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Study of Population Dynamics of Sugarcane Aphid *(Melanaphis sacchari)* in Rio Grande Valley, Texas

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STUDY OF POPULATION DYNAMICS OF SUGARCANE APHID (*MELANAPHIS SACCHARI*) IN RIO GRANDE VALLEY, TEXAS

A Thesis

by

NEETU KHANAL

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ABSTRACT

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Chapter 1: This chapter incorporates detailed information about the biotype concept, aphids, their types and their biotypes, importance of studying insect biotypes and their role in mediating host plant defenses.

Chapter 2: This chapter explains in detail about the biology, biotypes, feeding behavior, damage, and economic loss caused due to sugarcane aphid infestation. This chapter further elaborates on the need for studying population level differences and justifies the objectives and significance of this research study.

Chapter 3: This chapter provides information about the comprehensive work done on three different populations of sugarcane aphid collected from three different locations in Rio Grande Valley, Texas. This chapter includes detail information on the life history traits and feeding behavior of three different sugarcane aphid populations along with host plant defense responses against post infestation of sugarcane aphids.

Chapter 4: This chapter provides an overview of my major findings and possible future directions for research to understand the sugarcane aphids and the potential emergence of new biotype(s).

DEDICATION

I would like to dedicate my thesis to my grandparents, Mr. Nandi Keshar Khanal, Mrs. Bishnu Maya Khanal, Mrs. Ghana Kumari Bhattarai, Mr. Padam Prasad Bhattarai and Mr. Bhaktiram Sharma, my father and mother, Mr. Yogeswor Khanal and Mrs. Laxmi Devi Khanal, my father-in-law and mother-in-law, Mr. Lekhnath Acharya and Mrs. Bindu Acharya, my husband, Mr. Udit Acharya, and my brother, Mr. Nischal Khanal for all their love, blessings, support, and motivation.

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CHAPTER I

THE KNOWN AND UNKNOWNS OF APHID BIOTYPES, AND THEIR ROLE IN MEDIATING HOST PLANT DEFENSES

This chapter has already been published in the Diversity journal and this article belongs to the special issue Plant-Insect-Microbe Interaction and Diversity.

Khanal, N.; Vitek, C.; Kariyat, R. The Known and Unknowns of Aphid Biotypes, and Their Role in Mediating Host Plant Defenses. *Diversity* **2023**, *15*, 186. https://doi.org/10.3390/d15020186

Abstract

Insect species are subjected to disparate selection pressure due to various biotic and abiotic stresses. Management practices including the heavy use of chemical insecticides and introduction of insect-resistant plant cultivars have been found to accelerate these processes. Clearly, natural selection coupled with human intervention have led to insect adaptations that alter phenotypes and genetic structure over time, producing distinct individuals with specialized traits, within the populations, commonly defined as biotypes. Biotypes are commonly found to have better fitness in the new environment and, in the case of aphids, the most commonly studied system for biotypes, have the ability to successfully infest previously resistant host plants and new species of host plants. Although a large number of studies have explored biotypes, the

concept for defining biotypes varies among scientists, as we lack a consistency in estimating biotype behavior and their variation within and between biotypes. The concept of biotypes is even more complicated in aphid species (Aphidoidea), as they undergo parthenogenetic reproduction, making it difficult to understand the source of variation or quantify gene flow. In this review, we aim to illuminate the concept of biotype and how it has been used in the study of aphids. We intend to further elaborate and document the existence of aphid biotypes using sugarcane aphid (*Melanaphis sacchari*) as a model to understand their differences, level of variation, evolution, and significance in pest management.

Background

Insects are the most diverse group of organisms and have broad genetic variability that allows them to adapt to a wide array of less-than-ideal conditions, including their host plants, host animals and habitats (Saxena and Barrion 1987). Insect species feeding on different host plants experience different microclimatic conditions, presence of predators and natural enemies, variation in nutrient compositions, primary and secondary host plant metabolites, and different forms of plant defenses that consequently expose them to divergent selection (Nosil 2004; Ferrari et al. 2008; Guerrieri and Digilio 2008; Kaur et al. 2020; Singh et al. 2021). In addition, insect species are also vulnerable to abiotic stresses, such as sudden fluctuation in temperature and humidity, compounded by the scarcity of food sources. Management strategies such as the development of insect-resistant plant varieties and application of various insecticides and pesticides in agroecosystems may add to the intensity of selection pressure (Taggar and Arora 2017). Consequently, these selection pressures and divergent selection in insects lead to ecological adaptations (Ferrari et al. 2008; Carletto et al. 2009; Nosil 2012), leading to phenotypic and genotypic differences among populations (Nosil 2012). Although these

differences have been observed and studied in many insect species, this is predominantly observed within and among different species of aphids.

Aphids

About 5000 species of aphids (class Insecta, order Hemiptera) have been described, and they form one of the largest, most geographically widespread, and economically important insects around the globe (Blackman and Eastop 2000; Smith and Chuang 2014). Aphids are plant sap feeders, and they suck sap from the phloem by inserting their stylets on plant parts such as stems, leaves, panicles, and roots. During the process, they also inject toxic saliva into the plants, which causes leaf discoloration and leads to tissue death (Tjallingii 2006). Aphids also secrete a sticky substance called honeydew that favors the growth of black sooty mold that impairs photosynthesis, plant growth, and may ultimately kill plants (Pollard 1973; Dixon 1998). Besides direct damage through feeding, aphids also transmit a suite of viral diseases. Some of the common aphid-vectored diseases include maize dwarf mosaic virus, cucumber mosaic virus, potato leaf roll virus, barley yellow dwarf virus, potato virus Y, banana bunchy top virus, carrot mottle virus, lettuce necrotic yellow virus and sugarcane mosaic virus (Berger and Zeyen 1987; Gray et al. 2002; Hogenhout et al. 2008). All these traits have contributed to aphids, considered one of the most devastating pest groups of the major agricultural crops all over the world. Aphids have the dynamic ability to change into different forms (morphs) throughout their lifetime, which may specialize in feeding, reproduction, dispersal, and survival (Williams and Dixon 2007). The reproductive methods of aphids may vary even within the same species. They can reproduce asexually and form clones or reproduce sexually and produce eggs. They can combine these two methods of reproduction and may alternate between cyclical and obligate parthenogenesis (Simon et al. 2002; Williams and Dixon 2007). Under certain conditions, such as extreme

weather, scarcity of food and attack by natural enemies, aphids can produce winged or wingless males, which leads to sexual reproduction (Dixon 1985). Cyclical parthenogenesis, where they can alternate between asexual and sexual reproduction, is the most common mode of reproduction among many aphid species (Dixon 1985; Simon et al. 2002). Aphids also have a unique and interesting reproductive phenomenon referred to as telescoping of generations, where a female viviparous aphid has a daughter developing inside her, and that daughter has a parthenogenetic daughter developing inside her (Simon et al. 2002; Miura et al. 2003). These varied methods of reproduction highlight the great reproductive potential that aphids have in comparison to other than animals (Blackman and Eastop 2000; Powell et al. 2006).

Integrated pest management (IPM) has been considered the most sustainable way for combining and integrating various aspects of plant protection against aphids. IPM prioritizes physical, cultural, and biological control methods, with chemical control methods as the last resort (Stern et al. 1959; Barzman et al. 2015). Under IPM for aphids, host plant resistance has been established as the most practical solution. However, the colossal diversity, adaptable body structure, high fecundity, short generation time and innate plasticity of aphid species gradually overwhelm the resistance in cultivars by evolving new forms with increased ability to severely infest and damage previously known resistant host cultivars (Saxena and Barrion 1987; Gould and Nichols 1998; Rausher 2001). These new and distinct forms of insects isolated by host preferences, not yet considered a new species, are commonly referred to as biotypes (Thorpe 1930; Mayr 1999; Huxley 2010).

The Concept of Biotype

Benjamin Walsh (1864) (Walsh 1864) was the first entomologist who incorporated evolutionary concepts in his studies and recognized insect populations that are morphologically

similar but having different biological traits and named them “phytophagic varieties.” He found that 15 similar species of gall wasps differed primarily in their preference for varied species of willow plants. Cholodkovsky (1908) (Cholodkovsky 1908) used the term “biological species” for populations of adelgids who differed from each other in their biological activity. In 1951, Painter published a book (Painter 1951), Insect Resistance in Crop Plants, where he freely interchanged biotype with biological strains and races. Since then, entomologists and applied biologists have recognized different races and strains among insects, and many definitions on biotypes have been discussed. Some of the major ones are identified in Table 1.

Table 1. Commonly used definitions of biotypes.

S.N.	***Biotype Concept***	***Reference***
1.	Biotypes are the populations that can reproduce and survive on cultivars developed for resistance to a particular insect or can resist insecticides.	(Nielson *et al.* 1970)
2.	Biotype is a taxonomic concept mostly used by non-taxonomists and has been defined as consisting of all individuals of equal genotype. Biotypes are recognized by a biological function rather than by morphological characters. In practice, a biotype contains those individuals performing whatever biological feat interests the observer and thus may contain one or more races or strains.	(Eastop 1972)

Table 1, cont.

3.	Biotype is an individual or a population whose phenotype is determined by the interaction between plants having different genes for resistance and the larvae's ability or inability to survive on and stunt the plant.	(Gallun 1978)
4.	Biotype of insects are individuals or populations that are distinguished from the rest of its species by criteria other than morphology, for example, a difference in parasite ability.	(Gallun and GS 1980)
5.	Diverse biological differences have been used to designate populations as biotypes. They are (a) nongenetic polyphenisms, (b) polymorphic or polygenic variation within populations, (c) geographic races, (d) host races, and (e) species.	(Diehl and Bush 1984)
6.	Biotype is an intraspecific category referring to insect populations of similar genetic composition for a biological attribute. The biotype populations may be partially and temporarily sympatric, allopatric, or parapatric with other compatible populations, but differ in one or more biological attributes.	(Saxena and Barrion 1987)
7.	The concept of biotypes, strain, and host race: "strain designates a population arising from a single collection or clonal individual; biotype is a category designating shared	(Granett *et al.* 2001)

Table 1, cont.

	phenotypic traits; host race is a biotype that is better adapted to a specific host than are other biotypes."	
8.	Biotypes are populations within an arthropod species that differ in their ability to utilize a particular trait in a particular plant genotype.	(Smith 2005)

Clearly, these definitions designate biotypes based on their biological characteristics and differential performance on their host plants. However, Downie (2010) (Downie 2010) criticized the previous definitions listed in Table 1, emphasizing that the definitions are too basic and confusing. He further stated that race and species terms denote meaningful meaning of biotype and would be more appropriate to use and understand. Variations in views about biotype among scientists cannot be ignored, as the definitions are not unified and the meaning itself is not consistent either within or between biotypes. This confusion might have come up since a greater number of biotypes are seen in aphid species, which reproduce almost exclusively by parthenogenesis, and do not obey the gene for gene relationship/principle that many scientists have used as a basic explanation for evolution of insect biotypes (Diehl and Bush 1984; Taggar and Arora 2017). Though complex and complicated in nature, the existence of variation in factors that influence host choice within an insect population for various parameters cannot be ignored, and different populations with varied factors that influence host choice cannot just simply be labeled as races, clones, or species. Hence, the term biotype has served the purpose of defining the variations among different populations of arthropod species and that differentially affect their life history traits and host plant response.

Some parameters used in identifying biotypes are host preference, virulence, genetic composition, reproductive behavior, physiological response to biotic and abiotic conditions, disease vector capabilities, migration patterns, pheromone differences and insecticide resistance (Eastop 1972; Russell 1978; Stark et al. 1983), and in a few cases morphological variations (Saxena and Rueda 1982; Fargo et al. 1986; Inayatullah et al. 1987; Saxena and Barrion 1987). However, insect virulence on a particular host plant is a common parameter implicated in identifying insect biotypes (Maxwell and Jennings 1980). This biotype concept has been universally used to describe the differences among populations of insect species, mainly aphids. As discussed above, other factors include the continuous use of insect-resistant plant varieties, the change in morphological behavior and phenotype of insects, which may be due to various genetic and/environmental factors, or both might have led to the evolution of biotypes. Failure to recognize an existing biotype of an insect may also lead to the evolution of a more virulent biotype. Furthermore, to complicate the evolution of biotypes, the parasitic or mutualistic relationship of an insect pest with its endosymbiont has been found to spawn the variation and interdependence between and within species (Moran et al. 2008; Thompson 2009; Douglas 2009; Oliver et al. 2010). Natural enemies of herbivores, especially predators, may also be a causal factor in generating variation and change of host plant range. Thus, multitrophic interlinkages between host plant, herbivore, endosymbiont, predator, and other environmental factors and interference of various natural processes by human beings also contribute to initiate variation, and thus formation of biotypes.

Importance of Studying Insect Biotype

Studying biotypes is of prime importance for insect pest management involving resistance management and manipulating host attraction traits. It has been found critical to

incorporate the biotype concept in designing integrated pest management strategies involving host-plant resistance and biological control (Hoy and McKelvey 1979; Saxena and Barrion 1987). Insect populations with avirulent-dominant genes can be strategically released in populations with virulent-recessive genes, which might result in insect control by the production of biotypes with dominant genes for avirulence after a few generations (Hatchett and Gallun 1967; Foster and Gallun 1973; Foster and Lafayette 1976). For example, Foster and Gallun (1973) (Foster and Gallun 1973) studied two biotypes of Hessian flies (*Mayetiola destructor*)—Great Plains (GP) biotype and biotype B—which were released on a wheat cultivar susceptible to biotype B, but resistant to the GP biotype. The results from both greenhouse and field studies suggesting that the population of biotype B was completely suppressed. Thus, biotypes can be considered when deploying a strategy for genetic control of insects. Boller and Prokopy (1976) (Boller and Prokopy 1976) proposed the possibility of biological control of the European cherry fruit fly (*Rhagoletis cerasi*) by using and releasing their incompatible biotypes into the population of compatible ones. Knowledge of biotypes helps entomologists and plant breeders study diverse genetic and phenotypic plasticity in insects, quantify the effects of gene flow, and develop new insect-resistant crop varieties (Pathak and Saxena 2013). For example, new resistant cultivars of wheat against the Hessian fly (*Mayetiola destructor*) have been developed by using this analysis, as Hessian fly biotypes can differentiate resistant genes in different wheat varieties (Foster and Gallun 1973). Further, two biotypes of brown plant hopper (*Nilaparvata lugens*) have been selected for by rearing them on resistant rice varieties and are deployed in identifying brown plant hopper-resistant varieties of rice (Saxena and Barrion 1987). Multiple studies have been conducted on aphid biotypes, and subsequently that information has become handy in breeding programs and used to generate aphid-resistant plant cultivars. Comprehending aphid

biotypes and considering their response to insecticides can also guide the use, formulation, and production of insecticides (Stark et al. 1983). Clearly, the study of biotypes enhances our knowledge on evolution, evolutionary divergence in organism and speciation (Diehl and Bush 1984; Saxena and Barrion 1987).

Biotypes have been identified and studied in several insect orders (Thorpe 1930; Smith 1941; Painter 1951; Saxena and Rueda 1982; Diehl and Bush 1984; Taggar and Arora 2017). Initially, biotypes were listed into 36 arthropod species belonging to 17 families of 6 orders, with aphids contributing almost half to this list (Saxena and Barrion 1987). This biotype list was later updated and about 50 arthropod species belonging to 20 families from 7 orders have been documented to exist as biotypes (Smith 1941; Taggar and Arora 2017). Even with this update, about 50% of described biotypes are of aphids (Smith 2005; Smith and Chuang 2014; Taggar and Arora 2017), making it the most important and interesting group to explore biotypes in detail.

Aphid Biotypes

The concept of biotype apropos of aphids was first reviewed by (Eastop 1972), and he suggested that the term biotype in the case of aphids was synonymous with clone, as they are the individuals of same/similar genotypes. Aphids are mostly host specialized and are specific to one or two related plant species (Jean and Jean-Christophe 2010). It is for this reason that aphids are referred to as ecological specialists (Via 1999; Ferrari et al. 2008). For example, Ferrari et al. (2006) (Ferrari et al. 2006), found that pea aphid (*Acyrthosiphon pisum*) populations collected from alfalfa and red clover differed genetically and showed preference for the plant from which they were collected. Nibouche et al. (2015) (Nibouche et al. 2015) showed that different populations of sugarcane aphids had their genetic structure linked to their respective host plants.

