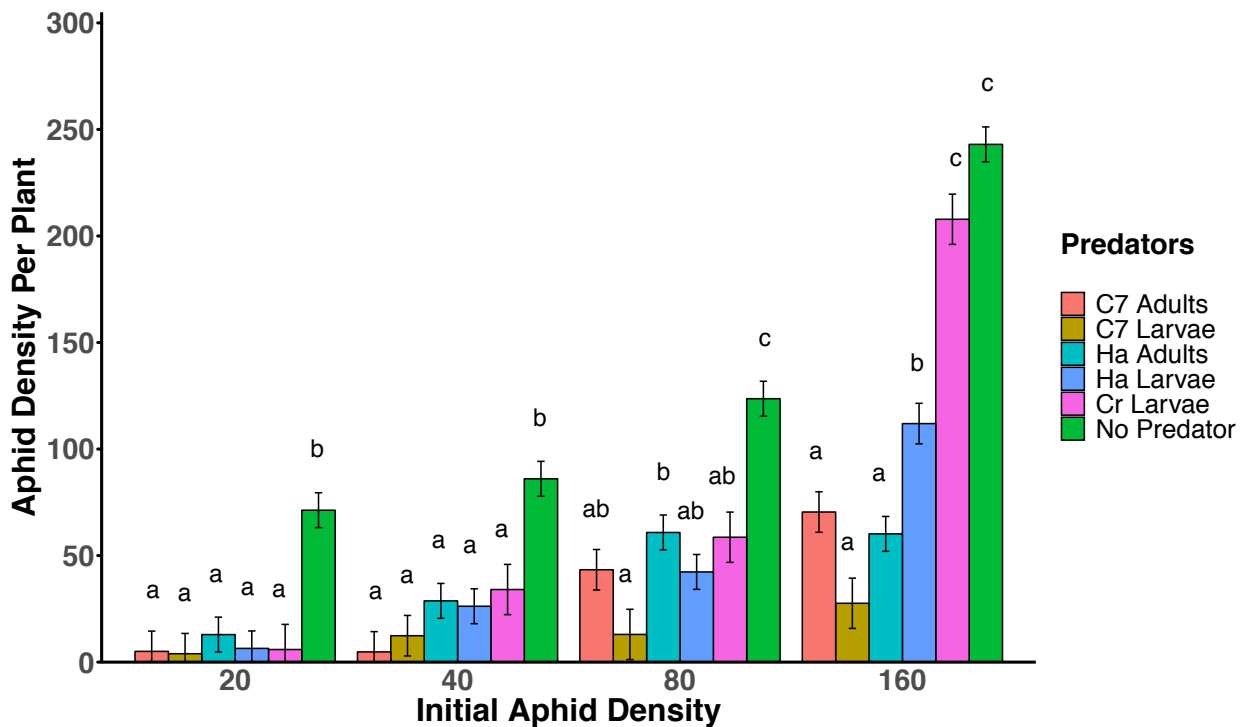


Fig. 1. Interactive effects of predators and initial sugarcane aphid density on sugarcane aphid suppression. Bars represent mean (\pm SEM) densities of aphids remaining on the plants after 48 h. Bars marked with different letters are significantly different ($P < 0.05$). Predators included adults and larvae of *C. septempunctata* (C7 Adults and C7 Larvae), and *H. axyridis* (Ha Adults and Ha Larvae), and larvae of *C. rufilabris* (Cr Larvae).

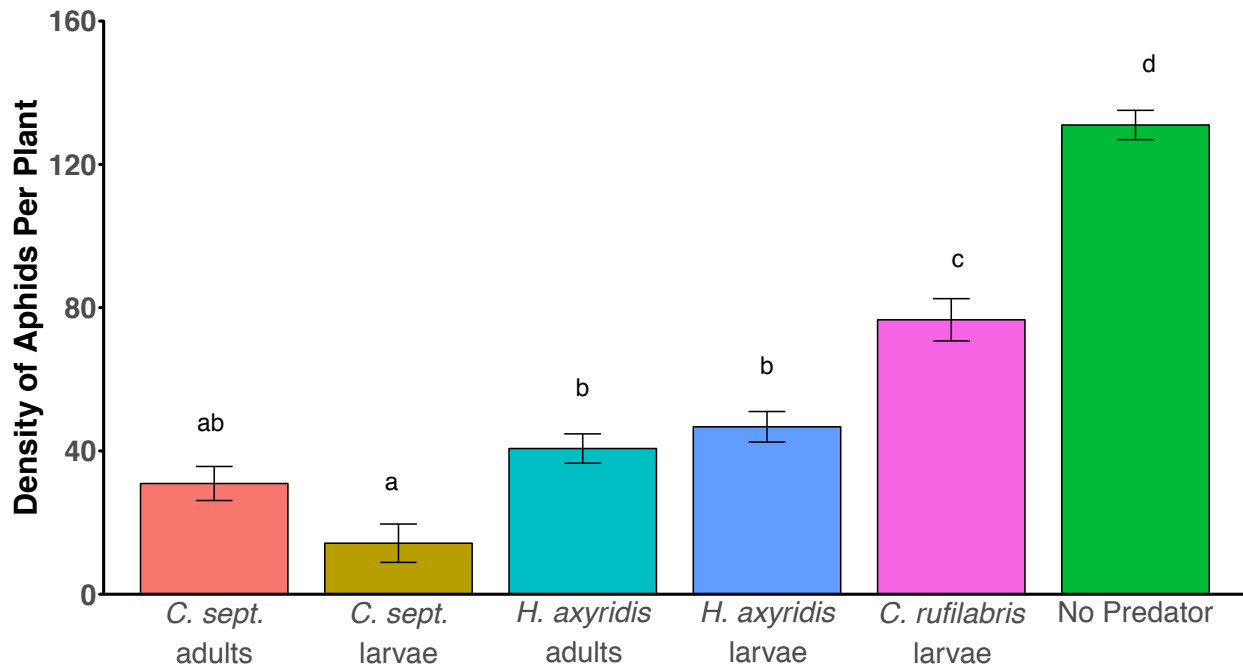


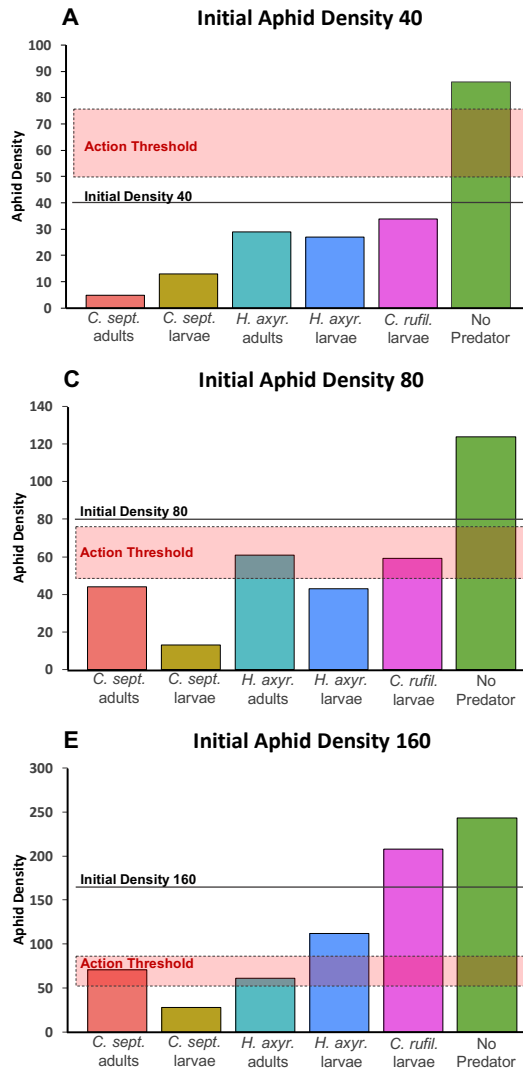
Fig. 2. The effect of predators on final sugarcane aphid abundance averaged across aphid densities. Bars represent the mean (\pm SEM) of aphids remaining after 48 hours for each predator. Bars marked with different letters are significantly different ($P < 0.05$). Predators included adults and larvae of *C. septempunctata* (C7 Adults and C7 Larvae), and *H. axyridis* (Ha Adults and Ha Larvae), and larvae of *C. rufilabris* (Cr Larvae).

2.3.1. Predator-Prey model

The predator-prey model predicted that all of the predator species and life stages were capable of providing significant suppression of sugarcane aphid population growth at low to medium densities (Figs. 3 and 4; aphid density of 20 not shown). For example, when initial aphid densities were 20 and 40 per plant all predator species and life stages suppressed sugarcane aphids below the action threshold after two (Fig. 3a) and four (Fig. 3b) days. When initial aphid density was 80, three of the predators decreased aphid abundance below the action threshold in two days (Fig. 3c) and all of the predator species and life stages suppressed aphids below the

action threshold after four days (Fig. 3d). Some predator species and life stages were less effective at relatively high aphid densities. For example, *C. rufilabris* larvae required four days to suppress aphids below the action threshold when there were 80 aphids per plant (Fig. 3d) and the larvae were unable to suppress sugarcane aphids at all when initial aphid density was 160 (Figs 3e and 3f, Fig. 4c). At 160 aphids per plant, *H. axyridis* larvae needed seven days to suppress aphids below the action threshold (Fig. 4c).