For example, the study compared four main isofemale lineages of sugarcane aphids, where Ms11 lineage was found mainly on sugarcane, Ms15 lineage was exclusively found on sorghum and Ms16 lineage were found on both sorghum (Ms16sorghum) and sugarcane (Ms16sugarcane). Furthermore, host transfer experiments showed both Ms16sorghum and Ms16sugarcane had fitness tradeoffs on alternate host plants. Aphids have characteristic features that may vary, resulting in different morphs. Aphids have alate and apterous forms, oviparous and viviparous forms, and different combinations of these forms where each form or morph has its own ecological function and are distinct in their response to various environmental factors (Agarwala 2006). In cotton aphid (*Aphis gossypii*), it has been found that a single individual can produce offspring with four different and distinct phenotypes—normal light green apterous aphid, normal dark green apterous aphid, dwarf yellow apterous aphid and alate aphid—as a response to the change in its environment and type and quality of host plants (Wall 1933; Kring 1959; Rosenheim et al. 1994; Watt and Hales 1996; Mondor et al. 2005). Thus, the inherent phenotypic plasticity, host-associated genetic divergence, underlying plasticity in gene expression (Wang, Liu, et al. 2020), and the ability to thrive in diverse environmental and geographic locations promotes the faster development of biotypes in aphids than any other insect groups (Moran 1992; Blackman and Eastop 2000; Simon et al. 2002; Huang et al. 2013; Wang et al. 2016, 2019).

Harrington (1943) (Harrington 1943) was the first to document the occurrence of biotype in aphid species. His study indicated the occurrence of four biotypes (referred to as physiological races) of pea aphid, which differed from one another significantly in size and virulence in the United States. Later, biotypes of the pea aphid were described, showing differences in morphology (Meier 1958; Thottappilly et al. 1972), life cycle (Frazer 1972; Srivastava and Auclair 1978), host plant preferences (Markkula and Roukka 1970; Srivastava and Auclair 1978;

Auclair 1978), growth rates (Cartier 1959; Srivastava and Auclair 1978) and nutrition (Srivastava and Auclair 1978). Cartier and Painter (1956) (Cartier and Painter 1956) worked on corn leaf aphid (*Rhopalosiphum maidis*) and documented the differential reaction of two biotypes of corn leaf aphid to resistant and susceptible varieties of sorghum. Later, Painter and Pathak (1962) (Painter and Pathak 1962) proposed four biotypes of corn leaf aphid based on their reproduction on different plants and plant reaction to aphid feeding. This was revised again by Wilde and Feese (1973) (Wilde and Feese 1973), who documented a fifth biotype of corn leaf aphid that differed significantly from those previously observed based on its ability to attack a plant species that had been considered resistant and its ability to reproduce well at higher temperatures. Nielson and Don (1974) (Nielson and Don 1974) studied four biotypes of spotted alfalfa aphid (*Therioaphis maculata*) on different varieties of alfalfa with varying resistance to different biotypes. In the case of greenbugs or wheat aphids (*Schizaphis graminum*), more than 10 biotypes have been reported, four of which are highly damaging (Harvey and Hackerott 1969a, 1969b; Saxena and Chada 1971).

Many aphid biotypes have been discovered and studied based on their behavior and characteristics on new or previously resistant host plant species or varieties, suggesting that a change in feeding preference and/or behavior will produce a new biotype. Saxena and Chada (1971) (Saxena and Chada 1971) studied two greenbug biotypes and found that they have differences in their ability to penetrate the plant tissue. They found that biotype A could penetrate its stylet up to the phloem, while biotype B ended its stylet penetration in the mesophyll parenchyma and could not reach the phloem tissue. Campbell et al. (1982) (Campbell et al. 1982) suggested that the differential feeding behavior of greenbug biotypes on different resistant and susceptible varieties of sorghum might be because of the difference in chemical

constituents of phloem between them. It has also been suggested that resistant host plants produce defensive chemical substances in response to the aphid stylet penetration (Nielson and Don 1974; Kennedy et al. 1978; Kariyat et al. 2019). Another, similar, study conducted by Montllor et al. (1983) (Montllor et al. 1983) on two greenbug biotypes found that they differed in time spent on phloem feeding, fecundity, longevity, post reproductive life, development time and larger size when monitored on a sorghum variety that was previously known for having resistance against greenbug (Weibel et al. 1972; Schuster and Starks 1975; Campbell et al. 1982; Kim et al. 2008). Kim et al. (2008) (Kim et al. 2008) confirmed two distinct soyabean aphid biotypes for the first time based on their unique virulence patterns on soybean genotypes.

In most cases, aphid biotypes have been known to evolve to break the host plant resistance and changing or expanding their host range. It is estimated that there are 26 aphid species known to have biotypes now. Aphid species with their respective host plants, number of known biotypes and the basis of classification are documented in Table 2.

Table 2. Detailed documentation of aphid biotypes across various host plants.

S.N.	***Aphid Species***	***Common Name***	***Crop***	***# of Biotypes***	***Biotypes based on***	***References***
1	*Acyrthosiphon kondoi* (Shinji)	Blue alfalfa aphid	Lucerne (*Medicago sativa*)	2	Virulence	(Zarrabi *et al.* 1995; Taggar and Arora 2017)
2	*Acyrthosiphon pisum* (Harris)	Pea aphid	Lucerne (*Medicago sativa*), winged	15	Genetic divergence and	(Harrington 1943; Cartier 1959; Sohi

Table 2, cont.

			broom (*G. sagittalis*), common sainfoin (*Onobrychis viciifolia*), white clover *(Trifolium repens)*, broad beans *(Vicia faba)* and horseshoe vetch (*Hippocrepis comosa*)	differential association with endosymbionts, virulence, body size, body color, differential survival rate, reproduction, mortality, virus transmission	and Swenson 1964; Frazer 1972; Auclair 1978; Peccoud *et al.* 2015; Taggar and Arora 2017)
3	*Amphorophora agathonica* (Hottes)	Large raspberry Aphid	Red raspberry (*Rubus idaeus*)	6	Colonizing ability on host plant and virulence	(Converse *et al.* 1971; Dossett and Kempler 2012)
4	*Amphorophora idaei* (Born)	Large raspberry aphid	Red raspberry (*Rubus idaeus*)	5	Genetic variation and virulence	(Gordon *et al.* 1997; Birch *et al.* 2002)
5	*Amphorophora rubi* (Kalt.)	Raspberry aphid	Red raspberry (*Rubus idaeus*)	4	Virulence and difference in reproductive rate	(Briggs 1959, 1965; Knight *et al.* 1960; Keep and Knight 1967; Keep *et al.* 1970;

Table 2, cont.

						Saxena and Barrion 1987)
6	*Aphis craccivora* (Koch)	Cowpea aphid	Cowpea (*Vigna unguiculata*)	2	Host plant preference, virulence	(Watson and Okusanya 1967; Ansari 1984; Saxena and Barrion 1987; Kusi *et al.* 2010; Aliyu and Ishiyaku 2013; Taggar and Arora 2017)
			Groundnut (*Arachis hypogaea*)	2	Differential ability to transmit viral strain	
			Bush sitao (*Vigna unguiculata sesquipedalis*)	5	Host preference, virulence	
7	*Aphis fabae* (Scopoli)	Bean aphid	Broad bean (*Vicia faba*)	2	Host preference, phenotypic plasticity	(Pathak 1991; Gorur *et al.* 2005; Taggar and Arora 2017)
8	*Aphis glycine* (Matsumura)	Soybean aphid	Soybean (*Glycine max*)	4	Virulence (ability to colonize on resistant plants)	(Kim *et al.* 2008; Hill *et al.* 2010; Michel *et al.* 2011; Alt and Ryan-Mahmutagic 2013)

Table 2, cont.

9	*Aphis gossypii* (Glover)	Cotton or melon aphid	Cotton (*Gossypium spp.)* cucumber *(Cucumis sativus)* and melon *(Cucumis melo)*	2	Host plant based genetic differentiation, host preference	(Vanlerberghe-Masutti and Chavigny 1998; Wang *et al.* 2004, 2016; Xu *et al.* 2014)
10	*Aphis nasturtii* (Kaltenbach)	Buckthorn aphid	Potato *(Solanum tuberosum)*	2		(Saxena and Barrion 1987; Taggar and Arora 2017)
11	*Aulacorthum solani* (Kaltenbach)	Foxglove aphid	Potato *(Solanum tuberosum)*	2	Difference in host use	(Saxena and Barrion 1987; Miller *et al.* 2009; Taggar and Arora 2017)
12	*Brevicoryne brassicae* (Linnaeus)	Cabbage aphid	Vegetables	2	Virulence	(Lammerink 1968; Dunn and Kempton 1972)
13	*Chaetosiphon fragaefolii* (Cockerell)	Strawberry aphid	Strawberry *(Fragaria ananassa)*	2	Host plant preference and aphid probing behavior	(Shanks and Chase 1976; Saxena and Barrion 1987;

Table 2, cont.

						Taggar and Arora 2017)
14	*Diuraphis noxia* (Kurdjumov)	Russian wheat aphid	Wheat *(Triticum spp.)*	10	Virulence	(Kiriac *et al.* 1990; Basky 2003; Haley *et al.* 2004; Smith *et al.* 2004; Tolmay *et al.* 2007; Jankielsohn 2011; Merrill *et al.* 2014)
15	*Dysaphis devecta*	Rosy leaf-curling apple aphid	Apple *(Malus spp.)*	3	Virulence	(Alston and Briggs 1977)
16	*Dysaphis plantaginea* (Passerini)	Rosy apple aphid	Apple *(Malus spp.)*	3	Virulence	(Rat Morris *et al.* 1999)
17	*Eriosoma lanigerum* (Hausmann)	Wooly apple aphid	Apple *(Malus spp.)*	3	Virulence and Life history traits	(Sen Gupta 1969; Gupta and Miles 1975; Young *et al.* 1982; Costa *et al.* 2014)

Table 2, cont.

18	*Macrosiphum euphorbiae* (Thomas)	Potato aphid	Tomato *(Solanum lycopersicum)* and Hairy nightshade *(Solanum sarrachoides)*	2	Virulence and host preference	(Goggin *et al.* 2001; Srinivasan and Alvarez 2011)
19	*Melanaphis sacchari*	Sugarcane Aphid	Sugarcane *(Saccharum officinarum),* sorghum *(Sorghum bicolor),* Johnsongrass *(Sorghum halepense),* Columbus grass *(Sorghum almum)*	6	Micro-locus lineages and host preference	(Nibouche *et al.* 2015, 2018; Paudyal, Armstrong, Harris-Shultz, *et al.* 2019)
20	*Myzus persicae* (Sulzer)	Green peach aphid	Tobacco *(Nicotiana tabacum),* cabbage *(Brassica oleracea var. capitata*), peach *(Prunus persica),* potato *(Solanum tuberosum)* and	3	Body color, life history traits, host plant preference and insecticide resistance,	(van Emden *et al.* 1969; Saxena and Barrion 1987)

Table 2, cont.

			sugar beet *(Beta vulgaris)*			
21	*Nasonovia ribisnigri* (Mosley)	Lettuce leaf aphid	Lettuce *(Lactuca sativa)*	2	Virulence	(Arendt *et al.* 1999; van der Arend 2003; Cid *et al.* 2012; Taggar and Arora 2017)
22	*Rhopalosiphum maidis* (Fitch)	Corn leaf aphid	Barley *(Hordeum vulgare)*, corn *(Zea mays)*, sorghum *(Sorghum bicolor)*	5	Differential reproduction, host plant response and virulence	(Cartier and Painter 1956; Painter and Pathak 1962; Singh and Painter 1964; Wilde and Feese 1973)
23	*Schizaphis graminum* (Rondani)	Greenbug or wheat aphid	Barley *(Hordeum vulgare)*, wheat *(Triticum spp.)*, oats *(Avena sativa)*, sorghum *(Sorghum bicolor)*	11	Virulence, a few morphological differences	(Wood Jr 1961; Harvey and Hackerott 1969a, 1969b; Teetes *et al.* 1975; Porter *et al.* 1982, 1997; Kindler and Spomer 1986;

Table 2, cont.

						Curvetto and Webster 1998; Kindler and Hays 1999; Kindler *et al.* 2001; Taggar and Arora 2017)
24	*Sitobion avenae* (Fabricius)	English grain aphid	Wheat *(Triticum spp.)*	6	Virulence, life history traits, body color	(Lowe 1981; Wang *et al.* 2019)
25	*Therioaphis maculata* (Buckton)	Spotted alfalfa aphid	Lucerne *(Medicago sativa)*	6	Biological activity and response to organophosphate insecticides.	(Nielson *et al.* 1970; Lehman *et al.* 1971; Panda and Khush 1995)
26	*Therioaphis* trifolii F. *maculata* (Buckton)	Spotted alfalfa aphid	Alfalfa *(Medicago sativa)*, clover *(Trifolium spp.)*	2	Host plant based genetic differentiation, host preference	(Nielson *et al.* 1971; Saxena and Barrion 1987; Sunnucks *et al.* 1997; Milne 1998a, 1998b; Taggar and Arora 2017)

Molecular Advances in Aphid Biotype Studies

Molecular methods have been well employed to study the biotypes in aphids. Aphids mainly undergo a parthenogenetic form of reproduction, due to which their gene flow is restricted, and are usually observed to have low genetic diversity. Most research findings show that the genetic divergence of aphid biotypes is linked to their host plants. This has also been studied as host-associated genetic makeup among aphid biotypes and host-associated genetic divergence between aphid biotypes. Microsatellite analyses, DNA markers, transcriptome profiling and analyses, and different mitochondrial sequences are commonly used to identify different biotypes of different aphids. Sunnucks et al. (1997) (Sunnucks et al. 1997) studied different populations of the spotted alfalfa aphid (*Therioaphis trifolii* F. *maculata*) collected from lucerne and subclover using RAPD-PCR techniques and mitochondrial DNA genetic markers. The result showed that there were significant differences in the genetic makeup of the spotted alfalfa aphid, where aphids collected from lucerne and subclover had different genetic makeup. The study concluded that these aphids are different host-associated biotypes of spotted alfalfa aphid and thus had host plant-based genetic differentiation. Similarly, using mitochondrial DNA sequences, host-adapted races of wheat aphid or greenbug (*Schizaphis graminum*) were confirmed and three different clades noted in a study conducted by Anstead et al. (2002) (Anstead et al. 2002). Wang et al. (2016) (Wang et al. 2016) found different mitochondrial sequences in two biotypes of cotton aphid (*Aphis gossypii*) where cotton aphids collected from cotton plant had a different five single-nucleotide polymorphisms when compared to the cotton aphids collected from cucumber plant, and further, they named the same aphid species cotton biotype and cucumber biotype based on their host plant specialization. Similarly, five genetic lineages, named Burk, C, Ivo, Auber and PsP4 of cotton aphids were observed using

microsatellite markers and the lineages found to be host specialized (Brévault et al. 2008). Simon et al. (2003) (Simon et al. 2003) studied the genetic differentiation of different populations of pea aphid (*Acyrthosiphon pisum*) collected from pea, clover, and alfalfa plants by using allozyme and microsatellite markers and found that the aphid populations collected from different host plants were genetically divergent. Frantz et al. (2006) (Frantz et al. 2006) conducted population genetic analyses on pea aphids collected from different pea, faba bean, red clover, and alfalfa where they observed three genetic clusters of pea aphid, and one from pea and faba bean, another from red clover and the third one from alfalfa. These results clearly indicate host-associated genetic difference in pea aphid biotypes. Genetic analysis of different biotypes of large raspberry aphid (*Amphorophora idaei*) has shown high genetic variability within and between its five biotypes (Birch et al. 2002). Furthermore, Wang et al. (2019) (Wang et al. 2019) studied genetic differentiation of different populations of English grain aphid (*Sitobion avenae*) collected from different wheat and barley plants using microsatellite markers. The study found that the populations collected from barley had higher genetic diversity than the populations collected from wheat. The results also showed low genetic differentiation among the populations from different geographic locations and hence provided an important insight to consider plant factors to be of relatively higher importance than geographical factors for stimulating genetic differentiation in aphid biotypes. In addition, the populations in different geographical locations having few or no phenotypic variations and some genetic variations are sometimes referred to as ecotypes (Diehl and Bush 1984).

Ecotypes and their Differences from Biotypes

Ecotypes are individuals or group of individuals of the same species that live in similar habitats, but different geographical regions or localities. They are also referred to as ecological

races. Ecotypes may share similar morphology and behavior, but still consist of distinct populations (Diehl and Bush 1984). While they have some genetic variation, they can breed among themselves, but do not do so because of geographical barriers. For example, sugarcane aphid biotypes are categorized as having different multiloci lineages (MLLs). Biotype MLL-A is found in East and West Africa, MLL-B in Australia, MLL-C in a wide region covering South America, the Caribbean, East Africa and the Indian Ocean, and other biotypes in another region (Nibouche et al. 2018). Here, MLL-A, MLL-B and MLL-C represent different SCA biotypes. However, MLL-C found in South America and West Africa are the same biotype but can be called ecotypes as they are in different environmental conditions prevalent in the different continents. Diverse environmental components can be held accountable for determining ecotypes from among the biotypes of a species (Von Kéler 1956). Over a prolonged period of evolution, the phenotypic differences among the biotypes may get genetically fixed and may also give rise to ecotypes. Some parameters useful in differentiating biotypes and ecotypes of insect species are described in Table 3.

Table 3. Commonly used parameters to differentiate biotypes and ecotypes.

Parameters	**Biotypes**	**Ecotypes**
Found in	Same or different geographical locations	Different geographical locations
Breeding	Cannot breed among themselves	Can breed among themselves

Table 3, cont.