Day 2 Aphid Population Growth



Day 4 Aphid Population Growth

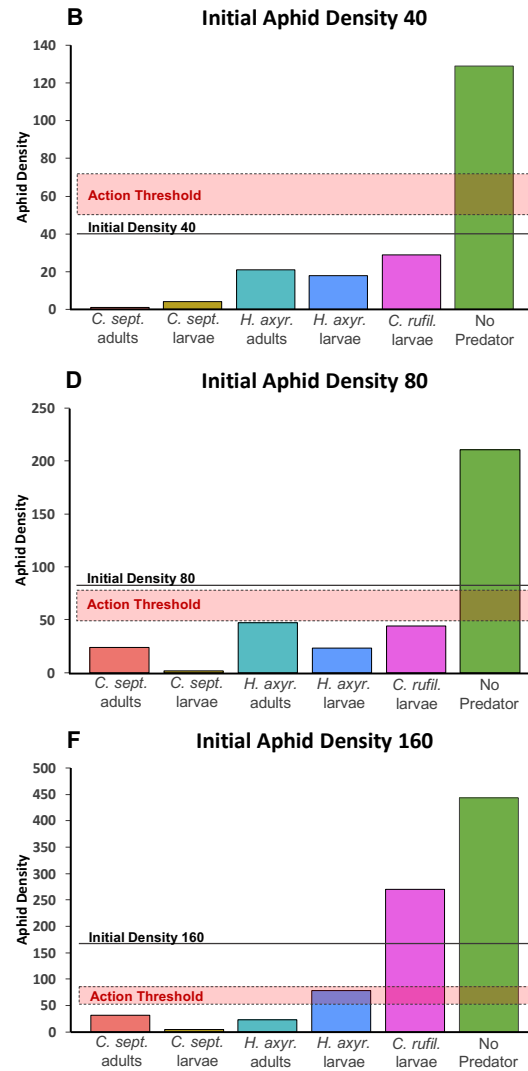


Fig. 3. Predicted aphid densities after two or four days when exposed to predators. Initial aphid densities (40, 80, 160) indicated by solid line and action threshold indicated by dashed box. The effects of predators on initial aphid densities of 40 (A), 80 (C), and 160 (E) on day two are shown in column-1, densities of 40 (B), 80 (D), and 160 (F) on day four in column-2. Initial aphid densities of 20 are similar to 40 and thus omitted.

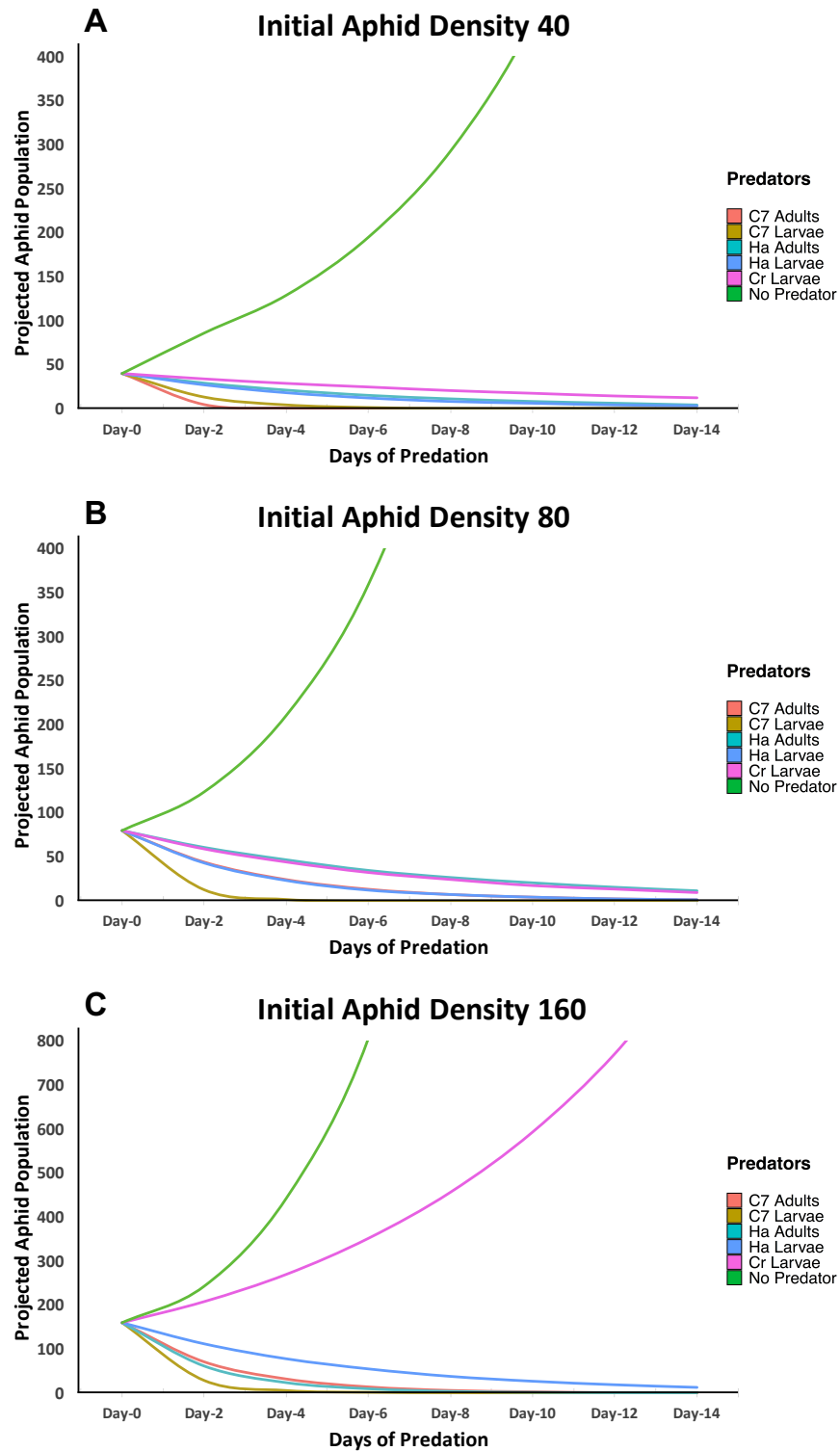


Fig. 4. The 14-day predator-prey model forecast for initial aphid densities. At low and intermediate densities (A, B), predators are able to continuously suppress aphid populations for as long as they are present. At high densities (C), all predators except lacewings can provide economic suppression. Predators included adults and larvae of *C. septempunctata* (C7 Adults and C7 Larvae), and *H. axyridis* (Ha Adults and Ha Larvae), and larvae of *C. rufilabris* (Cr Larvae).

2.4 Discussion

My study suggests that many common predators of sugarcane aphids found in sorghum are capable of providing economically important aphid suppression, often within 2-4 days. Specifically, I found that all predator species and life stages I studied were effective at suppressing sugarcane aphids at relatively low aphid densities. At higher aphid densities, *H. axyridis* larvae, and especially *C. rufilabris* larvae, were less effective. Overall, *C. septempunctata* larvae were the most effective predator at suppressing sugarcane aphids. Studies of lady beetles and green lacewings in other crops found similar results, confirming that these predators can significantly reduce aphid populations in agroecosystems (Honek 1979, Bode 1980, Fox et al. 2005, Costamagna et al. 2008, Hallett et al. 2014, Vandereycken et al. 2015). For example, Costamagna et al. (2007) found that *H. axyridis* and *C. septempunctata* were the primary predators responsible for suppression of soybean aphid in soybean. Strong predation pressure from these predators released soybean plants from herbivory and restored yield. Further, Fox et al. (2004, 2005) found that predators may be most effective in reducing smaller, newly established aphid populations, which is comparable to my results where predators were most effective at lower densities.

Integrating predators into pest management often depends on specific information regarding the densities of predators and pest that can have a meaningful, economically relevant impact on pest suppression. By creating dynamic action thresholds (e.g., Hallett et al. 2014) that incorporate natural enemies into the IPM decision-making process can delay or eliminate unnecessary insecticide applications. Reduced management costs can be substantial (Conway et al. 2006, Naranjo et al. 2015). For example, integrating predators into management programs targeting cotton fleahoppers in cotton decreased management costs by \$27/ha (Sterling et al.

1992). My results suggest that a dynamic action threshold for sugarcane aphids in sorghum that includes predators may be higher than the current action threshold. For example, my data suggests that a ratio of one *C. septempunctata* larvae to 160 aphids would result in rapid economic suppression of sugarcane aphids. I plan to use my results to develop and test in the field a dynamic action threshold that will include variation in the efficacy of different predators. I predict that my dynamic threshold will result in decreased insecticide use while maintaining yield when compared to sorghum managed with a conventional action threshold. This will save growers money and reduce the potential for the development of insecticide resistance.

Despite the presence of these predators in sorghum fields, sugarcane aphid outbreaks still occur. Factors such as sorghum growth stage (e.g., boot versus flowering), distribution of aphids colonizing fields (e.g., clumped versus random), and density of aphids could impact predator efficiency in several ways. For example, predators may not find aphids until they are at relatively higher densities (weak numerical response). Van Den Berg et al. (1997), for instance, found that low densities of soybean aphids did not attract enough predators to significantly reduce aphid numbers. Suppressive effects of predators are strongest when predators arrive early in the season and reduce aphid numbers before outbreaks occur (Takagi 1999, Fox et al. 2005, Koch and Costamagna 2016). Further, interactions among predators can affect biological control. When multiple predators occupy the same plant, predator interference, cannibalism, or intraguild predation (IGP) may severely limit predator efficacy (Sih et al. 1998, Stiling and Cornelissen 2005, Costamagna et al. 2008). For example, *H. axyridis* is a ubiquitous intraguild predator in many agroecosystems and readily preys on other coccinellids, including *C. septempunctata* (Yasuda et al. 2001, Costamagna and Landis 2006). Many generalist predators are known to inflict heavy mortality on lacewing larvae and can dramatically reduce their ability to suppress

aphids (Rosenheim and Wilhoit 1993). Sugarcane aphids may also be a poor quality prey for predators. For example, White et al. (2017) found that consumption of cowpea aphids originating from black locust trees by *H. axyridis* resulted in larval and adult death within three to seven days. Likewise, Chen and Liu (2001) found that *C. rufilabris* larvae fed turnip aphids took much longer to develop and had dramatically lower survival than when fed cotton or green peach aphids. Future research should explore if these or other factors limit sugarcane aphid predation in sorghum.

CHAPTER III
INTERACTIVE EFFECTS OF PREDATORS AND HOST PLANT RESISTANCE ON
SUGARCANE APHIDS IN SORGHUM

3.1 Introduction

Researchers have long desired to integrate host plant resistance (HPR) with biological control to achieve more effective pest suppression (Painter 1951, van Emden 1966, Schuster and Starks 1975, Bergman and Tingey 1979). For example, in a serendipitous study of natural enemies, Wyatt (1970) found there was a higher percentage of pest mortality on resistant plants than on susceptible plants. Starks et al. (1972) hints that this effect may be a synergistic interaction. He demonstrated that the combination of resistant cultivars and parasitoids significantly reduced pest populations and resulted in less plant damage. The simultaneous use of the resistant cultivar and parasitoids had a much greater suppressive effect together than they did separately. van Emden (1991) suggests similar results can be found in the field as well, where even on cultivars that were not completely resistant, there was a 60% reduction in pest populations in the presence of predators. While not directly stated, this may be because lower prey density favorably increased the predator-prey ratio, or because the resistant lines reduced prey reproduction rate and allowed predator consumption to outpace prey reproduction. In itself, host plant resistance should be an effective form of plant protection, especially when combined with biological control.

Resistant cultivars have been successfully utilized in the past as a means to reduce aphid populations (Settle et al. 1996, Pimentel et al. 2000, Pimentel et al. 2005, Dedryver et al. 2010, Desneux et al. 2010, Guedes and Cutler 2014). For example, sorghum germplasm identified as

resistant to greenbug (*Schizaphis graminum* Rondani) has been commercially used since the 1970s (Schuster and Starks 1973, Young and Teetes 1977), and Wood and Starks (1972) demonstrated that several greenbug biotypes exhibited reduced fecundity on resistant sorghum (PI 264453) as compared to susceptible (RS-610). Soybean varieties resistant to the soybean aphid (*Aphis glycines* Matsumura), a highly damaging pest of soybean, have also played an important role in managing this pest and reducing monetary losses (Ragsdale et al. 2011, Hodgson et al. 2012, McCarville and O'Neal 2013). Likewise, resistant varieties of wheat have been successful in managing the Russian wheat aphid (*Diuraphis noxia* Mordvilko). Messina and Sorenson (2001) found that even moderate reductions in population growth of the Russian wheat aphid on resistant wheat was enough to significantly alter the predation effects of green lacewings, *Chrysoperla plorabunda* (Fitch). The combined effects of predation and resistant cultivars were synergistic, resulting in a higher proportional reduction in aphid density on resistant plants. Lacewing larvae reduced aphid densities by 97% on resistant cultivars compared to only 20% on susceptible plants.

This interaction may not always produce the desired result. The food source of the pest dictates many of its life-history traits, such as size, fecundity, and survival, which may also affect the predator as well (Bottrell et al. 1998). Thus, the simultaneous use of resistant cultivars and biological control may cause interference, resulting in reduced overall predator efficacy and increased pest populations (Obrycki 1986, Price 1986, Messina and Sorenson 2001, Cai et al. 2009). Orr and Boethel (1986), for instance, demonstrated that the soybean looper moth (*Pseudoplusia includens* Walker), when consumed, could pass the toxic effects from its resistant soybean hosts to its predator, the spined soldier bug (*Podisus maculiventris* Say). These effects caused an increase in pre-imaginal development time, a reduction in female weight, and a

reduction in the estimated intrinsic rate of increase, hindering the overall efficacy of this predator. Efficacy can be inhibited indirectly as well. For example, in rice (*Oryza sativa*) cultivars resistant to the brown planthopper (*Nilaparvata lugens* Stål), reduced populations of planthoppers were shown to effectively remove strong density-dependent predators from the system (Stapley et al. 1979, Kenmore 1984). The introduction of resistant cultivars may also interrupt predator attraction to plant volatiles, which are an important factor in predator numerical response to prey (Vet and Dicke 1992, Bottrell et al. 1998, Vos et al. 2001). In one case, the parasitoid, *Diaeretiella rapae* (McIntosh), did not search for its prey, *Brevicoryne brassicae* (L.), on resistant Brussel sprouts because that variety produced less mustard oil volatiles than susceptible plants. Consequently, there were fewer parasitized aphids on the resistant variety, and eventually, higher populations of aphids (Read et al. 1970, van Emden 1978). Crops producing a weak numerical response by natural enemies may be at risk of colonization from secondary pests without an already established natural enemy community.

These studies show that integrating host plant resistance with biological control is complicated and emphasize the need for a thorough assessment. Similar to previous pest invasions in soybean and wheat, sugarcane aphids are a newly emerging pest of sorghum and have caused considerable yield loss (Bowling et al. 2016). Predictably, many studies have been conducted to identify resistant germplines to quantify suppressive effects on aphid population growth between resistant and susceptible hybrids (Armstrong et al. 2015, Brewer et al. 2017). In one study, susceptible hybrids (Tx 2752/Tx430 and DKS53-67) suffered significant yield loss, especially when aphid populations experienced rapid growth and peaked over 300 aphids per leaf. Conversely, on resistant sorghum (Tx2752/Tx2783), aphid populations either declined after establishment, or took 60% more time to double in size (Brewer et al. 2017). These data suggest

that resistant sorghum hybrids may improve the predator-aphid ratio. Indeed, data from a recent study by Szczepaniec (2018) indicate a higher predator-prey ratio was found on resistant cultivars than on susceptible cultivars. This is promising, because by hindering aphid populations on resistant cultivars, we can effectively delay or prevent explosive aphid population growth that can easily overtake predator action. The impact these cultivars have on predators, however, such as attractiveness or synergistic effects of the cultivar, are critically important to a successful IPM program and require further exploration. While there are many studies that aim to discover resistant cultivars and their effects on aphid populations, there are fewer studies that focus on the predators.

It is important to understand the overall effects across all trophic levels to develop a more effective IPM program (van Emden 1995). Understanding this interaction between cultivar, prey, and their natural enemies is vital for optimizing future management tactics (Bottrell et al. 1998, Cai et al. 2009). Thus, key to effective control of sugarcane aphid in sorghum is the interaction between sugarcane aphids, their predators, and the impact of resistant sorghum varieties. In this study I first examine the effects of green lacewing larvae (*Chrysoperla rufilabris*) on sugarcane aphids on both resistant (DKS-3707) and susceptible varieties (DKS-3808) of sorghum. I then use a choice experiment with both varieties, with and without aphids, to examine the effects of plant volatiles on adult lady beetle (*Coccinella septempunctata*) attraction.

3.2 Materials and Methods

3.2.1. Sorghum

Two widely-used hybrids of sorghum (*Sorghum bicolor* (L.) Moench) were used: DKS38-08 (Monsanto, St. Louis, Missouri), which is susceptible to aphid infestations, and DKS37-07 (Monsanto, St. Louis, Missouri), which displays resistance to aphid infestations

(Brewer et al. 2017, Szczepaniec 2018). One seed was planted per pot (15.24 cm diameter x 13.97 cm depth) (Dillen, Middlefield, OH) in Sunshine Mix #1 (Sungro, Agawam, MA) growing medium. Sorghum was cultivated in a greenhouse at 28°C-32°C with supplemental light on a L:D (light:dark) cycle of 12:12. Sorghum was grown in 60 x 60 x 60 cm insect-proof cages (MegaView Science Co., Ltd., Taichung, Taiwan) for two weeks to an approximate size of 20 cm (~six true leaves). All sorghum was top-watered ad libitum when the first ~2.5 cm of soil was dry.

3.2.2. *Aphid rearing*

Sugarcane aphid colonies were established using aphids originally collected from sorghum in Winnsboro, Louisiana in 2016. Colonies were maintained on sorghum in insect-proof enclosures (MegaView Science Co.) prior to the start of experiments. Sorghum used to support the colonies were grown under the previously described conditions above. A subset of aphids was moved by donor leaves to new plants approximately every two weeks to prevent overcrowding in the colonies.

3.2.3. *Aphid predators.*

Green lacewings (*C. rufilabris*) eggs were received from Beneficial Insectary Inc. (Redding, CA) and reared in an environmental chamber set to 27°C, 75% RH and 12:12 (L:D) photoperiod. Single eggs were placed into 60ml portion cups (Fabri-Kal, Kalamazoo, MI) and provided a damp cotton ball for moisture. Larvae were fed *ad libitum* with sugarcane aphids reared on sorghum. All larvae were reared to 3rd instar for this study. Adult lady beetles (*C. septempunctata*) were collected from sorghum fields and maintained together within several 3.8L, 25 cm tall wide-mouth plastic jars (Uline, Pleasant Prairie, WI), each with sides replaced

by fine mesh to allow for ventilation. Jars were placed into the same environmental unit as lacewings. Lady beetles were fed sugarcane aphids from sorghum clippings 2-3x per week.

3.2.4. Predation study design and analysis

3.2.4.1. Caged plant enclosures

Each cage was built from 3.8L, 25 cm tall wide-mouth plastic jars (Uline, Pleasant Prairie, WI) with the sides and bottom cut out and replaced with fine mesh to prevent arthropod ingress or egress. Each jar was turned upside down, placed over the plant, and the lip of the jar pressed into the topsoil. The jars were not removed once the experiment began.