Genetic variation	High (except for insects who reproduce mainly by parthenogenesis like aphids)	Low
Morphological variation	May or may not be present	Present
Variations due to	Mostly plant factors and to some extent environmental factors	Exclusively by environmental factors

Sugarcane Aphid (*Melanaphis sacchari*) and Sorghum (*Sorghum bicolor*)

Sorghum (*Sorghum bicolor* (L.) Moench) is a multipurpose crop grown for its food, fodder and fuel production and is rich in nutrients and bioactive phenolic compounds. Sorghum is also a nutrient-use efficient crop with high water and nitrogen use efficiencies and can further tolerate drought and elevated temperatures (Taylor et al. 2006; de Morais Cardoso et al. 2017; Kariyat et al. 2019; Kaur et al. 2020). However, sorghum is also susceptible to various insect pests and are a major target of aphids (Reddy 1988, 2017; Sharma 1993; Sharma et al. 2017). The most common aphid species feeding on sorghum are *Schizaphis graminum* (the previously mentioned greenbug), *Rhopalosiphum maidis* (corn leaf aphid), *Sipha flava* (yellow sugarcane aphid), and *Melanaphis sacchari* (sugarcane aphid) (Kariyat et al. 2019).

Melanaphis sacchari, the sugarcane aphid is tiny, soft-bodied, with a gray, tan, or yellow body color. It belongs to the order Hemiptera, suborder Sternorrhyncha, super-family Aphidoidea, and family Aphididae. They are globally distributed, and its host plant includes members of Poaceae family, including sugarcane, sorghum, rice, millet, corn, and wild grasses

(Singh et al. 2004). The sugarcane aphid has distinct dark-black cornicles, tarsi, and antennae, which distinguish it from other aphids. However, the feeding injury on sorghum appears similar to corn leaf aphid (Bowling et al. 2016). In the United States, *M. sacchari* was first reported in 1877 in Florida (Hall 1977; Mead 1978) and in 1999 in Louisiana on sugarcane (Saccharum officinarum L.) (Hall 1987; White et al. 2001). An outbreak of *M. sacchari* in sorghum was first reported near Beaumont, Texas in 2013 (Scott Armstrong et al. 2015; Bowling et al. 2016; Brewer et al. 2017; Zapata et al. 2018). By the end of 2013, it was reported from 38 counties from four states—Texas, Louisiana, Mississippi, and Oklahoma (Bowling et al. 2016) and has subsequently expanded its geographic range to 20 states (Nibouche et al. 2018). Among aphids, *M. sacchari* sucks copious amounts of sap from plant tissue and produces enormous amounts of honeydew, which favors growth of sooty mold on plants (Singh et al. 2004; Bowling et al. 2016; Zapata et al. 2018). The black sooty mold coats the leaf surface, due to which the leaves cannot receive adequate sunlight, and this impairs photosynthesis. The reduced photosynthetic capacity can lead to stunting in plants and can ultimately cause significant yield losses (van den Berg et al. 2003; Villanueva and Sekula 2014). In addition, it also vectors diseases including sugarcane yellow leaf virus (Rott et al. 2008). Since 2014, sorghum fields in Louisiana and Mississippi have been reported to be 100% infested with *M. sacchari*, costing approximately $10 million for aphid control alone (Brewer et al. 2017) and yield loss on susceptible sorghum hybrids can reach up to 60% (Gordy et al. 2019). During 2014 and 2015, *M. sacchari* caused an estimated loss of $64.53/ac primarily by increased production costs as well as reduced sorghum yields in the Rio Grande Valley, Texas (Zapata et al. 2018).

For a very long time, *M. sacchari* had contrasting feeding behavior and host choice in different continents. *M. sacchari* was not considered a pest of sugarcane and was a serious pest

of sorghum in Africa and Asia over a long period of time (van Rensburg 1973), which is opposite to what we observed in North America. In recent times, *M. sacchari* seems to have extended its host choice and feeding behavior within the same geographical region. The question, therefore, lies in whether this change in feeding behavior is due to the emergence of a new biotype of *M. sacchari* or the introduction of new genotypes of sorghum from Asia or Africa (Nibouche et al. 2018) or a combination of both. Genetic diversity has been examined worldwide for *M. sacchari*, and several multiloci lineages (MLL), including MLL-A, MLL-B, MLL-C, MLL-D, MLL-E, and MLL-F, have been identified (Harris-Shultz et al. 2020). Genotypic analysis using microsatellite markers suggested that MLL-F has been the lineage associated with the widespread outbreak of *M. sacchari* in the United States since 2013 (Nibouche et al. 2015, 2018; Harris-Shultz et al. 2017). In Brazil, lopes da Silva et al. (2014) (Lopes-da-Silva and Rocha 2014) showed that an aphid clonal lineage collected from sugarcane exhibited higher demographic parameters in terms of longer reproductive period, higher fecundity, and greater longevity of the aphid on sorghum than on sugarcane. In 2019, host plant specialization studies among *M. sacchari* by Paudyal et al. (Paudel et al. 2019) found that in the US, there exist two different host-specific biotypes where *M. sacchari* collected on sugarcane belonged to the multilocus lineage MLL-D, and *M. sacchari* collected from sorghum and Columbus grass belonged to MLL-F. Collectively, data from these studies indicate that there are host-associated genotypes of *M. sacchari* in the US, and should be explored further.

Conclusions and Future Directions

Collectively, studies on biotype and their emergence point out that the principle of biotype evolution relies on natural selection and human-mediated interference by manipulating the genome of host plants. They are coevolved with host plants, herbivores, parasitoids, and their

endosymbionts over time. Biotypes are derived from the survivors of resistant cultivars and other various biotic and abiotic stresses. A plant's resistance to pests is made vulnerable and threatened by the emergence of a new biotype. Based on our literature survey and synthesis, another consideration for a biotype definition could be: "Biotypes are the individuals and/or populations of insect species that demonstrate distinct characteristics and behavior influenced by the spatial and temporal variation of host plant species, biotic and abiotic factors, and human interventions." As new biotypes emerge, research about their similarities and differences inform the use of improved methods to produce healthy plants and ensure their sustainability. To progress the study of biotypes and their evolution ultimately leads to the question on how to disentangle the role of host plant among other biotic and abiotic factors that influence biotypes. Ultimately, as new biotypes emerge, the affected plants also adapt and evolve as a countermeasure, as observed in various crop species. The continuous use of resistant cultivars and heterogeneous methods applied to control pests also leads to the rise in biotypes and should be the basis and the subject of more research on them.

Insect management programs that incorporate host plant resistance are imperative and strategic in future pest control. To implement and make these strategies effective, there is a need to understand plant–insect interactions at both ecological and mechanistic levels. An effective surveillance program can also be developed to assess the gene mutation or population migration in pests/aphids that would provide results that could be used to improve strategies in growing stronger and resilient plants. An important feature of this surveillance program would include more time spent gathering data on insects from (PCR) techniques and DNA probes (Saxena and Barrion 1987). These efforts can be used as a springboard for further investigation of biotypes in the future. The electrical penetration graph (EPG) technique (which assesses the feeding

behavior of sap-sucking insects), PCR techniques (which can discriminate trivial differences in DNA between individual insects) and the development of molecular markers can better enable biotype identification and differentiation. This differentiation is important to implement biological control approaches to correctly match the right pest control agent with the right host biotype. For example., Wang et al. (2020) (Wang, Zhai, et al. 2020) studied defense-related genes of two biotypes of cereal aphid (*Sitobion avenae*), which indicated that the expression of these genes was plastic and related to the original and alternative host plants. Thus, study of host plant association and associated defensive genes of aphids might provide important insight into the adaptive evolution and differentiation mechanism of different biotypes on different host plants.

To decrease the potential development and/or outbreak of new insect biotype on new or previously resistant host plants, there is a need for the development of various short and long-term strategies. Plant breeding for insect-resistant cultivars should focus on broadening the genetic makeup for resistance in plants and thus diversifying the genetic base in terms of both major and minor genes. Gene pyramiding for resistance can be brought into effective use if thoroughly tested and evaluated for its efficacy. Also, horizontal resistance can be more effective and durable than single-gene resistance (Smith 2005). These abovementioned mechanisms of plant resistance might lower the probability of development of new biotype that is more virulent and robust than a previously existing biotype.

To conclude, the continuous use of resistant plant varieties along with the incremental use of chemical pesticides has caused the emergence of more virulent aphid biotypes. We should continue to study and quantify the phenotypic changes through life-history traits and correlate these with genetic diversity among aphid populations, which can contribute to a better

understanding of aphid population dynamics and pest status and thus will be useful in implementing various pest management strategies, even with the emergence of more biotypes in future.

CHAPTER II

PROPOSED RESEARCH

Introduction

Sugarcane aphid, *Melanaphis sacchari* (Zehntner) (Hemiptera: Aphididae) is an invasive pest distributed worldwide with its host plants mainly belonging to Poaceae/Gramineae family (Bowling, Brewer, Kerns, *et al.* 2016). Sugarcane aphids are economically important pest of sorghum (*Sorghum bicolor*) and sugarcane (*Saccharum officinarum*) but are also found feeding and damaging other cultivated plants such as rice (*Oryza sativa*), millet (*Setaria italica*), sorghum-sudangrass (*Sorghum × drummondii*) as well as wild grasses such as bermuda grass (*Cynodon dactylon)*, ornamental grass (*Miscanthus sinensis*), wild sudangrass (*Sorghum verticilliflorum*) (Singh *et al.* 2004). In North America, sugarcane aphids were considered a serious pest of sugarcane but not sorghum until 2013. In late summer of 2013, a sudden outbreak of sugarcane aphids on sorghum field was observed near Beaumont, Texas. Since then, several studies have been conducted to understand the difference in biology, feeding behavior and genetics of sugarcane feeding and sorghum feeding sugarcane aphids. However, limited studies have focused on understanding the population level differences among sorghum feeding sugarcane aphids in terms of their biology, feeding behavior and host-plant defense mechanisms.

Biology

Sugarcane aphids are tiny and soft bodied insect with their body size ranging from 1mm-2mm. The presence of distinct dark black colored cornicles, tarsi and antennae is the distinguishing feature of sugarcane aphids (Villanueva *et al.* 2014). Sugarcane aphids have body color ranging from gray to tan to light yellow depending on the time of the year influenced by host plant and weather conditions (Villanueva *et al.* 2014). Sugarcane aphids have predominantly anholocyclic life cycle and they reproduce mainly by parthenogenesis. In stressful situations such as cold weather, attack by predators, and overcrowding and lack of food source, aphids generally produce winged or wingless males and undergo sexual reproduction (Dixon 1985; Williams and Dixon 2007). No sexual form of sugarcane aphid has been reported yet in the United States (Paudyal, Armstrong, Harris-Shultz, *et al.* 2019) however, sexual forms have been previously observed in India, China, Japan and Mexico (Bowling, Brewer, Kerns, *et al.* 2016; Peña-Martínez *et al.* 2016). Sugarcane aphids can produce from 34 to 96 nymphs over her lifespan which ranges from 10 to 37 days depending on weather and food sources (Chang *et al.* 1982; Singh *et al.* 2004). The adults of sugarcane aphids can be either winged or wingless but they mostly produce wingless (apterous) females (Bowling, Brewer, Kerns, *et al.* 2016). The sugarcane aphid life cycle consists of four nymphal stages which takes between four to twelve days to complete (Chang *et al.* 1982). Furthermore, they have great ability to move long distances by forming winged or alate adults which contributes to its persistence and huge geographic spread (Bowling, Brewer, Kerns, *et al.* 2016; Esquivel *et al.* 2021). The short generation time along with high reproductive rate enables sugarcane aphid to severely infest and damage crop plants within short period of time impacting the farmers in field scouting and management decision process (Bowling, Brewer, Knutson, *et al.* 2016; Pekarcik and Jacobson

2021). Thus, additional research is required to understand the biology and life history traits of sugarcane aphid which will help in determining the survivorship, reproductive rate, and mortality through which effective management practices can be planned.

Feeding behavior and damage in plants

Sugarcane aphids commonly colonize on the abaxial surface of the leaves and generally start colonizing and feeding from the lower older leaves and slowly moves upward towards the younger leaves. In case of heavy infestation, sugarcane aphids can also colonize the sorghum grain heads and affect grain filling (Villanueva *et al.* 2014). Like other hemipterans, sugarcane aphids have piercing and sucking mouthparts and suck huge amount of sap from the plants. Their mouthparts include needle and tube-like structure called stylet which helps in the penetration of plant tissues and ultimately sucking plant sap (Singh *et al.* 2004). Sugarcane aphids cause direct damage to plants by feeding sugar and nutrients rich phloem which is often aggravated by the loss of water through xylem feeding process (Singh *et al.* 2004). Furthermore, sugarcane aphids secrete enormous amount of honeydew which favors the growth of black sooty mold in the leaves that hinders photosynthesis and thus reducing photosynthetic capacity of plants, ultimately reducing yield (Brewer *et al.* 2017). Moreover, honeydew makes leaves sticky which makes the movement of predators difficult in the leaf surface and also affects the harvesting procedures as honeydew coated leaves, stalks and grains get attached to the harvesting equipment causing loss of grains (Villanueva *et al.* 2014). In addition to direct feeding damage, sugarcane aphids cause indirect harm to plants by transferring plant virus such as sugarcane yellow leaf virus and sugarcane mosaic virus (Bowling, Brewer, Knutson, *et al.* 2016). Thus, during the period of sugarcane infestation, plants are under constant stress of nutrient, sugar, and water loss and pathogen infection stress.

Plant defense mechanisms against sugarcane aphid

Plants have developed various traits and mechanisms to defend themselves against various insect pests. These defense mechanisms are in the form of physical and chemical, direct, and indirect, constitutive, and induced defenses (Howe and Jander, 2008; Kaur and Kariyat 2020). Physical or structural defenses are the first line of defense in plants when herbivores come in close contact with the host plants. Plants have thorns, spines, trichomes, and epicuticular waxes as their physical defense mechanisms (Kariyat *et al.* 2017; Kaur and Kariyat 2020; Kaur *et al.* 2022; Watts and Kariyat 2022; Johnson *et al.* 2023). Plants also produce various chemical compounds like secondary metabolites and enzymes which affect the growth and development and hinders feeding by insect herbivores. Chemical compounds such as volatiles, flavonoids, terpenes, polyphenol oxidase to name a few, act as chemical defense mechanisms (Singh et al., 2021; Tayal et al., 2020). Plant physical defenses such as trichomes, waxes and tough leaves affect the landing, movement and feeding of insect herbivores are direct defenses whereas plant volatiles produced in response to herbivore feeding and nectars produced to attract natural enemies of those herbivore are indirect defense strategies of pants (Howe and Jander 2008; Watts and Kariyat 2021; Singh and Kariyat, 2020; Kariyat et al., 2012) Furthermore, the defense mechanisms which are always present on plants like thorns, spines, waxes, polyphenol oxidase are called constitutive defenses but these defenses are also induced by various biotic and abiotic stresses like extreme temperature, herbivore feeding and damage to plants and are called induced defenses (Kaplan 2007; Howe and Jander 2008; Johnson *et al.* 2023; Kaur and Kariyat, 2023). Sorghum has few bicellular trichomes and the effects of these trichomes to sugarcane aphids need additional research, but sorghum has high amount of epicuticular waxes. The presence of these trichomes and epicuticular waxes interferes with movement, feeding and settling behavior

of aphids (Powell *et al.* 1999; Chavana et al., 2021). The two-defense related chemical compounds found in wax namely α-amyrin and isoarborinone have been found to increase in response to sugarcane aphid feeding in six weeks old sorghum plants (Cardona *et al.* 2023). Furthermore, postinfestation by sugarcane aphid caused increase in the amount of triterpenoids in six week plants and trehalose in two week and six week old plants (Cardona *et al.* 2023). Infestation by sugarcane aphid led to the increase in the production of terpenes and methylnaphthalene, defense related volatile organic compounds, in sorghum plant when compared to the healthy plant with no sugarcane aphid infestation (Park et al. 2020). Similarly, sorghum also contains polyphenol oxidase which is an enzyme that catalyzes the oxidation of phenolic compounds producing reactive quinones which trigger plant defense responses against insect pests (Constabel and Barbehenn 2008; Kaur and Kariyat 2023). In sorghum, the polyphenol oxidase activity was found higher in shoot fly (*Atherigona soccata*) infested resistant and susceptible sorghum cultivars when compared with the control plants (Padmaja *et al.* 2014). Also, it has been reported that shoot fly infestation increased polyphenol oxidase and phenyl alanine ammonia lyase enzymes pathways which in turn elevated the amount of secondary metabolites like o-dihydroxyphenol and other phenolic compounds (Kumari *et al.* 2022). While the study on the activity and function of polyphenol oxidase on various plants (Watts and Kariyat 2022; Fajemisin *et al.* 2023) is plentiful, however, the data on sorghum and sugarcane aphid interaction is scarce and needs additional research.