3.2.4.2. Design

I conducted a 2x2x2 (two predators x two hybrids x two aphid densities) randomized factorial complete block design in a greenhouse to quantify the effects of green lacewing predation on aphid abundance (40 and 120 aphids per plant) on both resistant and susceptible sorghum after 48 hours of exposure to this predator. Each experimental unit consisted of one caged sorghum plant, which was randomly assigned to one of the eight treatment combinations. Aphids were introduced to each cage by transferring infested leaves from lab-reared aphid colonies and were allowed to acclimate for 24-48 hours. During the acclimation period, predators were maintained at 27°C without any prey. A final visual inspection was made to ensure aphids had established at the assigned densities and that there was a uniform population age structure between experimental units. A predator was then added to each cage with the exception of cages assigned to the no predator treatment. The number of aphids were counted once per day at 18:00h. The greenhouse was maintained at 28°C-33°C during the experiment. The entire experiment was replicated 11 times (n=88).

3.2.4.3. *Statistical analysis*

Data were tested for normality with (Shapiro–Wilks test) and variance (Levene’s test) to verify ANOVA assumptions. The effects of sugarcane aphid density on predation were then analyzed using repeated measures ANOVA with initial aphid density and variety as the fixed independent variables. Analyses were conducted using R v3.4.2 (R Core Team 2017).

3.2.5. *Choice bioassay design and analysis*

3.2.5.1. *Design*

I conducted a choice experiment using a y-tube olfactometer (ARS Gainesville, FL, OLFM-ADS-2AFM1C), which was connected to two 4L glass chambers. Air entering into the y-tube olfactometer was drawn through activated charcoal and purified before entering the y-tube. Airflow through each arm was maintained at 15 psi. Alternating combinations of resistant and susceptible sorghum plants with and without sugarcane aphids were placed in the two glass chambers. There were 26 replicates each of the following combinations: resistant-with-aphids vs resistant-without-aphids, and susceptible-with-aphids vs susceptible-without-aphids. There were also 17 replicates comparing the combination resistant-with-aphids vs susceptible-with-aphids. Sorghum plants were infested with aphids, allowed 48hrs to acclimate, and counted daily so that each plant contained 45 ± 5 aphids. Starved female lady beetles placed individually into the y-tube, and at the end of 500 seconds I recorded “no-choice” if the lady beetle had not yet selected a path through the y-tube. After each run, the chambers and the y-tube were rinsed with 70% ethanol, rinsed with distilled water, and blow-dried.

3.2.5.2. *Statistical analysis*

I used the chi-square test of independence to analyze the relationships between all plants both with and without aphids (cultivar agnostic), and between both cultivars with aphid

infestations. I also analyzed the relationships within each plant cultivar in the same fashion. These relationships were resistant plants with and without aphids, and susceptible plants with and without aphids. Analysis was performed with R v3.4.2 (R Core Team 2017).

3.2.6. Predator-Prey model

I used the predator effects from our experiment to parameterize a simple predator-prey model that estimates aphid population growth with and without predators on both resistant and susceptible sorghum varieties. The estimated rate of increase (λ) of aphid population per time step (two days) was estimated using Eq. (1), where N is aphid population density and t is time. I used two-day time intervals because our predation experiment lasted 48 hours.

$$\lambda_{experiment} = \frac{N_{t+1}}{N_t} \quad (1)$$

I then used a dynamic version of our model to simulate aphid colony growth with or without predator effects on both cultivars over a period of four days, Eq. (2). I was particularly interested in the interactive effects resistant and susceptible sorghum cultivars have on predator action and aphid densities. To simulate a field scenario, I used an action threshold of 50-75 aphids per leaf using data from Gordy et al. (unpublished), which was based on previous work by Brewer and Bowling (2014). Only the initial aphid density of 120 was used for this model.

$$\frac{dN}{dt} = \lambda_{experiment} \times N_{t-1} \quad (2)$$

3.3 Results

3.3.1. Predation study

Predators had a much larger effect on final aphid densities on susceptible plants rather than on resistant plants. While aphids reached much higher densities on susceptible varieties, overall, predators suppressed aphids to similar densities (~102 aphids) on both varieties. There was a significant interaction between predator and variety on final aphid densities ($F_{1,67}=4.637$, $P=0.035$; Fig. 5). On the resistant variety without a predator, there was a 1.8-fold increase in aphids (186 ± 13.4 aphids) vs a 2.4-fold increase (244 ± 13.4 aphids) on the susceptible variety without a predator. Interestingly, there was no difference in final aphid densities between resistant and susceptible cultivars when predators were present, suggesting that the larvae suppress aphids to the same final densities on resistant and susceptible varieties. I also found an interaction between density and variety ($F_{1,67}=5.029$, $P=0.028$; Fig. 6). At initial densities of 120 aphids per plant, aphid populations were significantly higher after 48hrs on susceptible (243 ± 14 aphids) plants vs resistant (182 ± 13.4 aphids) plants. At a lower initial density of 40, aphid population size was not significantly different between resistant and susceptible varieties after 48hrs. I did not find a significant interaction between predator and density at $\alpha 0.05$ ($F_{1,67}=2.784$, $P=0.100$; Table 1). I also did not find a 3-way interaction between predator, variety, and density ($F_{1,67}=0.813$, $P=0.370$; Table 1; Fig. 7 A and B).

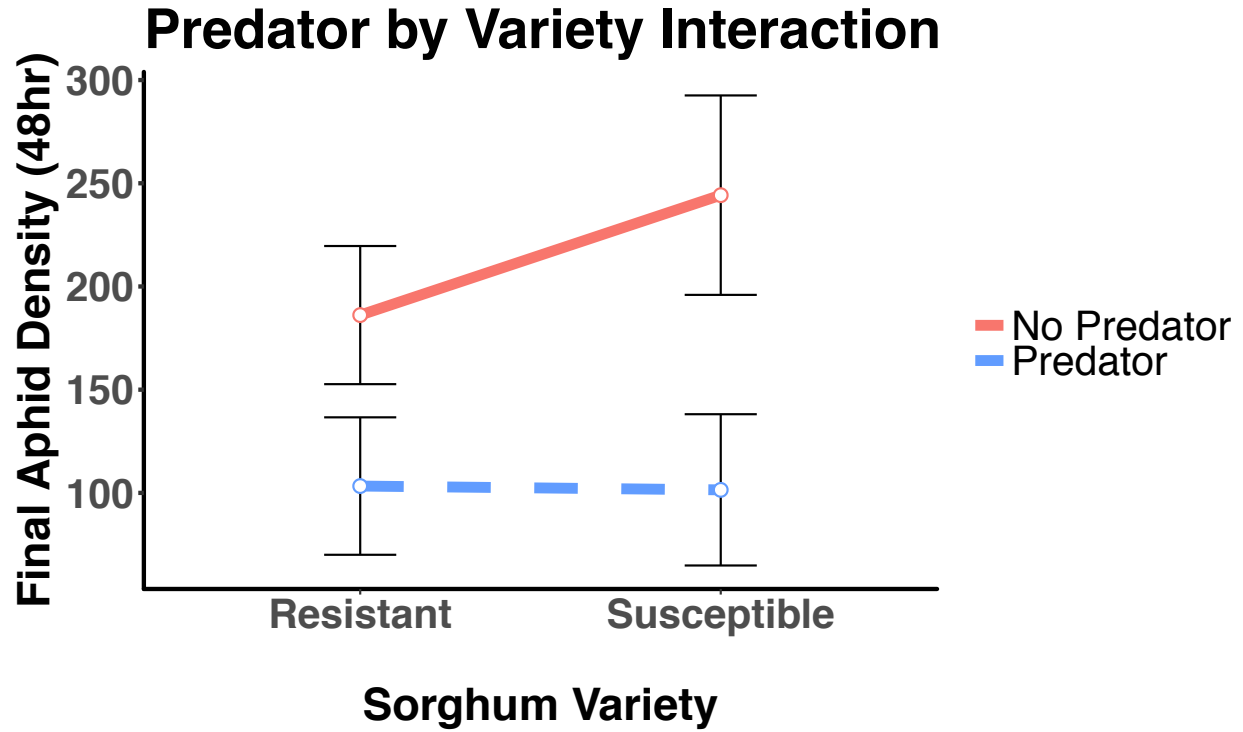


Fig. 5. Predator by variety interaction. Aphid colonies on resistant varieties, without a predator, show a 1.8-fold growth increase vs a 2.4-fold increase on the susceptible variety without a predator. However, predators suppress aphid populations to approximately the same final density on both. Cultivars are averaged across both densities (40, 120). Bars represent 95% CI.