Biotypes of Sugarcane aphid

Host plant resistance has been considered as the most practical and sustainable solution against the sugarcane aphids. However, the adaptable body structure, prolificity and short generation time of sugarcane aphids can gradually overwhelm the resistance in cultivars by

developing new sugarcane aphid biotypes (Nibouche *et al.* 2018; Paudyal *et al.* 2019). Based on the difference observed in multilocus genotypes, there are six biotypes of sugarcane aphid all around the world (Nibouche *et al.* 2015, 2018; Paudyal, Armstrong, Harris-Shultz, *et al.* 2019) and two biotypes of sugarcane aphid has been reported in the USA based on their host plant specialization (Nibouche *et al.* 2015; Paudyal, Armstrong, Harris-Shultz, *et al.* 2019). As sugarcane aphids are invasive and prolific pests of sorghum and sugarcane, more comprehensive research work focused on their biology, feeding behavior and host plant defense responses should be done to examine their population level differences and understand if there is any potential biotype(s) of sugarcane aphid based on these parameters.

Economic loss

Since 2013, almost all sorghum producing areas (≥98%) in the USA have reported sugarcane aphid infestation (Bowling, Brewer, Kerns, *et al.* 2016; Long *et al*. 2018; Pekarcik and Jacobson 2021; Martinez et al., 2020). Sorghum fields are reported to be infested by sugarcane aphids from early to mature stage, however, huge economic losses have been reported when sugarcane aphids infest at flowering and grain filling stages (Raetano and Nakano 1994). Yield losses in sorghum has been estimated at 100-400 lb/acre when infestation reach 50-500 sugarcane aphids per 15 leaves during pre-flowering stage severe (Bowling et al. 2016a). Since 2013, sorghum yield loss has been reported up to 60% (Gordy *et al.* 2019) and around $10 million (USD) has been used for sugarcane aphid control (Brewer *et al.* 2017). Zapata *et al.* (2018) reported that mean economic loss of $64.53/ac incurred between 2014 and 2015 in sorghum production due to sugarcane aphid infestations driven primarily by increased production costs as well as reduced sorghum yields.

In 2021, the infestation of sugarcane aphid in Rio Grande Valley has been light and under control as per sorghum growers and other sources (Russell 2021). This has been accounted to change in climatic condition and scientific breakthroughs which involved release of many resistant sorghum varieties. The winter storm in February,2021 which led to freezing conditions around the Rio Grande Valley might have knocked out aphid population and hindered their migration too. However, some sorghum producers still hesitate to plant sorghum, as SCA has become an annual threat causing severe economic losses. The temperature and other climatic patterns have favored the historic outbreak and spread of SCA in South Texas and hence, this region has always been kept as a very alert region by many biologists and entomologists. The use of resistant varieties and pesticides have been rapidly increasing and this has caused undeniable threat to the potential emergence of new sugarcane aphid biotype. More importantly, a regular study of aphid behavior is of great importance in agro-environment and agro-economics.

Objectives and Significance of this research

The objectives of this thesis were to investigate the population level differences through examining the life history traits of different sugarcane aphid populations feeding on sorghum plants, understand the feeding behavior of different sugarcane aphid populations and examine the post-infestation host plant defense response of sorghum-sudangrass against sugarcane aphid. Doing this research will allow us to identify the population level differences and understand the life history traits and feeding behavior of different sugarcane aphid populations. Thus, this study will contribute to a better understanding of the dynamics of different sugarcane aphid populations in the Rio Grande Valley, Texas. Findings from these studies will contribute to a better understanding of the pest status of aphid and will prove to be useful in implementing various pest management strategies.

CHAPTER III

VARIATION IN SUGARCANE APHID (*MELANAPHIS SACCHARI*) POPULATIONS TRANSLATES INTO LIFE HISTORY AND FEEDING BEHAVIOR ON SORGHUM-SUDANGRASS *(SORGHUM × DRUMMONDII)*

(This chapter is under consideration for Scientific Reports Journal)

Abstract

The sugarcane aphid (*Melanaphis sacchari*; SCA) is a new invasive pest of sorghum first reported in the USA in 2013. Since then, the use of chemical pesticides and resistant cultivars have increased, leading to the potential emergence of new SCA biotype(s). Most research, however, has concentrated on comparing SCA feeding on sorghum, sugarcane, and other hosts, which may have obscured any population differences that may exist within sorghum. To understand this, we collected three populations of SCA feeding on sorghum from three different locations around the Rio Grande Valley, Texas. We examined possible variations in life history traits, feeding behavior through electrical penetration graph and host plant defenses post infestation of SCA on sorghum-sudangrass. The results from life history traits showed significant difference in the net reproductive rate and intrinsic rate of increase among the three tested SCA populations. However, no significant difference on feeding behavior was observed. We also found that epicuticular wax varied significantly on sorghum-sudangrass when fed by the three

SCA populations, while total polyphenol oxidase activity was not. Altogether, we show that the population level differences of SCA translated into variation in specific traits, but further research is required to fully understand the presence of SCA biotype(s).

Introduction

The sugarcane aphid *(Melanaphis sacchari;* SCA*)* (Homoptera) is a tiny, phloem sucking insect (figure 1) that primarily feeds on plants of Gramineae family including sugarcane *(Saccharum officinarum)*, sorghum *(Sorghum bicolor)*, rice *(Oryza sativa)*, and wild grasses like johnsongrass *(Sorghum halepense)*, sorghum-sudangrass *(Sorghum × drummondii)* and Columbus grass (*Sorghum almum)* to name a few (Singh *et al.* 2004). The SCA is predominantly anholocyclic and parthenogenetic species, and no sexual form of SCA has been reported yet in the United States (Paudyal, Armstrong, Harris-Shultz, *et al.* 2019). Depending on the food availability and temperature, a female SCA can produce from 34 to 96 nymphs over her lifetime and the adult life span ranges from 10 to 37 days (Chang *et al.* 1982; Singh *et al.* 2004; Bowling, Brewer, Kerns, *et al.* 2016). The SCA feeds and thrives on sorghum and sugarcane during the summer and spring growing seasons, while in the winter season it can survive on alternate host plants like johnsongrass or Columbus grass (Scott Armstrong *et al.* 2015; Paudyal, Armstrong, Harris-Shultz, *et al.* 2019). The SCAs are found to persist under harsh winter in alternate host plants and increase population rapidly during warm weather with a population doubling time of four to twelve days (Bayoumy *et al.* 2016; Brewer *et al.* 2017; Pekarcik and Jacobson 2021). Such high fecundity along with the ability to spread rapidly has impact on the scouting and timely management of SCA in sorghum fields (Bowling, Brewer, Knutson, *et al.* 2016; Pekarcik and Jacobson 2021). During the year 2013, SCA caused an estimated sorghum profit loss of $66.56/ac in Louisiana (Kerns *et al.* 2015) and in 2015, a mean economic loss of $64.53/ac was

estimated in Rio Grande Valley, Texas, mainly due to reduced yield and increased production cost (Zapata *et al.* 2018). As SCA is a highly destructive pest with great dispersal ability and rapid population growth, it can break the resistance in cultivars and insecticides rapidly leaving the control methods unsuccessful (Khanal *et al.* 2023). Taken together, SCA behavior, fitness and SCA mediated interactions with host plants warrants more empirical studies.

The SCA had been previously regarded as a serious pest of sugarcane but not of sorghum in North America. In 2013, an abrupt outbreak of SCA was seen in sorghum for the first time near Beaumont, Texas (Scott Armstrong *et al.* 2015; Bowling, Brewer, Kerns, *et al.* 2016; Brewer *et al.* 2017; Zapata *et al.* 2018). Since 2013, SCA has spread to almost all (≥98%) sorghum producing regions in the USA (Bowling, Brewer, Kerns, *et al.* 2016; Pekarcik and Jacobson 2021), and has now established as a detrimental pest of sorghum. Since 2013, the cost of aphid control is estimated to be $10 million (USD) (Brewer *et al.* 2017) and yield loss on susceptible sorghum hybrids reaching up to 60% (Gordy *et al.* 2019). The diversification of host plant choice has led to several questions regarding the sudden change in feeding habit of SCA, including studies to estimate the genetic diversity of SCA worldwide. Six multilocus lineages (MLL) of SCA have been reported which includes MLL-A, MLL-B, MLL-C, MLL-D, MLL-E, and MLL-F (Harris-Shultz *et al.* 2020; Nibouche *et al.* 2021). Molecular analysis suggested that MLL-F is the lineage which invaded and caused heavy loss of sorghum in the United States in 2013 (Nibouche *et al.* 2015, 2018). Before 2013, MLL-D lineage of SCA was the lineage associated with feeding and causing loss of sugarcane plants in the United States (Nibouche *et al.* 2018; Paudyal, Armstrong, Harris-Shultz, *et al.* 2019). The rapid shift in host preference behavior associated with the genetic diversity of SCA in the United States raises the possibility that a new biotype of SCA having preference for sorghum may have emerged. Paudyal et al.

(2019) (Paudyal, Armstrong, Harris-Shultz, *et al.* 2019) studied host plant specialization and their data supports the fact that there exists two different host associated biotypes of SCA in the United States. In another host transfer experiments, demographic parameters of SCA collected from sugarcane were studied on sorghum and SCA collected from sorghum were studied on sugarcane. These studies suggested that SCA has fitness tradeoffs when their host plants are alternated (Lopes-da-Silva and Rocha 2014; Nibouche *et al.* 2015). Clearly, to complement molecular developments on SCA, studies that examine population growth and feeding behavior from multiple populations and host plants are clearly needed.

Host plant resistance is considered as the most practical and sustainable way for the management of SCA as it will reduce the use of pesticide, slowdown the development of insecticide resistance in aphid and also increase the activity of beneficial organisms (Sharma and Ortiz 2002; Limaje *et al.* 2018; Armstrong *et al.* 2018). As it has a sucking and piercing mouthparts, SCA uses its stylet for probing into the plant tissue and feeding on phloem or sap (Souza and Davis 2021; Grover *et al.* 2022). The electrical penetration graph (EPG) is a technique for the assessment of feeding behavior of piercing and sucking insect group and thus facilitating the assessment of plant resistance and plant-insect interaction (McLean and Kinsey 1964; McLean and Kinsey 1965; Tjallingii 1978). Sorghum and its related plant species are known to have epicuticular waxes as first line of surface defense which deters settling and movement of aphids on their leaves (Kariyat *et al.* 2019; Grover *et al.* 2022; Cardona, Grover, Busta, *et al.* 2023) and various secondary metabolites such as flavonoids, terpenoids, and polyphenols as chemical defense mechanisms which affects survival, reproduction and growth of aphids and various other insect pests (Kariyat *et al.* 2019; Singh *et al.* 2021). Although quite a few studies have examined

the role of these plant defenses in pre-infestation period however, the post-infestation impact on amount and activity of these plant defense mechanisms, in relation to SCA, are limited.

While a few studies have examined the biology of SCA on various host plants (Lopes-da-Silva and Rocha 2014; Nibouche *et al.* 2015), most of them have been limited to comparisons of SCA populations based on their activity in susceptible and resistant plant cultivars (Limaje *et al.* 2018; Paudyal, Armstrong, Giles, *et al.* 2019; Paudyal *et al.* 2020). Another important aspect of SCA biology- the possible emergence of biotypes and their ability to overcome or circumvent host defenses is less understood. More specifically, we lack knowledge on how population level behavior or traits may differ between SCA from the same host plant but from different locations. In addition, pesticides and insecticides use on sorghum fields has increased since 2013 as has the development and use of resistant sorghum cultivars (Bowling, Brewer, Kerns, *et al.* 2016; Brewer *et al.* 2017; Limaje *et al.* 2018). These factors suggest the development of new biotype(s) of SCA may be occurring (Nibouche *et al.* 2018).

To bridge this knowledge gap, we asked three questions using SCA populations collected from the same host plant that vary spatially: 1. Is there variation among SCA populations for life history traits?; 2. Is there any difference in the feeding behavior among SCA populations?, and 3. Can variation in SCA populations affect host plant defense response? We hypothesized that geographical variation of SCA populations will translate into variation for life history traits in aphids leading to differential defense response in the host sorghum-sudangrass.

Materials and Methods

1. *Aphid Collection*: Three populations of SCA were collected from three different locations in Rio Grande Valley (figure 2). All three SCA populations were collected from sorghum

(Sorghum bicolor) plants. The SCAs were collected from Edcouch, Texas (26.2961252, -97.9592991); population 1 in April 2022, from Santa Rosa, Texas (26.29911261613136, -97.83730125063829); population 2 in May 2022 and from Penitas, Texas (26.40936614396429, -98.46627627489697); population 3 in May 2022. All three SCA populations were reared on sorghum-sudangrass (Super sugar sudex variety, Green Cover Seed Company, USA) in separate environmental chambers with same growing conditions as described in Kaur et al., (2020) (Kaur *et al.* 2020). The environmental chambers were kept at a temperature of 25± 2°C, the relative humidity of 65± 5% and the light: dark period of 16:8 hours.

2. *Life Table Demography:* To assess life history traits, a cohort life table was constructed. Ten one day old SCA nymphs were placed on one of the mature leaves of the sorghum-sudangrass. Three replications per SCA population was used in this experiment. The nymphs were observed daily, and data was recorded for survival, mortality and number of offspring produced. Each day the number of new nymphs were counted and removed from the plant whereas the initial or old nymphs were retained in the plant. Using this data, a cohort life table was made and net reproductive rate, cohort generation time and intrinsic rate of increase were calculated (Birch 1948; Du *et al.* 2018). The net reproductive rate (R_0) refers to the mean number of offspring that a female could produce if she passed through her lifetime conforming to the age specific fecundity and death rates of a given year (Yu *et al.* 2005; Chi and Su 2006). The cohort generation time (Tc) refers to the period of time between the birth of an individual and the birth of its first offspring. The intrinsic rate of increase (r) is the average rate of increase of a population

per individual and this tells us about the rate at which the population of pest/insect increases (Birch 1948; Neupane *et al.* 2020).

3. *Feeding behavior assessment:* We used Electrical Penetration Graph (EPG) technique to assess the feeding behavior of different SCA populations. All EPG experiments were conducted inside the Faraday cage to prevent the interference of any external electrical noise. In EPG, the dorsum of a phloem feeding insect like aphid is attached to a gold wire (2cm long and 10 μm in diameter) which is an insect electrode using conductive silver glue and is placed on the adaxial surface of leaf allowing aphid to freely move around. A second electrode called plant electrode which is a copper wire (10 cm long and 0.2 cm in diameter) is inserted into the soil of pot of the experimental plant. Now, these two electrodes were connected to a GIGA-8 direct current amplifier having 10^9 -Ω input resistance (manufactured by Wageningen University, Wageningen, The Netherlands). An electrical circuit is formed when the aphid inserts its stylet into the host plant and the feeding pathways are visualized in the monitor in the form of graph with different waveforms based on the plant tissue the insect is feeding (Mutti *et al.* 2008; Lei *et al.* 2016). For EPG, we used 45 days old sorghum-sudangrass and 11 adult aphids from each of the three different SCA populations. The aphids were starved for one hour on a petri dish before starting the experiment. The EPG experiment was carried out at room temperature (22°C-24°C) and was run for four hours. We assessed five major variables which are pathway phase, phloem phase, xylem phase, non-probing phase, and number of potential drops (Louis *et al.* 2012; Schwarzkopf *et al.* 2013; Souza and Davis 2021).
4. *Plant defense traits assessment*: For plant defense assessment, we measured the total wax content (Watts and Kariyat 2022) and Polyphenol Oxidase (PPO) (Fajemisin *et al.* 2023)

content in the plants fed by different SCA populations and compared with the control plants. For both experiments, 10 adult aphids were kept in the lower or older leaf of the sorghum-sudangrass and were left undisturbed for five days. A total of six replications were done for each SCA population and six healthy plants which were not fed by any aphids or insects were used as the control plants.

i) *Epicuticular wax quantification*: Epicuticular wax is an important surface defense against herbivores (Johnson *et al.* 2023). For the wax measurement, two leaves from each experimental plant were used. One, on which we placed the aphids, and the other one, which was protected from aphid feeding using a mesh bag. This additional leaf is considered a control leaf to assess changes in epicuticular wax in leaves not directly exposed to SCA. We used hole puncher to make leaf punch holes. 16 leaf punch holes from each leaf were used. These leaf punch holes were placed in a pre-weighed 2ml Eppendorf tubes having 1.5ml of chloroform for one minute followed by gentle shaking. After one minute, the leaf punch holes were removed and the tubes containing wax + chloroform solution were kept in fume hood for 24 hours to allow the chloroform to evaporate completely. After 24 hours, the tubes were reweighed and the total wax content was determined (Kariyat *et al.* 2019; Watts and Kariyat 2022).

ii) *Polyphenol Oxidase Assay (PPO):* PPO is an enzyme that catalyzes reactions leading to chemical plant defenses (Constabel and Barbehenn 2008; Watts and Kariyat 2022). For PPO, we excised a single fresh leaf from each experimental plant and the assay was performed following the polyphenol oxidase assay kit manual (Catalog #: MBS822343; MyBioSource) (Watts and Kariyat 2022). The following equation from

the Polyphenol Oxidase Assay Kit manual (Catalog #: MBS822343; MyBioSource) was used for the quantification of PPO.

$$PPO\ (U/g) = (ODSample - ODControl) \times VTotal / (W \times VSample / VAssay) / 0.01 / T$$

$$= 233.3 \times (ODSample - ODControl) / W$$

In this equation, OD_{sample} and $OD_{control}$ refers to the calorimetric readout of optical density at 410nm for the sample and control respectively, W represents the weight of the sample (0.1g), V_{Total} represents the total volume of the sample (0.35ml), V_{Sample} represents the volume of the sample (0.05ml), V_{Assay} represents the volume of the Assay buffer (1ml) and T represents the reaction time (3 minutes) (Catalog #: MBS822343; MyBioSource) (Watts and Kariyat 2022; Fajemisin *et al.* 2023).

Statistical Analysis

The three SCA populations were used as an independent explanatory variable in all the analysis. One-way ANOVA followed by Tukey's post hoc test was used to analyze the net reproductive rate, cohort generation time and intrinsic rate of increase of the three SCA populations. The data for different feeding phases i.e., mean time spent by each aphid population on different feeding activities did not follow a normal distribution, so the data was analyzed by using nonparametric Kruskal-Wallis test. The data for total wax production was log transformed for analysis because the data did not follow a normal distribution. For wax analysis, one-way ANOVA with Tukey's post hoc test was used where the total wax production was used as a dependent response variable. The PPO data was normally distributed, and thus one-way ANOVA was used for analyzing the data where total PPO content was the dependent response variable. All the analyses were carried out using the JMP Pro 15 statistical software (SAS Institute, Cary, NC, USA).

Results

1. *Life Table Demography:* For life history traits, three major parameters were assessed which are net reproductive rate (R_0), cohort generation time (T_c), and intrinsic rate of increase (r). The net reproductive rate was found to be significantly different (ANOVA, d.f. =2, $p = 0.0183$; fig 4; table 4) among the three SCA populations. Similarly, the intrinsic rate of increase was found to be significantly different (ANOVA, d.f. = 2, $p = 0.0013$; fig 6; table 4) among the three SCA populations. Furthermore, Tukey's post hoc test suggested that population 3 is the population which is significantly different from the other two populations. However, cohort generation time was not significantly different among the three SCA populations (ANOVA, d.f. = 2, $p = 0.3522$; fig 5; table 4).

Table 4. Details of statistical analyses calculated using ANOVA test to assess the life history traits of sugarcane aphid on sorghum-sudangrass.

Parameter	*Population*	*Mean ± SE*	*d.f.*	*F*	*P*
Net Reproductive Rate (R_0)	Population 1	32.933 ± 9.100	2	8.3881	**0.0183***
	Population 2	36.567 ± 5.293			
	Population 3 *	4.333 ± 0.800			
Cohort Generation Time (T_c)	Population 1	14.164 ± 1.342	2	1.2479	0.3522
	Population 2	14.453 ± 0.408			
	Population 3	12.381 ± 1.029			

Table 4, cont.

Intrinsic Rate of Increase (r)	Population 1	0.10430559 ± 0.000119989	2	24.3126	**0.0013***
	Population 2	0.10779135 ± 0.005806961			
	Population 3 *	0.05099933 ± 0.009554212			

(Significant differences are represented in bold at $p < 0.05$ and * in population column represents the SCA population which is significantly different from the other populations.)

2. *Feeding behavior assessment*: The time spent by SCA on different feeding activities in each of 4 hours of recording period (n=11) were assessed in the form of five major variables based on the plant part the aphid was feeding. The five variables assessed were non-probing phase, pathway phase, xylem phase, phloem phase and number of potential drops. The results indicated no significant differences in non-probing phase (Kruskal-Wallis test, d.f. = 2, $p = 0.6228$; fig 7), pathway phase (Kruskal-Wallis test, d.f. = 2, $p = 0.6156$; fig 8), xylem phase ((Kruskal-Wallis test, d.f. = 2, $p = 0.1156$; fig 9), phloem phase (Kruskal-Wallis test, d.f. = 2, $p = 0.9201$; fig 10) and number of potential drops (Kruskal-Wallis test, d.f. = 2, $p = 0.6623$; fig 11). The average time spent by each SCA population in each feeding phases are given in Table 5.

Table 5. Details of statistical analyses calculated using Kruskal-Wallis to assess feeding behavior of sugarcane aphid on sorghum-sudangrass.

Parameter	Population	Mean ± SE	d.f.	*ChiSq*	P
Non-Probing Phase	Population 1	41.389 ± 13.681	2	0.9470	0.6228
	Population 2	64.374 ± 16.654			
	Population 3	57.126 ± 16.485			
Pathway Phase	Population 1	166.780 ± 15.193	2	0.9703	0.6156
	Population 2	148.782 ± 16.235			
	Population 3	135.155 ± 19.444			
Xylem Phase	Population 1	12.362 ± 12.362	2	4.3152	0.1156
	Population 2	10.824 ± 5.749			
	Population 3	26.5 ± 11.611			
Phloem Phase	Population 1	20.377 ± 11.694	2	0.1665	0.9201
	Population 2	16.020 ± 9.837			
	Population 3	21.220 ± 13.345			
	Population 1	7.818 ± 2.017	2	0.8242	0.6623

Table 5, cont.

Number of Potential Drops	Population 2	11.182 ± 4.624			
	Population 3	6.182 ± 2.101			

(Significant differences are represented in bold at p <0.05.)

3. *Plant defense traits assessment*: The total wax content and the total PPO activity of sorghum-sudangrass was examined to assess the host plant defense traits as a response to post infestation by three different SCA populations. The total wax content was found to be significantly different (ANOVA, d.f. = 2, $p < 0.0001$; fig 12; table 6) among the plants fed by the three SCA populations and the control plants. Furthermore, Tukey's post hoc test suggested that the plants fed by population 1 had significantly higher wax content than the plants fed by the other two SCA populations and the control plants. However, we didn't find any significant difference in the total PPO activity (ANOVA, d.f. = 2, p = 0.3613; fig 13; table 6) among the plants fed by the three SCA populations and the control plants.

Table 6. Details of statistical analyses calculated using ANOVA test to assess the sugarcane aphid post-infestation defense traits of sorghum-sudangrass.

Parameter	Population	Mean ± SE	d.f.	F	P
Total Wax Content	Population 1*	0.04725 ± 0.000559017	2	77.1916	**<0.0001***
	Population 2	0.007 ± 0.001149534			
	Population 3	0.006375 ± 0.000625			

Table 6, cont.

	Control	0.00725 ± 0.000881354			
Total PPO activity	Population 1	654.5815 ± 97.89253	2	1.1110	0.3613
	Population 2	628.3061 ± 91.22168			
	Population 3	574.5013 ± 203.1169			
	Control	847.229 ± 299.5407			

(Significant differences are represented in bold at p <0.05 and * in population column represents the SCA population which is significantly different from the other populations.)

Discussion

Collectively, we found evidence which suggests that there are differences in both SCA populations and the host, however, these differences are not sufficient enough to consider these three populations of SCA as biotypes. While our data suggests that there are differences within the variables we tested, these differences need additional research and should be explored further.

To develop successful integrated pest management (IPM) strategies, it is important to understand the life cycle and life history traits of insect species. Fertility is one of the most important biological factors that determines the success, failure and population growth rate of an insect species at a particular environment (Hentz and Nuessly 2004). We observed one population of SCA (population 3) had a significantly different net reproductive rate and intrinsic rate of increase compared to the other two populations. This implies that they differ in their

reproductive ability, as all the factors of the experiment including experimental plants were same for all three populations. The SCA population 3 produced fewer nymphs when compared to populations 1 and 2. The average net reproductive rate of population 3 was 4.333±0.8 which is very low when compared to population 1 (32.933±9.1) and population 2 (36.567±5.293). Although, the population of population 3 seems increasing but it is increasing at a very high decreasing rate. If this population 3 is left to feed on sorghum-sudangrass which is our experimental plant, this population might eventually collapse after a few generations. Also, feeding by population 3 caused drying of the leaves as they progressed their feeding from tip to base of the leaf. The presence of chlorotic and necrotic patches in leaves and stunting of plant is a common injury observed due to aphid feeding behavior (Quisenberry and Ni 2007) but the SCA population 3 was mostly found to colonize at the tip of the leaf and slowly move towards the base causing dryness in the leaf surface. This characteristic was unique and was not observed in the other two populations which suggests possible evolutionary divergence. The drying of the leaves could be possibly because the SCA population 3 was found to spend highest amount of time feeding xylem, plant tissue responsible for the movement of water and nutrients from plant-soil interface to different parts of the plant (Bollard 1960; Brodersen *et al.* 2019), than population 1 and 2 (fig 9). However, the cohort generation time was not significantly different among the three SCA populations. We observed that all three SCA populations started producing offspring at similar age and had similar life span. This suggests that SCA population 3 possibly underwent reproductive tradeoff for growth and survival which indicates that the Rio Grande Valley may not have homogenous distribution of the SCA populations. Hence, we speculate that when new host plants are offered, population 1 and population 2 might behave in a relatively similar way for their growth and reproduction and might have similar selection for host plant whereas

population 3 might have differential host plant choice (Kariyat et al., 2019) and the mechanisms underlying (Grover et al., 2022) this should be explored further.

A host switching experiment conducted to study survival and reproduction of SCA on sugarcane, sorghum, Columbus grass and Johnsongrass collected from different host plants including sorghum (SoSCA), sugarcane (SuSCA) and Columbus grass (CoSCA) indicated that the SCA from sorghum and Columbus grass behaved in a relatively similar fashion for survival and reproduction whereas SCA collected from sugarcane had significantly lower survival and reproduction on Johnsongrass. This result showed that the two SCA populations collected from sorghum (SoSCA) and Columbus grass (CoSCA) are likely to behave similarly for host plant selection (Paudyal, Armstrong, Harris-Shultz, *et al.* 2019). Although, the SCA populations in our study were collected from the same host plant, it is likely that the difference we observed in the reproductive characteristics in a new host plant is helpful to predict that the two populations (P1 and P2) will behave in a similar fashion in a new host plant whereas P3 will likely behave in a different pattern, an area we plan to explore further. The intrinsic rate of increase is one of the major parameter in determining the population differences of an insect species which reflects many major population characteristics like fecundity rate, survival rate and mean generation time (Du *et al.* 2018). Hence, the significant difference we observed in the intrinsic rate of increase also suggests differences in reproductive performance and behavior among the three SCA populations.

The feeding behavior of three SCA populations was investigated by using the EPG technique as it is an excellent tool to study aphid probing and feeding behavior and also identifying plant part/tissues that influence the host-plant's resistance and susceptibility (Schwarzkopf *et al.* 2013). We assessed five major variables/phases using the EPG technique

which are non-probing phase, pathway phase, xylem phase, phloem phase and number of potential drops. The results from the feeding behavior assessment indicated no significant difference in any of the five variables assessed. The probing activity of an aphid is an important behavioral trait through which the aphid can collect required information to decide if the plant/plant part is suitable for feeding (Louis *et al.* 2012).

Furthermore, the repetitive probing in the same leaf surface may cause hydrolysis of the plant tissues through the action of aphid's saliva and its enzymes. Ultimately, the tissues (epidermal and mesophyll layer) will be unable to resist the penetration of aphid's stylet and eventually reach the phloem/sieve elements. Hence, other plant tissues/factors should also be considered for investigating the resistance of plant against insect pests (Montllor *et al.* 1983). As the aphids penetrate the epidermal and mesophyll cells, the pathway phase begins and this phase involves salivation of the sheath, puncturing the cell and movement of stylet towards the sieve elements (Schwarzkopf *et al.* 2013). Thus, pathway phase is the most important phase for an aphid to select the suitable host plant and secretion of its saliva to initiate the required steps for reaching sieve element and the establishment of successful feeding procedure. The xylem and phloem phase indicates the ingestion of xylem and phloem sap from the plant (Alvarez *et al.* 2006). Also, the variable "number of potential drops" indicates the number of times the sugarcane aphids became successful to locate and reach the phloem but couldn't undergo sustained feeding or only fed phloem for less than three seconds. Successful and sustained feeding of phloem indicates the susceptibility of aphid to that plant cultivar. One possible reason on why we didn't observe any difference in the feeding behavior may be because we used experimental plants of same age and variety, although we expected that the difference in

behavior and life history traits of the three populations would have consequences for feeding behavior.

Plants have developed a suite of traits to defend themselves against insect pests. These include physical and chemical, direct, and indirect, constitutive, and induced defense traits (Howe and Jander 2008; Kaur *et al.* 2020). For example, epicuticular wax and leaf trichomes have been found to act as a physical defense (Kaur *et al.* 2020) while secondary metabolites such as flavonoids function as chemical defense mechanisms (Alvarez *et al.* 2006; Schwarzkopf *et al.* 2013; Tayal *et al.* 2020; Singh *et al.* 2021). These defenses can affect host plant selection, landing, movement and feeding decision for aphid and other insect species (Powell *et al.* 1999; Chavana *et al.* 2021). Epicuticular wax and trichomes have been found to not only affect the settling behavior of aphids but also the allelochemicals present in them affect the initial decision and first initiation of stylet penetration in plant tissues (Eigenbrode *et al.* 1996; Musetti and Neal 1997; Powell *et al.* 1999). The two compounds called α-amyrin and isoarborinone found in wax have been reported to increase in six weeks old sorghum plants after feeding by SCA (Cardona, Grover, Bowman, *et al.* 2023). Our results from wax experiment showed significant difference in the total wax content post-feeding by different SCA populations and control plants. The results showed that the plants fed by population 1 had significantly higher wax content when compared to the plants fed by populations 2, 3 and control plants. Although the results from the feeding experiment were non-significant, on closer look, (fig 8 and 10) the SCA population 1 had relatively higher phloem phase and pathway phase than the population 2 and population 3. This might be due to a possibility that the SCA population 1 (or may be succeeded) fed more on phloem than the other two populations which might have resulted in the increased defense response of plant through higher wax production. However, no significant difference was

observed in the total wax production post-infestation by the other two SCA populations (P2 and P3) and the control plants.

We further assessed the total polyphenol oxidase (PPO) activity post-infestation by the SCA. However, no significant difference was observed in the total PPO content between the plants fed by different SCA populations and the control plants. This might be because the PPO activity in sorghum-sudangrass may not be directly associated with the SCA feeding and defense against it. Studies have found that in most plant species, the leaf PPO is mostly located in mesophyll cells and in glandular trichomes (almost 45% in *Solanum* spp.) (Kowalski *et al.* 1992; Constabel and Barbehenn 2008). However, sorghum and sorghum-sudangrass have a very few bicellular trichomes and especially lack glandular trichomes on leaf (Chester G. McWhorter *et al.* 1995), this indicates that the PPO mediated defense through trichomes might be missing in sorghum-sudangrass. As our feeding behavior analysis shows that all the three SCA populations spent highest amount of time in the pathway phase, this indicates that the mesophyll factors including mesophyll PPO couldn't restrict the penetration and movement of SCA's stylet to the sieve element. Hence, the action and pathway of other phytochemicals such as anthocyanins, phenolic acids, phytosterols, to name a few should be examined in sorghum-sudangrass – SCA system (Kariyat *et al.* 2019; Tayal *et al.* 2020; Singh *et al.* 2021).

Various other studies have been conducted to study behavior of new SCA biotype specialized in feeding sorghum plant (Nibouche *et al.* 2015, 2018; Paudyal, Armstrong, Harris-Shultz, *et al.* 2019; Paudyal, Armstrong, Giles, *et al.* 2019). However, all these studies have compared the SCA collected from different host plants and have analyzed their feeding behavior on resistant and susceptible sorghum cultivars. In our study we have studied and examined the population difference of SCA feeding on same host plant in the light of possible biotype(s)

development. The biotype concept is of great importance in breeding programs to develop insect resistant cultivars (Khanal *et al.* 2023). For example, SCA biotypes can be used to examine the life history traits and feeding behavior in newly developed sorghum and sugarcane varieties before releasing them to farmers. The biotype concept and its knowledge have been used for developing resistant wheat cultivars against Hessian fly (*Mayetiola destructor*) and resistant rice cultivars against Brown plant hopper (*Nilaparvata lugens*) by using their different biotypes, respectively (Foster and Gallun 1973; Saxena and Rueda 1982). The SCA is a noxious pest having ability to develop and spread rapidly which makes management efforts difficult and challenging. Furthermore, the established host expansion ability of SCA with emergence of new biotype has threatened not only sorghum fields but other closely related and economically important grain and grass species.