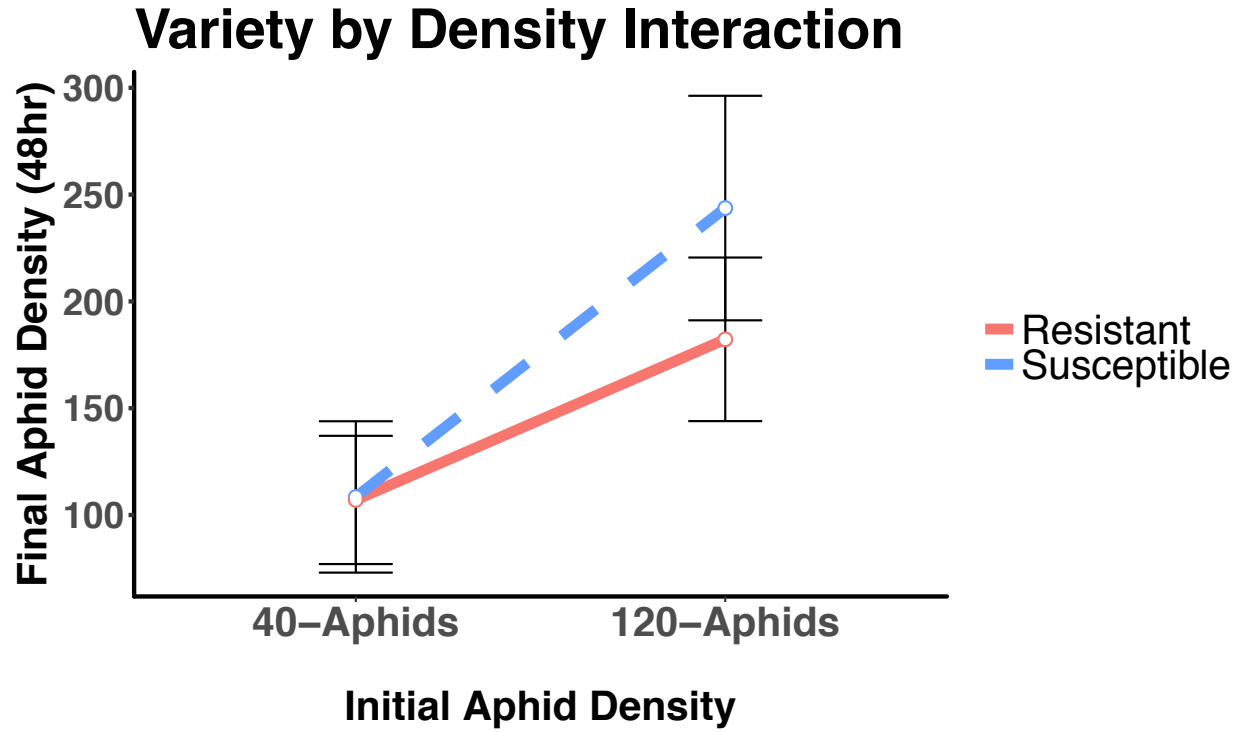


Fig. 6. Variety by density interaction. At high initial densities (120), aphid populations on the susceptible variety have a greater final density than on the resistant variety. Predators have a larger suppressive effect at initial aphid densities of 120 rather than at 40. There was no difference between final aphid densities when initial aphid densities were low (40). Densities are averaged across both cultivars. Bars represent 95% CI.

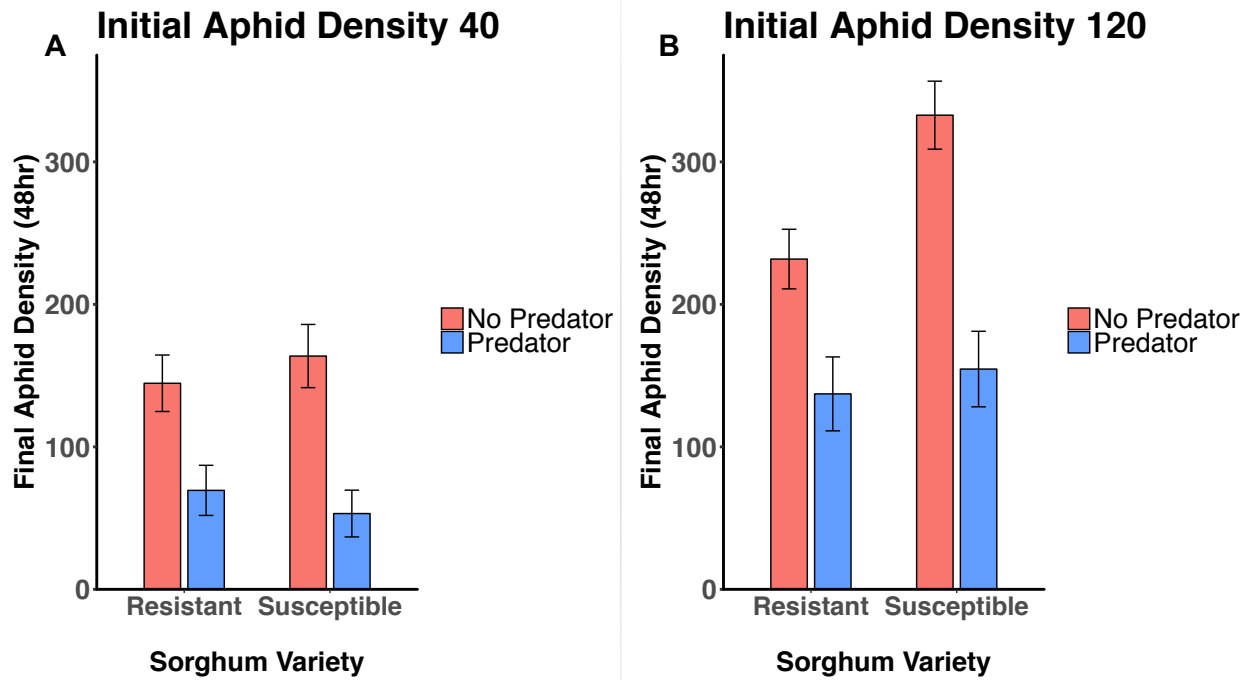


Fig. 7. The effects of predator on both cultivars at low (40) and high (120) aphid densities. Aphid populations are significantly higher on susceptible varieties when no predator is present, however there was no significant difference between cultivars when predators were present. Bars represent \pm SEM.

Table 1. Analysis of variance for the effects of predator, variety, and density on aphid population growth.

Source of Variation	Df	Mean Sq	F value	
Predator	1	270209	72.366	***
Variety	1	16397	4.391	*
Density	1	239347	64.101	***
Block	10	13763	3.686	***
Predator x Variety	1	17313	4.637	*
Predator x Density	1	10394	2.784	.
Variety x Density	1	18778	5.029	*
Predator x Variety x Density	1	3035	0.813	
Error	67	3734		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

3.3.2. Choice bioassay

Overall, plant volatiles from aphid-infested sorghum plants, regardless of cultivar, was a significant determinant ($\chi^2 (1) = 11.077, P=0.001$) in the choices made by each lady beetle. When choosing between resistant-with-aphids vs resistant-without-aphids, female lady beetles were more attracted to volatiles isolated from the resistant, aphid-infested plants rather than the uninfested resistant plants ($\chi^2 (1) = 9.783, P=0.002$; Fig. 8). In this, lady beetles chose the resistant aphid-infested plant 19 times, and the resistant uninfested plant 4 times. There was no significant difference in choice between susceptible-with-aphids vs susceptible-without-aphids ($\chi^2 (1) = 2.793, P=0.095$). There was also no distinction between resistant-with-aphids vs susceptible-with-aphids as lady beetles chose each option an equal number of times.

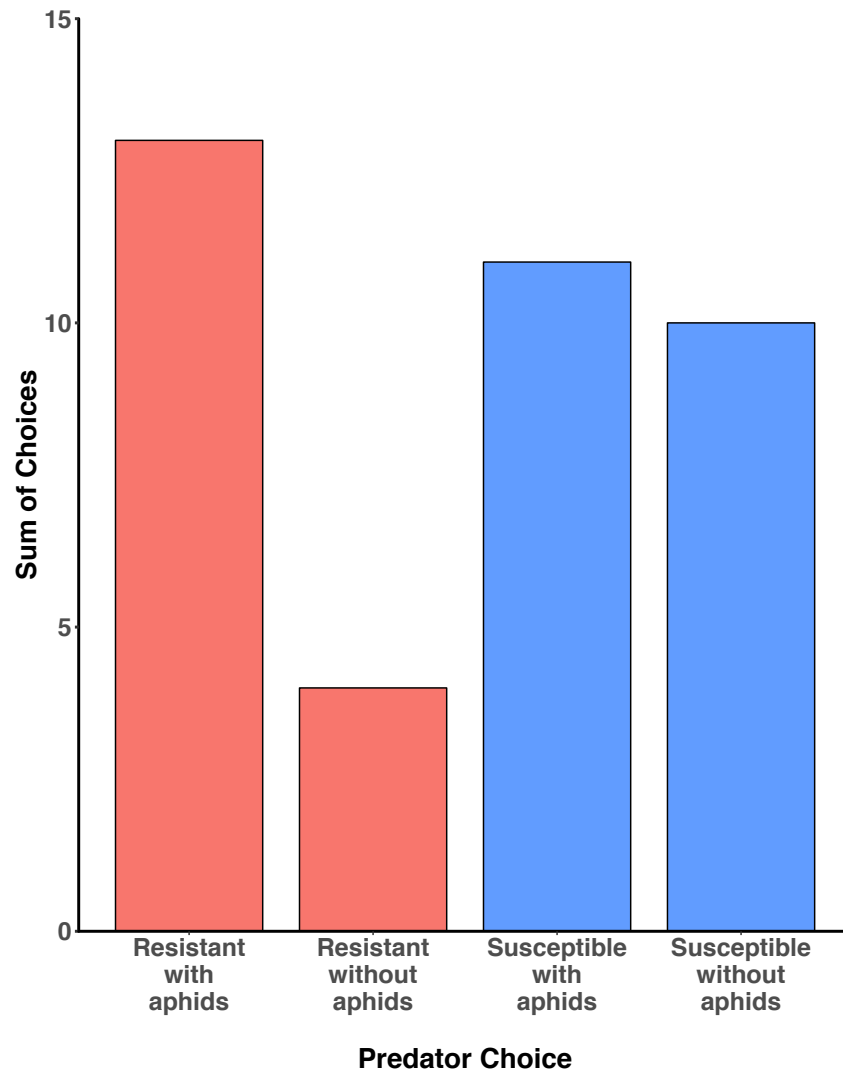


Fig. 8. Volatile preference of lady beetles. On resistant cultivars with and without aphids, lady beetles chose with aphids significantly more often than without. This effect was not seen on susceptible varieties, where there was no difference in preference between the two options. No-choice is not shown.