In conclusion, our results provide evidence that suggest differences in behavior and possible evolutionary divergence in SCA populations, however, additional research is required before concluding the presence of different biotypes of SCA in the Rio Grande Valley, Texas. For any study to conclusively determine that there is an emergence of new biotype of an aphid or other insect species, multiple empirical studies should be conducted that correlates population growth, feeding behavior and the underlying behavioral mechanisms. The differences we observed in life history traits, host plant defense, and feeding pattern should be considered and follow up experiments should be conducted in different host plants like sugarcane, johnsongrass and susceptible and resistant sorghum. Results from our study can be used as a baseline information and genetic analysis can be followed up to examine possible biotype emergence. Indeed, the population level differences that we observed in the reproductive traits and plant defense traits shouldn’t be ignored. These differences should be considered and explored further

so that we can have better information on behavior of different sugarcane aphid populations and/or potential biotype(s). This information will be helpful in developing management strategies including the development of resistant plant cultivars so that there won't be a sudden surprising outbreak of new sugarcane aphid biotype specialized in feeding new grain or other closely related crops. For example., host specialization study conducted by Nibouche (2015) (Nibouche *et al.* 2015) in showed that different multilocus genotypes of SCA existed on both sorghum and sugarcane fields with difference in their numbers present in each field. This indicates that SCA of different multilocus genotypes can coexist in the same host plant field. Although sexual forms of the SCA has not been reported yet in the USA (Paudyal, Armstrong, Harris-Shultz, *et al.* 2019), they have been previously reported in Mexico, Japan, India and China (Bowling, Brewer, Knutson, *et al.* 2016; Peña-Martínez *et al.* 2016). As the Rio Grande Valley shares the border with Mexico, there remains the possibility that the sorghum fields in the Rio Grande Valley (Davis *et al.* 2020) might have sexual forms of SCA with different multilocus genotypes or will possibly have a sexual form of the SCA in the near future. The presence of sexual forms will provide us with more information on behavior and will greatly increase our ability to understand and differentiate if the populations are different or are separating from each other. Furthermore, the presence of sexual forms will allow us to explore the idea of reproductive isolation between the SCA populations which will potentially tell us if these populations are different from each other. In addition, sorghum-sudangrass is popular as a forage grass for livestock and is getting more popular among the farmers as it has dense canopy and can act as a natural weed suppressant and as a cover crop in the field (Urbano *et al.* 2006; Martinez 2020; Soti and Racelis 2020). Hence, the different phytohormones of sorghum-sudangrass should be examined thoroughly and how SCA (and/or different SCA populations) feeding changes the

synthesis and pathways of these hormones should be examined. As SCA is an invasive pest which can change its host plant range causing considerable yield loss, regular and timely studies of SCA behavior and its population dynamics in a particular geographical location/s is of prime importance for agroecosystem health and agroeconomics.

(a) (b)

Figure 1. Sugarcane aphids (a, b).

(Images by: Neetu Khanal)

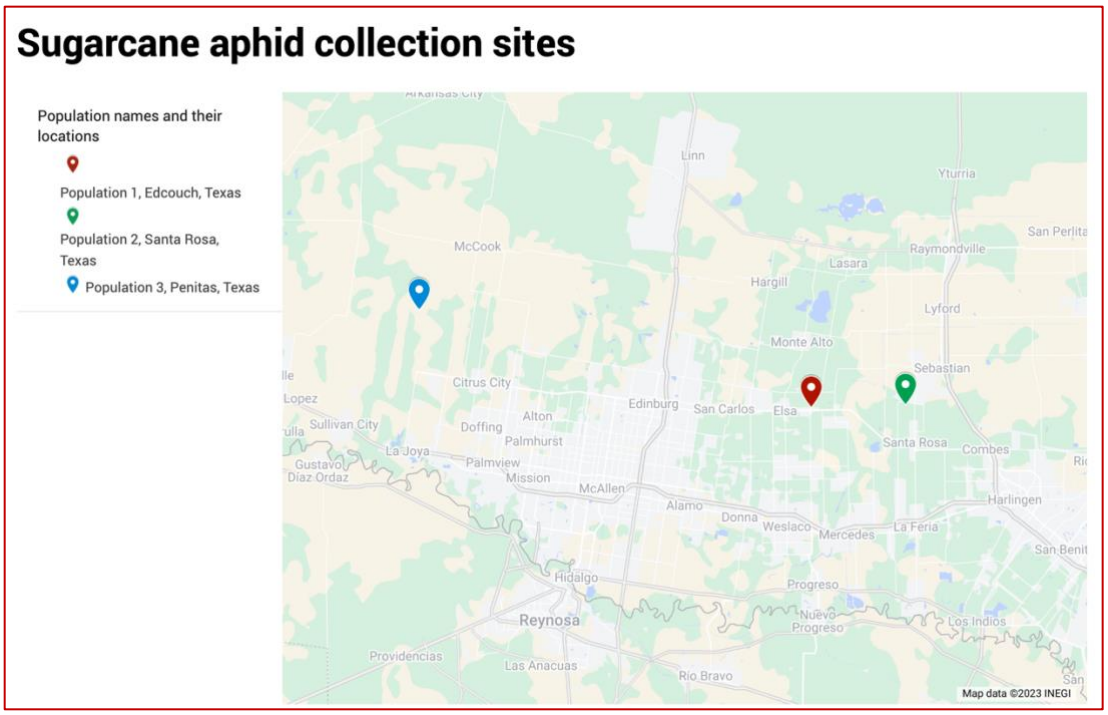
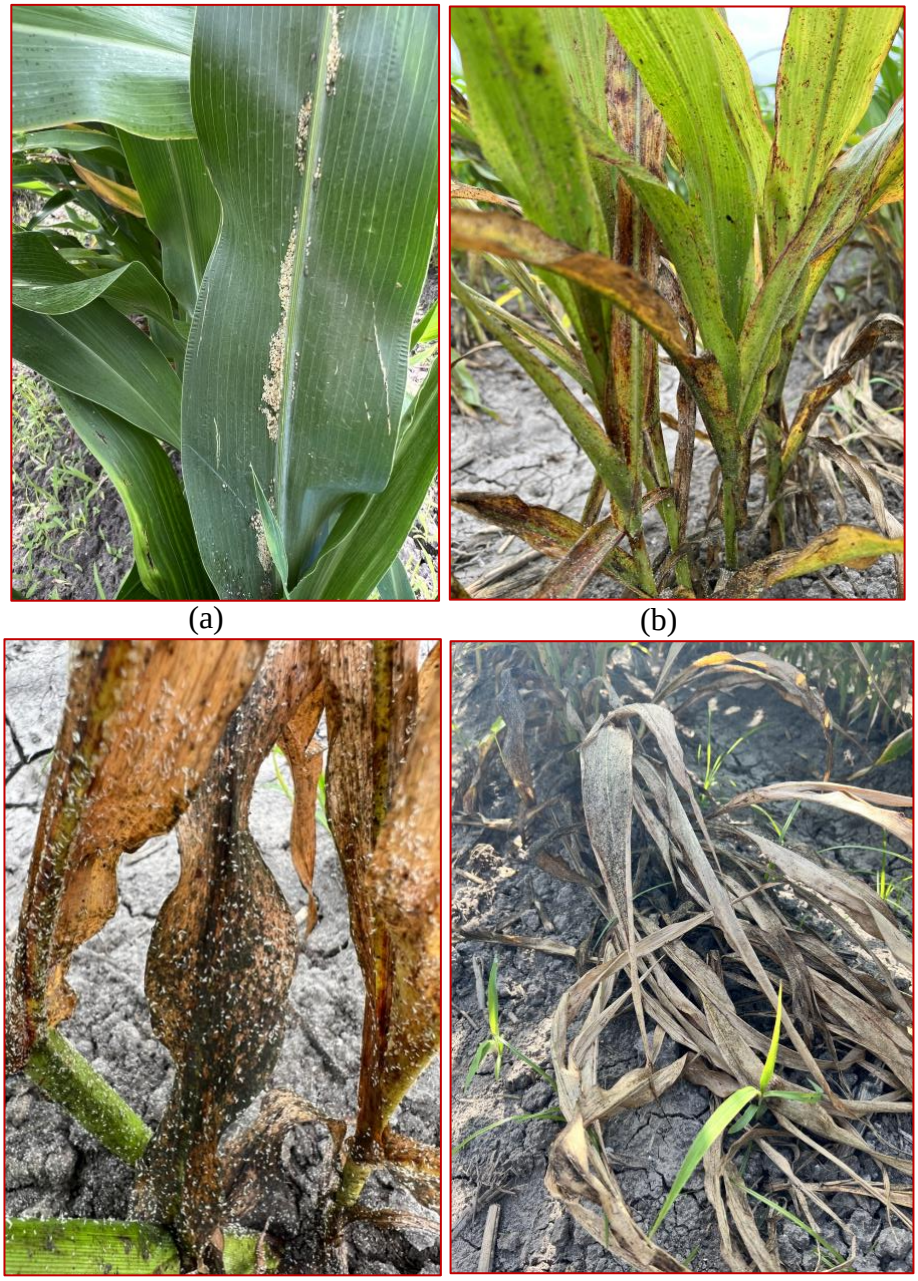


Figure 2. Google map showing the three different locations from where we collected the three different populations of sugarcane aphids. The pinning points with different colors indicate different locations. The red pinning point represent Edcouch, Texas; the green pinning point represents Santa Rosa, Texas and the blue pinning point represents Penitas, Texas.

(Google map by Neetu Khanal)



(a) (b)

(c) (d)

Figure 3. Sugarcane aphid infestation and damage in Sorghum field in Rio Grande Valley, Texas. (a) Initial stage of sugarcane aphid infestation, (b) Increased sugarcane aphid infestation, (c) black sooty mold development in leaf surface due to heavy sugarcane aphid infestation and (d) sorghum plant death due to heavy sugarcane aphid infestation.

(Images by Neetu Khanal)

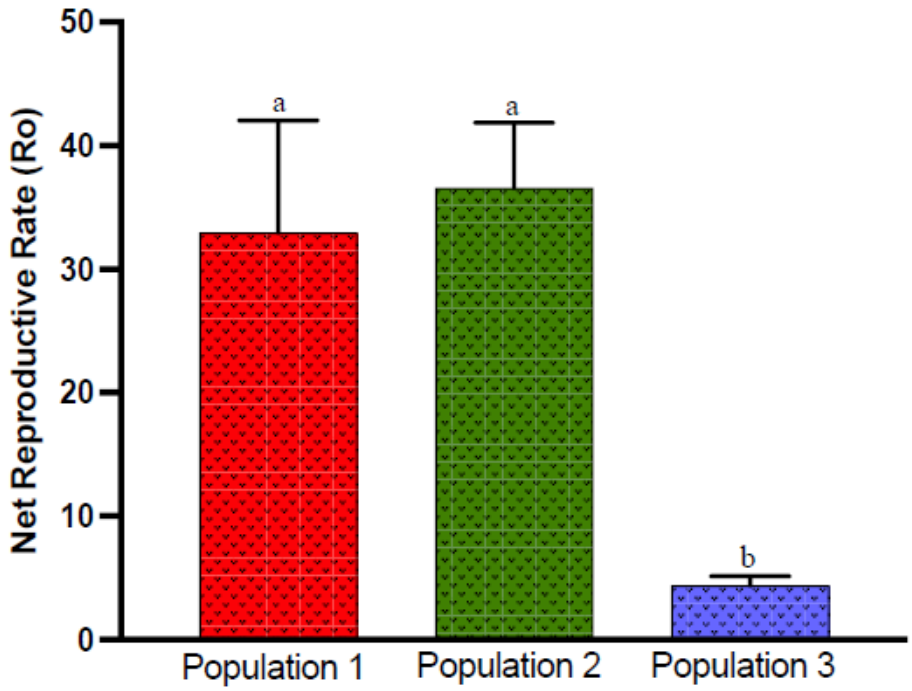


Figure 4. Net reproductive rate of three populations of sugarcane aphid (ANOVA, d.f. =2, $p = 0.0183$. The different lowercase alphabetical letters indicate statistically significant differences at $p < 0.05$.

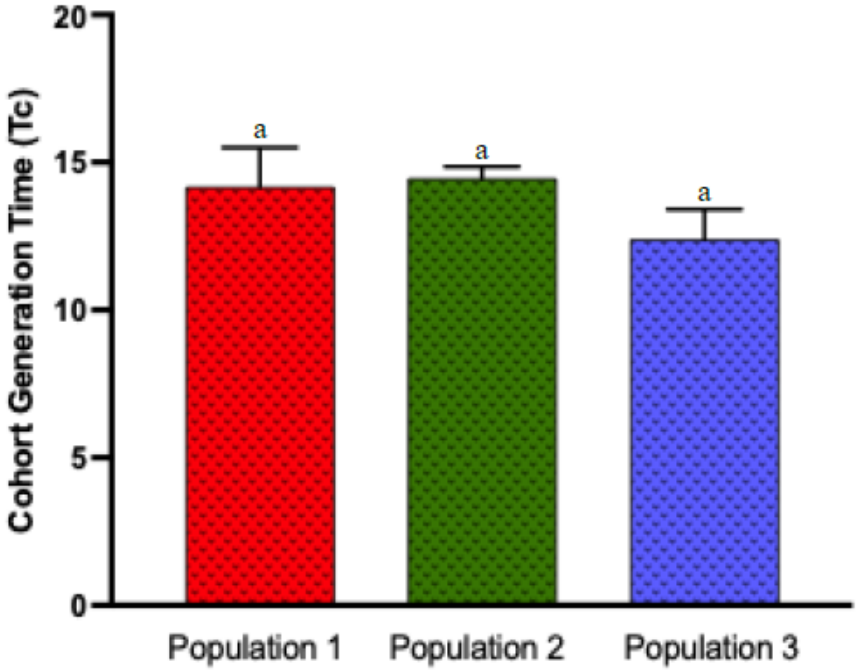
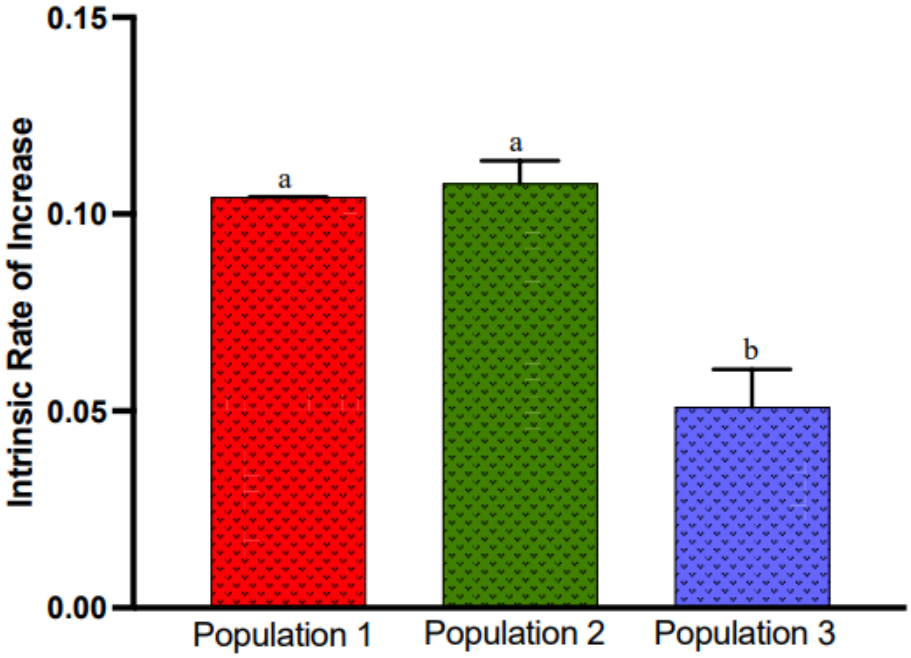


Figure 5. Cohort generation time of three populations of sugarcane aphid ANOVA, d.f. = 2, p = 0.3522). The different lowercase alphabetical letters indicate statistically significant differences at p <0.05.



(c)

Figure 6. intrinsic rate of increase of three populations of sugarcane aphid (ANOVA, d.f. = 2, p = 0.0013). The different lowercase alphabetical letters indicate statistically significant differences at p <0.05.

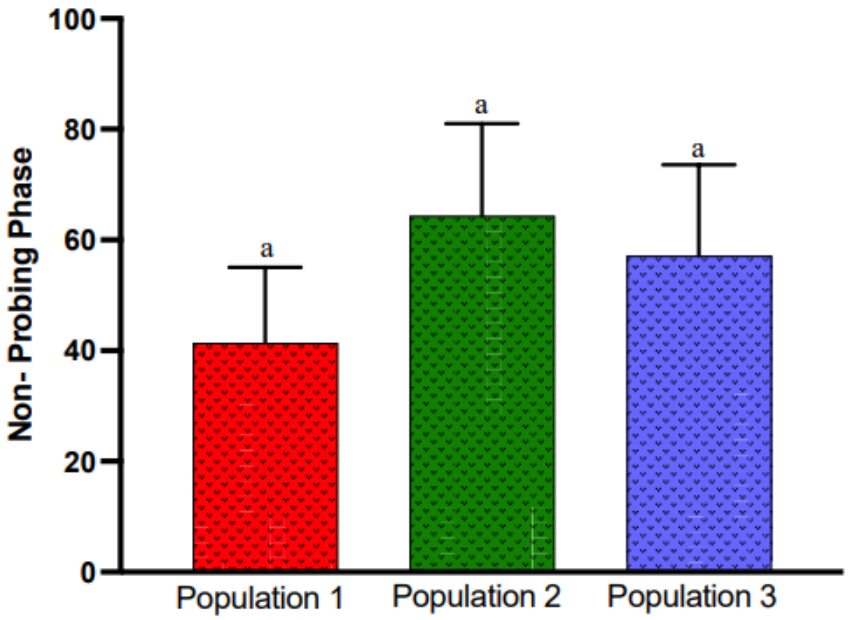


Figure 7. Time spent on non-probing phase by three populations of sugarcane aphid (Kruskal-Wallis test, d.f. = 2, p = 0.6228). The different lowercase alphabetical letters indicate statistically significant differences at p <0.05.

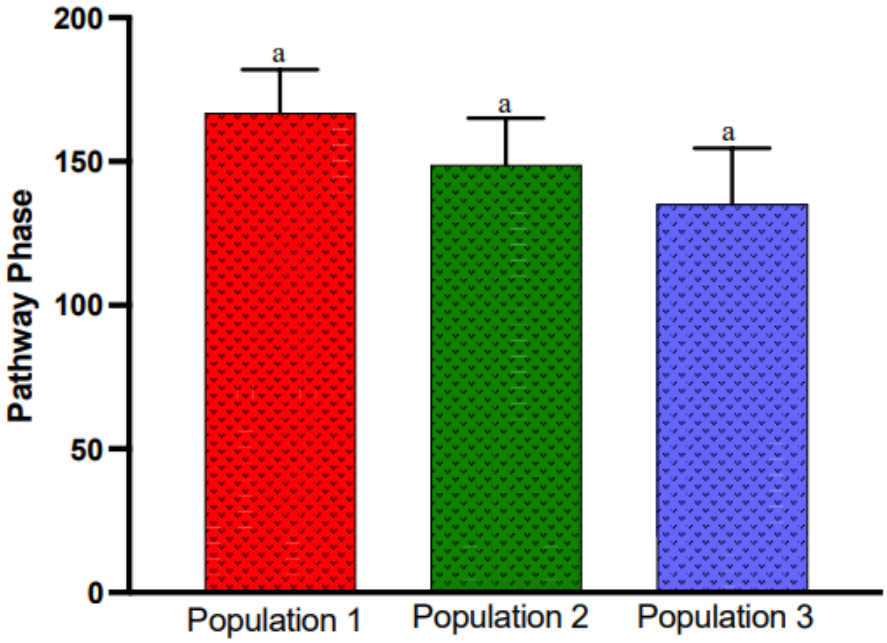


Figure 8. Time spent on pathway phase by three populations of sugarcane aphid (Kruskal-Wallis test, d.f. = 2, $p = 0.6156$). The different lowercase alphabetical letters indicate statistically significant differences at $p < 0.05$.

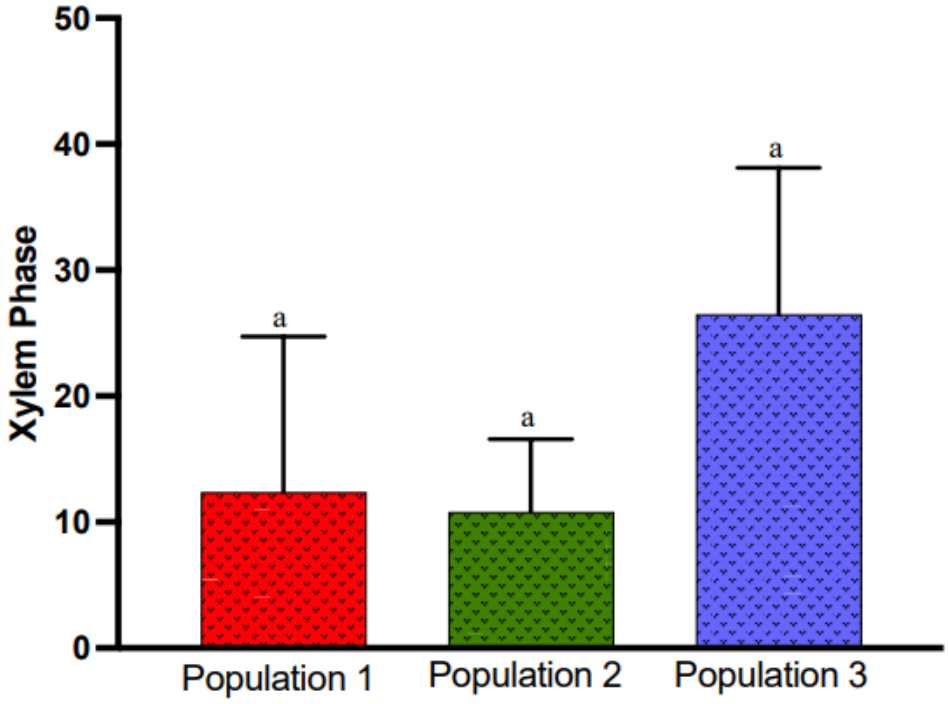


Figure 9. Time spent on xylem phase by three populations of sugarcane aphid (Kruskal-Wallis test, d.f. = 2, $p = 0.1156$). The different lowercase alphabetical letters indicate statistically significant differences at $p < 0.05$.

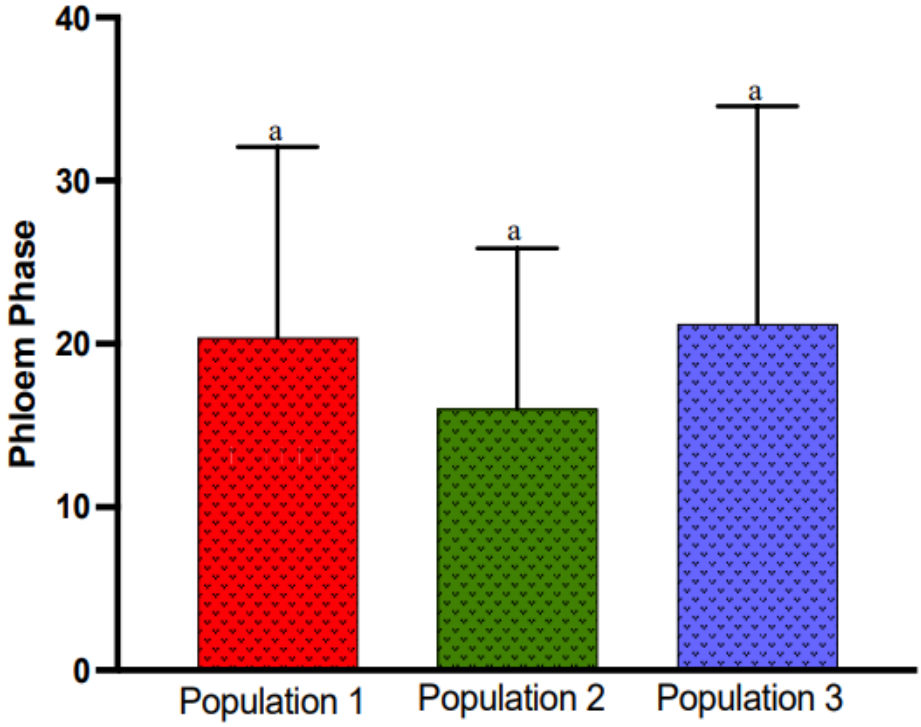


Figure 10. Time spent on phloem phase by three populations of sugarcane aphid (Kruskal-Wallis test, d.f. = 2, p = 0.9201). The different lowercase alphabetical letters indicate statistically significant differences at p <0.05.

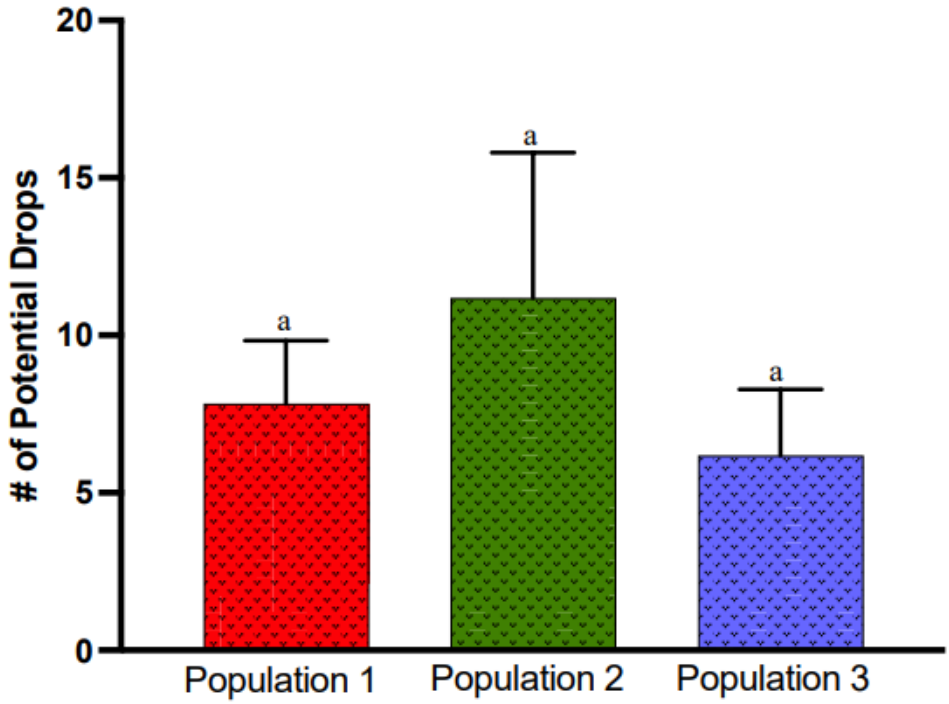
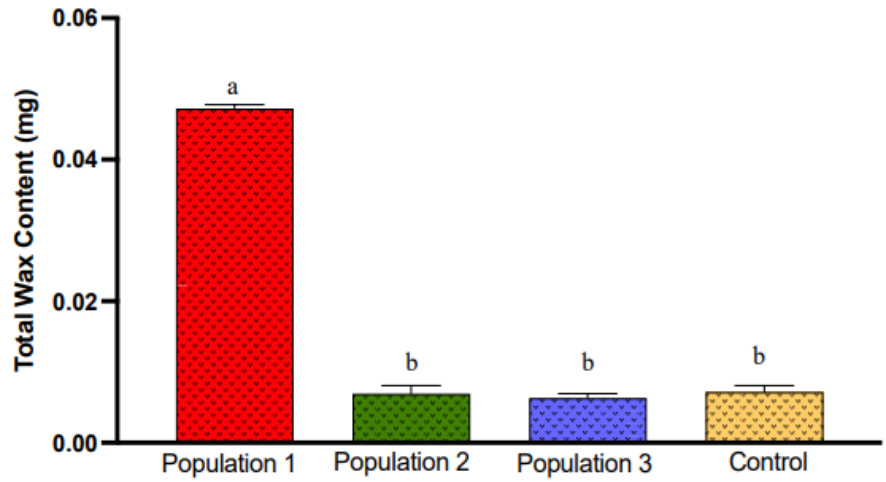


Figure 11. Number of potential drops observed in feeding behavior of three populations of sugarcane aphid (Kruskal-Wallis test, d.f. = 2, p = 0.6623). The different lowercase alphabetical letters indicate statistically significant differences at p <0.05.



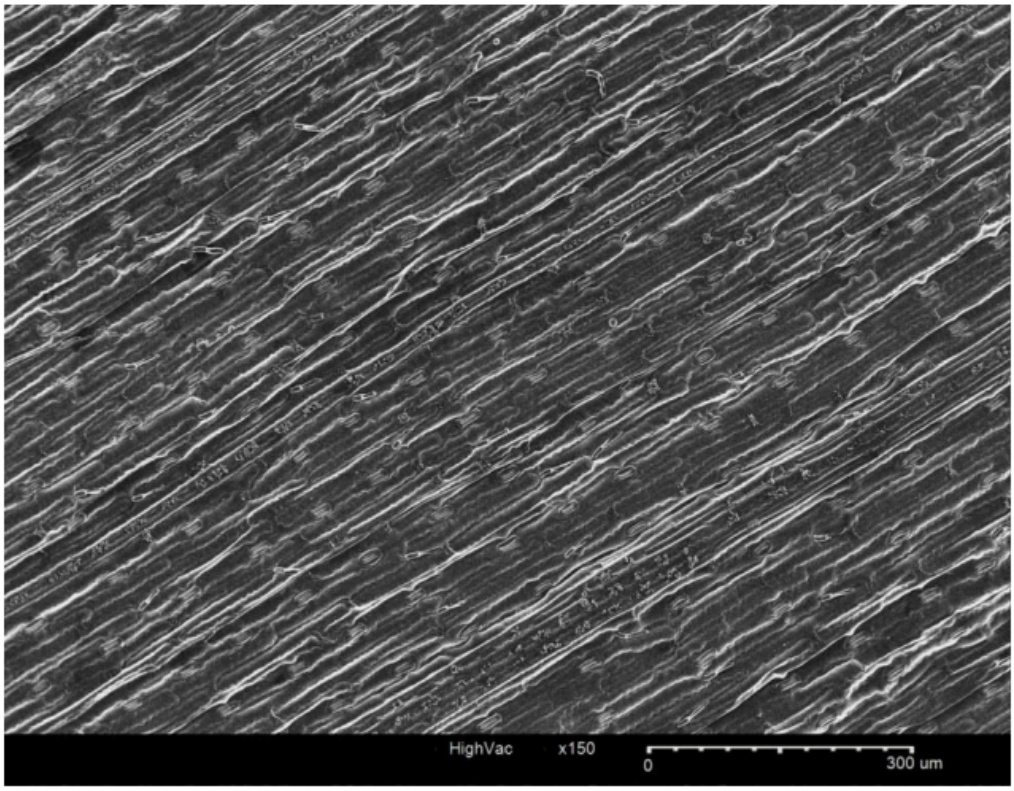


Figure 12. Host plant defense traits examined for total wax content post infestation by three different sugarcane aphid populations and compared with the control plants (ANOVA, d.f. = 2, $p < 0.0001$). (inset electron micrograph image of leaf surface that shows stomates, trichomes and wax at 150X magnification captured using a tabletop DSEM (SNE 450; Watts et al., 2022) The different lowercase alphabetical letters indicate statistically significant differences at $p < 0.05$. (DSEM Image by Dr. Rupesh Kariyat).

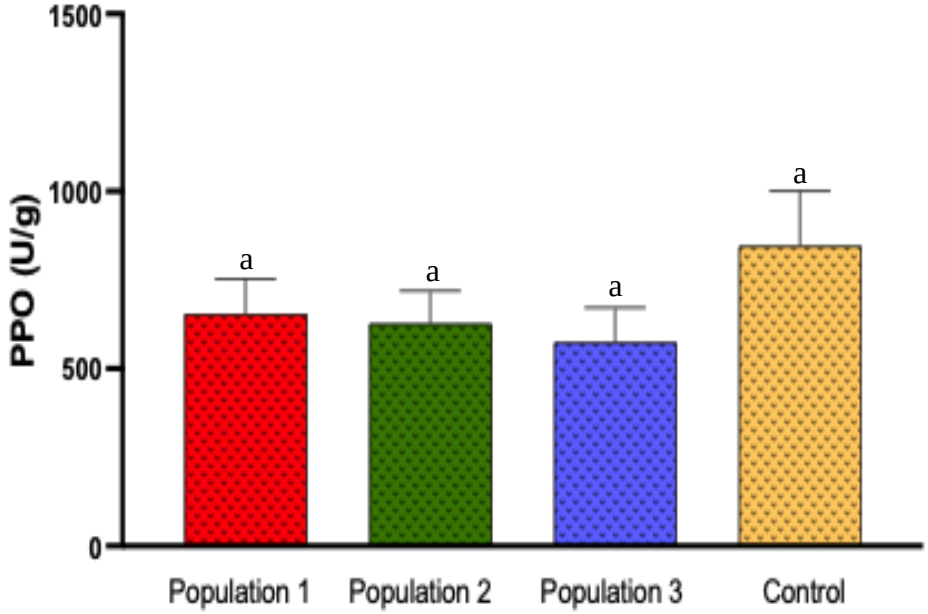


Figure 13. Host plant defense traits examined for total polyphenol oxidase (PPO) activity post infestation by three different sugarcane aphid populations and compared with the control plants (ANOVA, d.f. = 2, $p = 0.3613$). The different lowercase alphabetical letters indicate statistically significant differences at $p < 0.05$.

CHAPTER IV

CONCLUSIONS AND FUTURE DIRECTIONS

This thesis incorporates in-depth information about the concept of biotype, aphids and their existing biotypes and importance of studying them. Furthermore, this thesis also examined how sugarcane aphids and their possible differences that exist among different populations could potentially lead to biotype development. Sugarcane aphids are invasive pest of sugarcane and sorghum all around the world and thus, understanding their population level differences in terms of their biology, life history traits, feeding behavior and host plant defense responses is an important aspect for the development of sustainable control measures against them.