3.3.3 Predator-Prey model

Predators consumed approximately the same number of aphids on both resistant and susceptible sorghum varieties and there was little difference in final aphid densities. After two days, final aphid densities on resistant and susceptible was 137 and 155, respectively (Fig 9A). Day four densities were 157 and 199, respectively (Fig. 9B). On varieties without predators, aphid populations were much slower to expand than on the susceptible varieties. By the end of day four, the aphid populations had nearly a 2-fold increase in size over populations on the resistant variety (Fig. 9B).

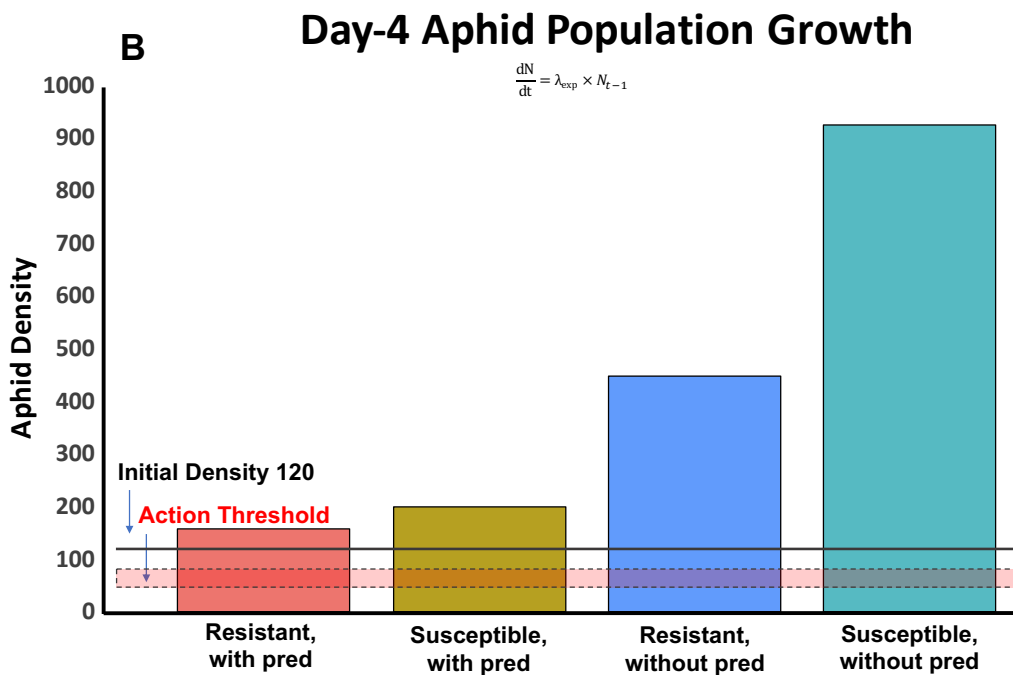
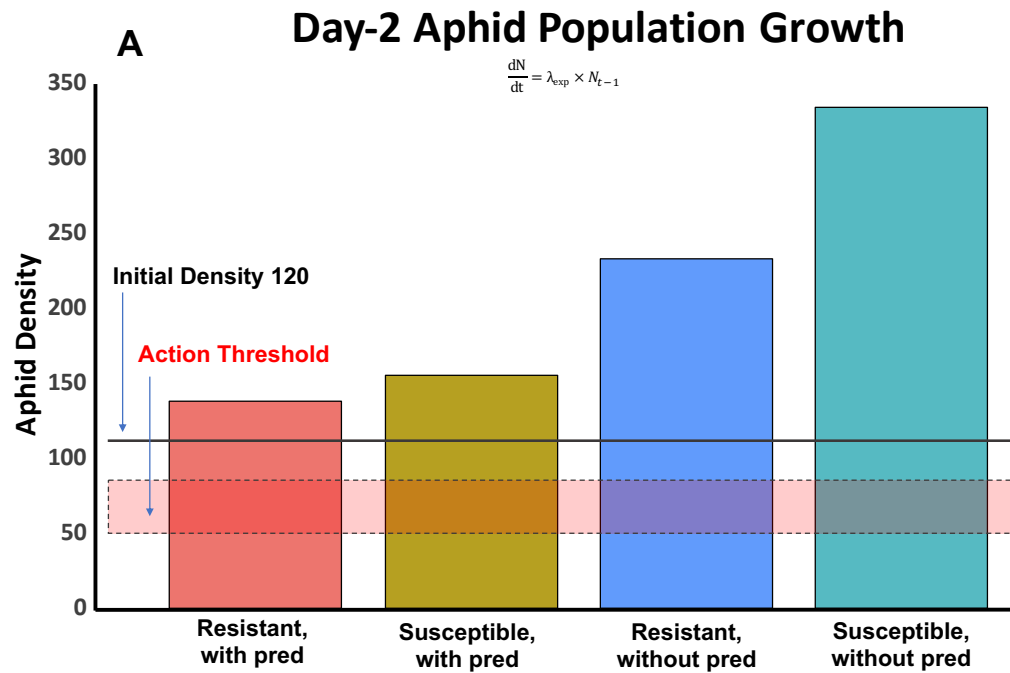


Fig. 9. Predator-Prey model of aphid population growth on resistant and susceptible sorghum. Final aphid populations show little difference on either cultivar when predators are present. Aphid populations grew considerably faster on susceptible sorghum when predators were absent, suggesting predators could be more important on susceptible varieties.

3.4 Discussion

My data show that lacewing larvae have a much larger effect on aphids when initial densities are higher (density 120 vs 40). I found that when no predator was present, aphid populations were considerably higher (~30% more) on the susceptible variety than on the resistant plants. Interestingly, lacewing larvae suppressed aphids to approximately the same density on both varieties (Fig. 1), suggesting that lacewing larvae are more important on susceptible varieties than on resistant (Fig. 9). This result is similar to van Emden (1986), in which he describes field studies where predators were more frequent and effective on susceptible, rather than resistant cultivars. Brewer et al. (1998) also show that the parasitoid *Diaeretiella rapae* (McIntosh) was more effective at parasitizing aphids on susceptible barley with higher pest densities.

These studies support the predator-prey theory that predation tends to be density dependent (i.e., type II functional response), and I would predict that predators should have a higher consumption rate as aphid densities increase (Holling 1961). However, susceptible sorghum varieties are likely to suffer rapid increases in aphid densities, resulting in predators quickly becoming overwhelmed (and satiated) by aphids and unable to provide any effective suppression (Holling 1961). Thus, in the field where aphid densities are much higher and outbreaks are possible, resistant sorghum is likely to play a larger role by delaying explosive aphid population growth and providing more time for predator action.

Resistant varieties may also provide a subtler, unanticipated benefit. Szczepaniec (2018) shows that resistant varieties have an effect on the predator to aphid ratio. While the absolute density is higher on susceptible, the predator to aphid ratio is higher on resistant. Thus, resistant varieties of sorghum had a significant suppressive effect because of the more favorable ratio.

These data show an interactive effect where an aphid on a resistant plant is more attractive to predators than an aphid on susceptible varieties. Predators orienting to the odors of infested plants is a relatively common phenomenon and has been previously observed in aphid-infested plants (Tingey and Bergman 1979, van Emden 1991, Du et al. 1998). I observed a similar effect in my bioassay study (Fig. 8). Considering a hypothetical field with resistant cultivars, my data show that lady beetles would be far less likely to choose the uninfested resistant plants. This suggests that odor cues of resistant sorghum may be unique and the resistant with aphids is more attractive to predators. The resistant volatiles with aphids attracting more predators, rather than fewer (as in the mustard oil volatiles study), may be a worthwhile positive outcome that is worth exploring in future studies. Particular importance should be placed on situations where major aphid-predators with behavioral plasticity could change preferences as a result of the learning process.

As in the Szczepaniec study, I did find there was a higher proportional reduction in aphid density on resistant than susceptible varieties. This reduction was only at initial densities of 120 aphids though, indicating that the resistance effect, at least for DKS37-07, could be aphid-density dependent. In any case, the effect was not significant. This may be because DKS37-07, while widely used for the resistance effects, is considered to be on the weaker side of resistance compared to other hybrids that have been screened (Trostle 2016). I may have also underestimated the effects of resistant hybrids of sorghum in this study because of the short timeframe. There may not have been enough time for interactive effects to occur (Van der Westhuizen et al. 1998). I expect that with some minor alterations, the differences between susceptible and resistant cultivars in this study would become more differentiated. Specifically, aphid populations should be permitted to mature for several generations to allow for the

antibiosis effects of resistant cultivars to become more apparent. I must also allow the predators more time with their prey, which would tease apart the three-way interaction that should exist. This, however, would necessitate using utilizing all of the lacewing larval instars (1st – 3rd) instead of only the 3rd, as each larval instar persists for only a few days. These changes are aligned with another similar study on the effectiveness of *Chrysoperla plorabunda* (Fitch) on resistant and susceptible wheat. Messina and Sorenson (2001) allowed their aphid (*Diuraphis noxia* Mordvilko) populations to persist for nearly two weeks before adding predators, and while their design also included more lacewings (five) per plant, their results show a significant synergistic, higher proportional reduction in aphid density on resistant than susceptible wheat varieties.

A common view is that resistant cultivars and biological control are compatible, and when merged together, can provide enhanced pest suppression (Emden and Wearing 1965, van Emden 1986, Bottrell et al. 1998). Indeed, I can conclude that there was no interference from resistant plants on predator consumption. However, I did not observe any interactive effects leading to enhanced suppression of aphids as I would expect. This is different than the results of some of the other studies mentioned, but the potentially abnormal results could be an artifact of the short-duration of the predation experiment. Despite that, my data do show the effects of resistant cultivars versus the effects of one lacewing larva on the density of sugarcane aphids. Natural enemies have a much larger suppressive effect on aphid populations than do resistant cultivars. For example, with no predators, final aphid densities are ~180 on resistant varieties and ~250 on susceptible, but one introduced predator will reduce final densities to ~100. Predators are simply more effective at reducing aphid densities. Further, if cultivars do differ in their attraction of predators to aphid infested plants, the resistant plants will have a higher predator to

aphid density. The combined synergistic effects will significantly slow the growth rate of aphid colonies on resistant sorghum as compared to susceptible.

CHAPTER IV

CONCLUSION

Predators have a significant negative effect on sugarcane aphid colony growth. Predators commonly found in sorghum are capable of preventing sugarcane aphids at low-medium initial densities from ever reaching their action threshold. These predators are also able to reduce established aphid populations to below threshold in two to four days, but this effect is largely dependent on predator identity. For example, *Coccinella septempunctata* is the most voracious, providing quick suppression of aphids at all densities studied. However, *Chrysoperla rufilabris* is the weakest predator and cannot provide effective economic suppression at higher densities. The simultaneous use of biological control and aphid-resistant sorghum should be an effective way to further increase predator efficacy by increasing the predator-prey ratio. My data does not show any negative interaction that would prevent successful integration of biological control and host plant resistance. There may also be a synergistic interaction attracting predators to the resistant, aphid-infested sorghum. With more predators arriving to an infestation, this has the potential to quickly bring infested fields back to below action thresholds. This key concept should be more fully explored in future studies in the field where dynamic action thresholds can be manipulated. Nevertheless, my data show that predators can be significantly more effective at reducing aphid populations than the sole use of resistant cultivars. As aphids show rapid population growth on susceptible varieties, predators may be more important on susceptible plants rather than resistant plants.

Biological control is a key component of IPM, and as more tools are integrated, the results can become complicated and produce unexpected results. It is important to fully

incorporate the relative contribution of predators and cultivars for effective sugarcane aphid management in sorghum. Successful integration of natural enemies into IPM programs, particularly those with the simultaneous use of host plant resistance, will require field studies of predator colonization of infested fields, the impact of intraguild predation, and prey quality on predator survival and efficacy.

REFERENCES

- Armstrong, J. S., W. L. Rooney, G. C. Peterson, R. T. Villenueva, M. J. Brewer, and D. Sekula-Ortiz. 2015. Sugarcane Aphid (Hemiptera: Aphididae): Host Range and Sorghum Resistance Including Cross-Resistance From Greenbug Sources. *J Econ Entomol* **108**:576-582.
- Bergman, J. M., and W. M. Tingey. 1979. Aspects of interaction between plant genotypes and biological control. *Bulletin of the ESA* **25**:275-279.
- Bode, E. 1980. Aphids in winter wheat: abundance and limiting factors from 1976 to 1979. *Bulletin SROP* **3**:49-57.
- Bottrell, D. G., P. Barbosa, and F. Gould. 1998. Manipulating natural enemies by plant variety selection and modification: a realistic strategy? *Annu Rev Entomol* **43**:347-367.
- Bowling, R., M. Brewer, D. Kerns, J. Gordy, N. Seiter, N. Elliott, G. D. Buntin, M. O. Way, T. A. Royer, S. Biles, and E. Maxson. 2016. Sugarcane Aphid (Hemiptera: Aphididae): A New Pest on Sorghum in North America. *Journal of integrated pest management* **7**:12.
- Brewer, M., and R. Bowling. 2014. Sugarcane Aphid on Sorghum: Distribution, Damage, Thresholds, and Insecticides. Texas A&M AgriLife Research.
- Brewer, M., J. Struttman, and D. Mornhinweg. 1998. *Aphelinus albipodus* (Hymenoptera: Aphelinidae) and *Diaeretiella rapae* (Hymenoptera: Braconidae) Parasitism on *Diuraphis noxia* (Homoptera: Aphididae) Infesting Barley Plants Differing in Plant Resistance to Aphids. *Biological control* **11**:255-261.
- Brewer, M. J., and J. Gordy. 2016. Sugarcane aphid economic threshold and sampling update. Texas A&M AgriLife Research and Texas A&M AgriLife Extension.
- Brewer, M. J., J. W. Gordy, D. L. Kerns, J. B. Woolley, W. L. Rooney, and R. D. Bowling. 2017. Sugarcane Aphid Population Growth, Plant Injury, and Natural Enemies on Selected Grain Sorghum Hybrids in Texas and Louisiana. *Journal of Economic Entomology* **110**:2109-2118.
- Cai, Q.-N., X.-M. Ma, X. Zhao, Y.-Z. Cao, and X.-Q. Yang. 2009. Effects of host plant resistance on insect pests and its parasitoid: A case study of wheat-aphid-parasitoid system. *Biological control* **49**:134-138.

- Chen, T. Y., and T. X. Liu. 2001. Relative consumption of three aphid species by the lacewing, *Chrysoperla rufilabris*, and effects on its development and survival. *BioControl* **46**:481-491.
- Colares, F., J. P. Michaud, C. Bain, and J. Torres. 2015a. Indigenous Aphid Predators Show High Levels of Preadaptation to a Novel Prey, *Melanaphis sacchari* (Hemiptera: Aphididae). *Journal of Economic Entomology* **108**:2546-2555.
- Colares, F., J. P. Michaud, C. L. Bain, and J. B. Torres. 2015b. Recruitment of aphidophagous arthropods to sorghum plants infested with *Melanaphis sacchari* and *Schizaphis graminum* (Hemiptera: Aphididae). *Biological control* **90**:16-24.
- Conway, H. E., D. C. Steinkraus, J. R. Ruberson, and T. J. Kring. 2006. Experimental treatment threshold for the cotton aphid (Homoptera: Aphididae) using natural enemies in Arkansas cotton. *Journal of entomological science* **41**:361-373.
- Cortez-Mondaca, E., M. Lopez Buitimea, G. Herrera Rodriguez, M. López Buitimea, J. López Arroyo, F. Orduño Cota, and G. Herrera Rodríguez. 2016. Especies de Chrysopidae Asociadas al Pulgón del Sorgo en el Norte de Sinaloa, México. *Southwestern Entomologist* **41**:541-545.
- Costamagna, A. C., and D. A. Landis. 2006. Predators Exert Top-Down Control of Soybean Aphid across a Gradient of Agricultural Management Systems. *Ecological Applications* **16**:1619-1628.
- Costamagna, A. C., D. A. Landis, and M. J. Brewer. 2008. The role of natural enemy guilds in *Aphis glycines* suppression. *Biological control* **45**:368-379.
- Costamagna, A. C., D. A. Landis, and C. D. Difonzo. 2007. Suppression of soybean aphid by generalist predators results in a trophic cascade in soybeans. *Ecological Applications* **17**:441-451.
- Dedryver, C.-A., A. Le Ralec, and F. Fabre. 2010. The conflicting relationships between aphids and men: A review of aphid damage and control strategies. *Comptes Rendus Biologies* **333**:539-553.
- Desneux, N., E. Wajnberg, K. A. Wyckhuys, G. Burgio, S. Arpaia, C. A. Narváez-Vasquez, J. González-Cabrera, D. C. Ruescas, E. Tabone, and J. Frandon. 2010. Biological invasion of European tomato crops by *Tuta absoluta*: ecology, geographic expansion and prospects for biological control. *Journal of pest science* **83**:197-215.

- Du, Y., G. M. Poppy, W. Powell, J. A. Pickett, L. J. Wadhams, and C. M. Woodcock. 1998. Identification of Semiochemicals Released During Aphid Feeding That Attract Parasitoid *Aphidius ervi*. *Journal of Chemical Ecology* **24**:1355-1368.
- Emden, H. F. V., and C. H. Wearing. 1965. The role of the aphid host plant in delaying economic damage levels in crops. *Annals of Applied Biology* **56**:323-324.
- Fox, T. B., D. A. Landis, F. F. Cardoso, and C. D. Difonzo. 2004. Predators Suppress *Aphis glycines* Matsumura Population Growth in Soybean. *Environmental Entomology* **33**:608-618.
- Fox, T. B., D. A. Landis, F. F. Cardoso, and C. D. Difonzo. 2005. Impact of predation on establishment of the soybean aphid, *Aphis glycines* in soybean, *Glycine max*. *BioControl* **50**:545-563.
- Giles, K. L., B. P. McCornack, T. A. Royer, and N. C. Elliott. 2017. Incorporating biological control into IPM decision making. *Current Opinion in Insect Science* **20**:84-89.
- Guedes, R. N. C., and G. C. Cutler. 2014. Insecticide-induced hormesis and arthropod pest management. *Pest Management Science* **70**:690-697.
- Hallett, R. H., C. A. Bahlai, Y. Xue, and A. W. Schaafsma. 2014. Incorporating natural enemy units into a dynamic action threshold for the soybean aphid, *Aphis glycines* (Homoptera: Aphididae). *Pest Management Science* **70**:879-888.
- Hodgson, E. W., B. P. McCornack, K. Tilmon, and J. J. Knodel. 2012. Management Recommendations for Soybean Aphid (Hemiptera: Aphididae) in the United States. *Journal of integrated pest management* **3**:E1-E10.
- Holling, C. S. 1961. Principles of Insect Predation. *Annu Rev Entomol* **6**:163-182.
- Honek, A. 1979. Plant density and occurrence of *Coccinella septempunctata* and *Propylaea quatuordecimpunctata* (Coleoptera, Coccinellidae) in cereals. *Acta Entomologica Bohemoslovaca*.
- Kenmore, P. E. 1984. Population regulation of the rice brown planthopper (*Nilaparvata lugens* Stål) within rice fields in the Philippines. *Journal of plant protection in the tropics* **1**:19-37.

- Knutson, A., R. Bowling, B. M., E. Bynum, and P. Porter. 2016. The Sugarcane Aphid: Management guidelines for grain and forage sorghum in Texas. Page 8 Texas A&M AgriLife Extension: ENTO-035 Texas A&M AgriLife Extension and Research, College Station, TX.
- Koch, R. L., and A. C. Costamagna. 2016. Reaping benefits from an invasive species: role of *Harmonia axyridis* in natural biological control of *Aphis glycines* in North America. *BioControl* **62**:331-340.
- Losey, J. E., and M. Vaughan. 2006. The Economic Value of Ecological Services Provided by Insects. *BioScience* **56**:311-323.
- McCarville, M. T., and M. E. O'Neal. 2013. Soybean Aphid (Aphididae: Hemiptera) Population Growth as Affected by Host Plant Resistance and an Insecticidal Seed Treatment. *Journal of Economic Entomology* **106**:1302-1309.
- Messina, F. J., and S. M. Sorenson. 2001. Effectiveness of Lacewing Larvae in Reducing Russian Wheat Aphid Populations on Susceptible and Resistant Wheat. *Biological control* **21**:19-26.
- Musser, F. R., J. P. Nyrop, and A. M. Shelton. 2006. Integrating Biological and Chemical Controls in Decision Making: European Corn Borer (Lepidoptera: Crambidae) Control in Sweet Corn as an Example. *Journal of Economic Entomology* **99**:1538-1549.
- Naranjo, S. E., P. C. Ellsworth, and G. B. Frisvold. 2015. Economic Value of Biological Control in Integrated Pest Management of Managed Plant Systems. *Annu Rev Entomol* **60**:621-645.
- Obrycki, J. J. 1986. The influence of foliar pubescence on entomophagous species. Ellis Horwood.
- Orr, D. B., and D. J. Boethel. 1986. Influence of Plant Antibiosis through Four Trophic Levels. *Oecologia* **70**:242-249.
- Painter, R. 1951. Insect resistance in crop plants. Univ. Kansas Press, Lawrence.
- Pimentel, D., L. Lach, R. Zuniga, and D. Morrison. 2000. Environmental and Economic Costs of Nonindigenous Species in the United States. *BioScience* **50**:53-65.

- Pimentel, D., R. Zuniga, and D. Morrison. 2005. Update on the environmental and economic costs associated with alien-invasive species in the United States. *Ecological Economics* **52**:273-288.
- Price, P. W. 1986. Ecological aspects of host plant resistance and biological control: interactions among three trophic levels. *Interactions of plant resistance and parasitoids and predators of insects*:11-30.
- R Core Team. 2017. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- Ragsdale, D. W., D. A. Landis, J. Brodeur, G. E. Heimpel, and N. Desneux. 2011. Ecology and management of the soybean aphid in North America. *Annu Rev Entomol* **56**:375-399.
- Read, D. P., P. P. Feeny, and R. B. Root. 1970. Habitat selection by the aphid parasite *diaeretiella rapae* (hymenoptera: Braconidae) and hyperparasite charips brassicae (hymenoptera: Cynipidae). *The Canadian Entomologist* **102**:1567-1578.
- Rosenheim, J. A., and L. R. Wilhoit. 1993. Why lacewings may fail to suppress aphids … Predators that eat other predators disrupt cotton aphid control. *California Agriculture* **47**:7-9.
- Schuster, D., and K. Starks. 1973. Greenbugs: components of host-plant resistance in sorghum. *Journal of Economic Entomology* **66**:1131-1134.
- Schuster, D., and K. Starks. 1975. Preference of *Lysiphlebus testaceipes* for greenbug resistant and susceptible small grain species. *Environmental Entomology* **4**:887-888.
- Settle, W. H., H. Ariawan, E. T. Astuti, W. Cahyana, A. L. Hakim, D. Hindayana, and A. S. Lestari. 1996. Managing Tropical Rice Pests Through Conservation of Generalist Natural Enemies and Alternative Prey. *Ecology* **77**:1975-1988.
- Sih, A., G. Englund, and D. Wooster. 1998. Emergent impacts of multiple predators on prey. *Trends in Ecology & Evolution* **13**:350-355.
- Stapley, J., Y. Y. May-Jackson, and W. Golden. 1979. Varietal resistance to the brown planthopper in the Solomon Islands. *Brown planthopper: threat to rice production in Asia*. International Rice Research Institute, Los Banõs:233-239.

- Starks, K., R. Muniappan, and R. Eikenbary. 1972. Interaction between plant resistance and parasitism against the greenbug on barley and sorghum. *Annals of the Entomological Society of America* **65**:650-655.
- Sterling, W. L., A. Dean, and N. M. Abo El-Salam. 1992. Economic Benefits of Spider (Araneae) and Insect (Hemiptera: Miridae) Predators of Cotton Fleahoppers. *Journal of Economic Entomology* **85**:52-57.
- Stiling, P., and T. Cornelissen. 2005. What makes a successful biocontrol agent? A meta-analysis of biological control agent performance. *Biological control* **34**:236-246.
- Szczepaniec, A. 2018. Interactive effects of crop variety, insecticide seed treatment, and planting date on population dynamics of sugarcane aphid (*Melanaphis sacchari*) and their predators in late-colonized sorghum. *Crop Protection* **109**:72-79.
- Takagi, M. 1999. Perspective of practical biological control and population theories. *Researches on population ecology* **41**:121-126.
- Tingey, W. M., and J. M. Bergman. 1979. Aspects of interaction between plant genotypes. *Bulletin of the Entomological Society of America* **25**:275-279.
- Trostle, C. 2016. Evaluating if Grain Sorghum Hybrids with Seed Company Designation of Tolerance/Resistance to Sugarcane Aphid Are Right for You in 2016. Lubbock, TX.
- Van Den Berg, H., D. Ankasah, A. Muhammad, R. Rusli, H. A. Widayanto, H. B. Wirasto, and I. Yully. 1997. Evaluating the Role of Predation in Population Fluctuations of the Soybean Aphid *Aphis glycines* in Farmer's Fields in Indonesia. *Journal of applied ecology* **34**:971-984.
- Van der Westhuizen, A., X.-M. Qian, and A.-M. Botha. 1998. Differential induction of apoplastic peroxidase and chitinase activities in susceptible and resistant wheat cultivars by Russian wheat aphid infestation. *Plant Cell Reports* **18**:132-137.
- van Emden, H. 1966. Plant insect relationships and pest control. *World Rev. Pest Control* **5**:115-123.
- van Emden, H. 1978. Insects and secondary plant substances—an alternative viewpoint with special reference to aphids. *Biochemical aspects of plant and animal coevolution*. Academic Press, London, UK:309-323.

- van Emden, H. 1986. The interaction of plant resistance and natural enemies: effects on populations of sucking insects. Chichester: Ellis Horwood Limited.
- van Emden, H. F. 1991. The Role of Host Plant Resistance in Insect Pest Mis-management. *Bulletin of entomological research* **81**:123-126.
- van Emden, H. F. 1995. Host plant-Aphidophaga interactions. *Agriculture, Ecosystems & Environment* **52**:3-11.
- Vandereycken, A., D. Durieux, E. Joie, F. Francis, E. Haubruge, and F. Verheggen. 2015. Aphid species and associated natural enemies in field crops: what about the invasive ladybird *Harmonia axyridis* (Coleoptera: Coccinellidae)? *Entomologie Faunistique – Faunistic Entomology* **68**.
- Vet, L. E., and M. Dicke. 1992. Ecology of infochemical use by natural enemies in a tritrophic context. *Annu Rev Entomol* **37**:141-172.
- Vos, M., S. M. Berrocal, F. Karamaouna, L. Hemerik, and L. E. M. Vet. 2001. Plant-mediated indirect effects and the persistence of parasitoid–herbivore communities. *Ecology Letters* **4**:38-45.
- Walker, G. P., T. J. B. Herman, A. J. Kale, and A. R. Wallace. 2010. An adjustable action threshold using larval parasitism of *Helicoverpa armigera* (Lepidoptera: Noctuidae) in IPM for processing tomatoes. *Biological control* **52**:30-36.
- White, J. A., J. S. McCord, K. A. Jackson, A. C. Dehnel, P. A. Lenhart, and A. Biere. 2017. Differential aphid toxicity to ladybeetles is not a function of host plant or facultative bacterial symbionts. *Functional ecology* **31**:334-339.
- Wood, E., and K. Starks. 1972. Effect of temperature and host plant interaction on the biology of three biotypes of the greenbug. *Environmental Entomology* **1**:230-234.
- Wyatt, I. J. 1970. The distribution of *Myzus persicae* (Sulz.) on year-round chrysanthemums. *Annals of Applied Biology* **65**:31-41.
- Yasuda, H., T. Kikuchi, P. Kindlmann, and S. Sato. 2001. Relationships between attack and escape rates, cannibalism, and intraguild predation in larvae of two predatory ladybirds. *Journal of Insect Behavior* **14**:373-384.

Young, W., and G. Teetes. 1977. Sorghum entomology. *Annu Rev Entomol* **22**:193-218.

Zhang, W., and S. M. Swinton. 2009. Incorporating natural enemies in an economic threshold for dynamically optimal pest management. *Ecological Modelling* **220**:1315-1324.

Zhang, W., and S. M. Swinton. 2012. Optimal control of soybean aphid in the presence of natural enemies and the implied value of their ecosystem services. *Journal of Environmental Management* **96**:7-16.