The results from the life history traits experiment show that there are significant differences in the net reproductive rate and intrinsic rate of increase among the three sugarcane aphid populations whereas populations were not significantly different for the cohort generation time. One of the sugarcane aphid populations (population 3) had significantly lower number of offspring and hence significantly low net reproductive rate and intrinsic rate of increase. This indicates that there exists difference in the reproductive traits among the different sugarcane aphid populations. The feeding behavior assessment experiment was done to assess the time spent by each sugarcane aphid populations in the different feeding phases including non-probing phase, pathway phase, xylem phase, phloem phase and number of potential drops. However, the results indicate no significant differences in the time spent by the three different sugarcane aphid

populations in each of the five phases studied. While there were variations in the amount of time spent in each phase by the three different populations, those variations weren't sufficient to indicate significant differences in the result. We also studied host plant defense mechanisms through the assessment of total wax content and total polyphenol oxidase (PPO) activity post infestation by three sugarcane aphid populations and compared it with the control plants. We observed significant difference in the total wax content of sorghum-sudangrass infested by the three sugarcane aphid populations and the control plants where plants infested by population 1 had significantly higher total wax content. However, no significant difference was observed in the total PPO activity between the control plants and plants infested with three sugarcane aphid populations.

Collectively, our results show evidence to support that there are differences in the three sugarcane aphid populations, however, these differences are not adequate to consider these three sugarcane aphid populations as biotypes. While we did not observe significant differences in all the variables we hypothesized and examined, the differences we observed are important and needs further exploration to conclude the presence of different biotypes of sugarcane aphid in the Rio Grande Valley, Texas. The differences we observed in the life history traits are strong evidence that the reproductive behavior of different sugarcane aphid populations is different despite feeding on same host plant on same environmental conditions. This result should be further explored, and reproductive behavior should be examined on the various host plants mainly susceptible and resistant sorghum cultivars. This information will assist us in the development of sugarcane aphid management strategies and further, the development of resistant plant cultivars so that there won't be a sudden surprising outbreak of new sugarcane aphid biotype. It has been found that temperature has large effect on the development and reproduction

of sugarcane aphids (Peña-Martínez *et al.* 2018). Hence, the development and reproduction of different sugarcane aphid populations and traits beyond growth and development, including dispersal, and reproductive traits (Kariyat and Portman, 2016) should be studied to investigate how different populations behave under different temperatures. Similarly, the feeding behavior assessment of sugarcane aphid populations should be studied in other host plants such as sugarcane, Johnsongrass, Columbus grass and various susceptible and resistant sorghum cultivars. Also, the feeding behavior of different sugarcane aphid populations should be examined in different plant growth stages and environmental conditions. Furthermore, plant defense experiments need qualitative assessment of wax content to examine the chemical compounds found in wax of sorghum and sorghum-sudangrass and studies should be done to determine the change in those chemical compounds in response to sugarcane aphid feeding. Detailed study of secondary metabolites like terpenes, phytanes, flavonoids, etc. found in sorghum (Kariyat et al., 2019) and sorghum-sudangrass should be done and how their level and signaling increase or decrease after sugarcane aphid infestation should be examined. Also, the research on biology, feeding behavior and host plant response should further be complemented with molecular analysis and morphological characterization.

REFERENCES

Agarwala B (2006) Phenotypic plasticity in aphids (Homoptera: Insecta): Components of variation and causative factors. Curr Sci 93:308–313.

Aliyu H, Ishiyaku MF (2013) Identification of Novel Resistance Gene Sources to Cowpea Aphid (*Aphis craccivora* Koch) in Cowpea (*Vigna unguiculata* L.). *Pakistan J of Biological Sciences* **16**:743–746.

Alston FH, Briggs JB (1977) Resistance genes in apple and biotypes of *Dysaphis devecta*. *Annals of Applied Biology* **87**:75–81.

Alt J, Ryan-Mahmutagic M (2013) Soybean Aphid Biotype 4 Identified. *Crop Science* **53**:1491–1495.

Alvarez A, Tjallingii W, Garzo E, Vleeshouwers V, Dicke M, Vosman B (2006) Location of resistance factors in the leaves of potato and wild tuber-bearing Solanum species to the aphid *Myzus persicae*. *Entomologia Experimentalis et Applicata* **121**:145–157.

Ansari AK (1984) Biology of *Aphis craccivora* (Koch.) and Varietal Resistance of Cowpeas. University of Reading, Department of Agriculture and Horticulture.

Anstead JA, Burd JD, Shufran KA (2002) Mitochondrial DNA sequence divergence among *Schizaphis graminum* (Hemiptera: Aphididae) clones from cultivated and non-cultivated hosts: haplotype and host associations. Bulletin of Entomological Research 92:17–24.

Arend AJ van der (2003) The possibility of *Nasonovia ribisnigri* resistance breaking biotype development due to plant host resistance: a literature study. *Eucarpia leafy vegetables* 75–81.

Arendt AJM van der, Ester A, Schijndel JT van (1999) Developing an aphid resistant butterhead lettuce 'Dynamite'. Palacky University.

Armstrong JS, Paudyal S, Limaje A, Elliott N, Hoback W (2018) Plant Resistance in Sorghums to the Sugarcane Aphid (Hemiptera: Aphididae). *Journal of Entomological Science* **53**:478–485.

Auclair JL (1978) Biotypes of the pea aphid, *Acyrthosiphon pisum*, in relation to host plants and chemically defined diets. *Entomologia Experimentalis et Applicata* **24**:212–216.

Barzman M, Bàrberi P, Birch ANE, et al. (2015) Eight principles of integrated pest management. Agron Sustain Dev 35:1199–1215.

Basky Z (2003) Biotypic and pest status differences between Hungarian and South African populations of Russian wheat aphid, *Diuraphis noxia* (Kurdjumov) (Homoptera: Aphididae). *Pest Management Science* **59**:1152–1158.

Bayoumy MH, Perumal R, Michaud JP (2016) Comparative Life Histories of Greenbugs and Sugarcane Aphids (Hemiptera: Aphididae) Coinfesting Susceptible and Resistant Sorghums. *Journal of Economic Entomology* **109**:385–391.

Berg J van den, Pretorius AJ, Loggerenberg M van (2003) Effect of leaf feeding by *Melanaphis sacchari* (Zehntner) (Homoptera: Aphididae), on sorghum grain quality. South African Journal of Plant and Soil 20:41–43.

Berger PH, Zeyen RJ (1987) Effects of sustained immobilisation on aphids. Annals of Applied Biology 111:247–256.

Birch L (1948) The intrinsic rate of natural increase of an insect population. *The Journal of Animal Ecology* 15–26.

Birch A, Jones A, Fenton B, *et al.* (2002) Resistance-breaking raspberry aphid biotypes: constraints to sustainable control through plant breeding. *Acta Horticulturae*.

Blackman RL, Eastop VF (2000) Aphids on the world's crops: an identification and information guide. 2. ed. Chichester Weinheim: Wiley.

Bollard E (1960) Transport in the xylem. *Annual Review of Plant Physiology* **11**:141–166.

Boller EF, Prokopy RJ (1976) Bionomics and Management of Rhagoletis. Annual Review of Entomology 21:223–246.

Bowling RD, Brewer MJ, Kerns DL, *et al.* (2016) Sugarcane Aphid (Hemiptera: Aphididae): A New Pest on Sorghum in North America. *J Integr Pest Manag* **7**:12.

Bowling R, Brewer M, Knutson A, Biles S, Way M, Sekula-Ortiz D (2016) Scouting sugarcane aphids in south, central, and west Texas. *NTO-043 Texas A&M AgriLife Extension Service, College Station, TX Accessed on* **21**.

Brévault T, Carletto J, Linderme D, Vanlerberghe-Masutti F (2008) Genetic diversity of the cotton aphid *Aphis gossypii* in the unstable environment of a cotton growing area. Agricultural and Forest Entomology 10:215–223.

Brewer MJ, Gordy JW, Kerns DL, Woolley JB, Rooney WL, Bowling RD (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.

Briggs JB (1959) Three new Strains of *Amphorophora rubi* (Kalt.) on cultivated Raspberries in England. *Bulletin of Entomological Research* **50**:81–87.

Briggs JB (1965) The Distribution, Abundance, and Genetic Relationships of Four Strains of the Rubus Aphid (*Amphorophora rubi* (Kalt.)) in Relation To Raspberry Breeding. *Journal of Horticultural Science* **40**:109–117.

Brodersen CR, Roddy AB, Wason JW, McElrone AJ (2019) Functional status of xylem through time. *Annual review of plant biology* **70**:407–433.

Campbell BC, Mclean DL, Kinsey MG, Jones KC, Dreyer DL (1982) Probing behavior of the greenbug (*Schizaphis graminum*, biotype c) on resistant and susceptible varieties of sorghum. Entomologia Experimentalis et Applicata 31:140–146.

Cardona JB, Grover S, Bowman MJ, *et al.* (2023) Sugars and cuticular waxes impact sugarcane aphid (*Melanaphis sacchari*) colonization on different developmental stages of sorghum. *Plant Science* **330**:111646.

Cardona JB, Grover S, Busta L, Sattler SE, Louis J (2023) Sorghum cuticular waxes influence host plant selection by aphids. *Planta* **257**:22.

Carletto J, Lombaert E, Chavigny P, Brévault T, Lapchin L, Vanlerberghe-Masutti F (2009) Ecological specialization of the aphid (*Aphis gossypii*) Glover on cultivated host plants. Molecular Ecology 18:2198–2212.

Cartier JJ (1959) Recognition of Three Biotypes of the Pea Aphid from Southern Quebec. *Journal of Economic Entomology* **52**:293–294.

Cartier JJ, Painter RH (1956) Differential Reactions of Two Biotypes of the Corn Leaf Aphid to Resistant and Susceptible Varieties, Hybrids and Selections of Sorghums1. *Journal of Economic Entomology* **49**:498–508.

Chang C, Fang M, Tseng H (1982) Studies on the life history and varietal resistance in grain sorghum aphid, *Melanaphis sacchari* Zehntner in central Taiwan. *Chinese Journal of Entomology* **2**:70–81.

Chavana J, Singh S, Vazquez A, Christoffersen B, Racelis A, Kariyat RR (2021) Local adaptation to continuous mowing makes the noxious weed *Solanum elaeagnifolium* a superweed candidate by improving fitness and defense traits. *Scientific reports* **11**:6634.

Chester G. McWhorter, Paul RN, J. Clark Ouzts (1995) Bicellular Trichomes of Johnsongrass (*Sorghum halepense*) Leaves: Morphology, Histochemistry, and Function. *Weed Science* **43**:201–208.

Chi H, Su H-Y (2006) Age-Stage, Two-Sex Life Tables of *Aphidius gifuensis* (Ashmead) (Hymenoptera: Braconidae) and Its Host *Myzus persicae* (Sulzer) (Homoptera: Aphididae) with Mathematical Proof of the Relationship Between Female Fecundity and the Net Reproductive Rate. *Environmental Entomology* **35**:10–21.

Cholodkovsky N (1908) Zur Frage über die biologischen Arten. Biol Zentralbl 28:769–782.

Cid M, Ávila A, García A, Abad J, Fereres A (2012) New sources of resistance to lettuce aphids in *Lactuca* spp. *Arthropod-Plant Interactions* **6**:655–669.

Constabel CP, Barbehenn R (2008) Defensive roles of polyphenol oxidase in plants. *Induced plant resistance to herbivory* 253–270.

Converse R, DAUBENY HA, Stace-Smith R, Russell LM, Koch E, Wiggans S (1971) Search for biological races in *Amphorophora agathonica* Hottes on red raspberries. *Canadian Journal of Plant Science* **51**:81–85.

Costa A, Williams DG, Powell KS (2014) Discovery of three woolly apple aphid *Eriosoma lanigerum* (Hemiptera: Aphididae) biotypes in Australia: the role of antixenosis and antibiosis in apple tree resistance. *Austral Entomology* **53**:280–287.

Curvetto RO, Webster J (1998) Resistance mechanisms of PI 240675 rye to biotype F greenbug. *The Southwestern entomologist (USA)*.

Davis HN, Goolsby JA, Thomas DB, *et al.* (2020) Review of major crop and animal arthropod pests of South Texas. *Subtropical Agriculture and Environments* **71**:36.

Diehl SR, Bush GL (1984) An Evolutionary and Applied Perspective of Insect Biotypes. *Annu Rev Entomol* **29**:471–504.

Dixon AFG (1985) Structure of Aphid Populations. *Annu Rev Entomol* **30**:155–174.

Dixon AFG (1998) Aphid ecology: an optimization approach. 2nd ed. London Weinheim New York: Chapman & Hall.

Dossett M, Kempler C (2012) Biotypic Diversity and Resistance to the Raspberry Aphid *Amphorophora agathonica* in Pacific Northwestern North America. *J Amer Soc Hort Sci* **137**:445–451.

Douglas AE (2009) The microbial dimension in insect nutritional ecology. Functional Ecology 23:38–47.

Downie DA (2010) Baubles, Bangles, and Biotypes: A Critical Review of the use and Abuse of the Biotype Concept. Journal of Insect Science 10:1–18.

Du J, Zhan Q, Huang B, *et al.* (2018) Biological characteristics and life table parameters of the sorghum aphid (*Melanaphis sacchari*) on different sorghum and *Sorghum bicolor*× *Sorghum sudanense* cultivars. *J Yunnan Agric University (Natural Science)* **33**:191–197.

Dunn JA, Kempton DPH (1972) Resistance to attack by *Brevicoryne brassicae* among plants of Brussels sprouts. *Ann Applied Biology* **72**:1–11.

Eastop VF (1972) Deductions from the present day host plants of aphids and related insects. *Roy Entomol Soc London Symp*.

Eigenbrode S, Castagnola T, Roux M, Steljes L (1996) Mobility of three generalist predators is greater on cabbage with glossy leaf wax than on cabbage with a wax bloom. *Entomologia Experimentalis et Applicata* **81**:335–343.

Emden HF van, Eastop VF, Hughes RD, Way MJ (1969) The Ecology of *Myzus persicae*. *Annu Rev Entomol* **14**:197–270.

Esquivel IL, Faris AM, Brewer MJ (2021) Sugarcane aphid, *Melanaphis sacchari* (Hemiptera: Aphididae), abundance on sorghum and johnsongrass in a laboratory and field setting. *Crop Protection* **148**:105715.

Fajemisin A, Racelis A, Kariyat R (2023) Cascading Effects of Cover Crops on the Subsequent Cash Crop Defense against the Polyphagous Herbivore Fall Armyworm (*Spodoptera frugiperda*). *Insects* **14**:177.

Fargo WS, Inayatullah C, Webster JA, Holbert D (1986) Morphometric variation within apterous females of *Schizaphis graminum* biotypes. Res Popul Ecol 28:163–172.

Ferrari J, Godfray HCJ, Faulconbridge AS, Prior K, Via S (2006) Population differentiation and genetic variation in host choice among pea aphids from eight host plant genera. Evolution 60:1574–1584.

Ferrari J, Via S, Godfray HCJ (2008) Population Differentiation and Genetic Variation in Performance on Eight Hosts in the Pea Aphid Complex. Evolution 62:2508–2524.

Foster J, Gallun R (1973) Control of Hessian fly race B on resistant wheat by the release of a dominant avirulent race.

Foster J, Lafayette W (1976) Current status of genetic control of Hessian fly populations with the dominant great plains race. 157–163.

Frantz A, Plantegenest M, Mieuzet L, Simon J-C (2006) Ecological specialization correlates with genotypic differentiation in sympatric host-populations of the pea aphid. Journal of Evolutionary Biology 19:392–401.

Frazer B (1972) Population dynamics and recognition of biotypes in the pea aphid (Homoptera: Aphididae). *The Canadian Entomologist* **104**:1729–1733.

Gallun RL (1978) Genetics of Biotypes B and C of the Hessian Fly1. *Annals of the Entomological Society of America* **71**:481–486.

Gallun RL, GS K (1980) Genetic factors affecting expression and stability of resistance.

Goggin FL, Williamson VM, Ullman DE (2001) Variability in the Response of *Macrosiphum euphorbiae* and *Myzus persicae* (Hemiptera: Aphididae) to the Tomato Resistance Gene Mi. *Environ Entomol* **30**:101–106.

Gordon S, Woodford J, Birch A (1997) Arthropod pests of Rubus in Europe: pest status, current and future control strategies. *Journal of Horticultural Science* **72**:831–862.

Gordy JW, Brewer MJ, Bowling RD, *et al.* (2019) Development of Economic Thresholds for Sugarcane Aphid (Hemiptera: Aphididae) in Susceptible Grain Sorghum Hybrids. *Journal of Economic Entomology* **112**:1251–1259.

Gorur G, Lomonaco C, Mackenzie A (2005) Phenotypic plasticity in host-plant specialisation in *Aphis fabae*. *Ecological Entomology* **30**:657–664.

Gould WR, Nichols JD (1998) Estimation of Temporal Variability of Survival in Animal Populations. Ecology 79:2531–2538.

Granett J, Walker MA, Kocsis L, Omer AD (2001) Biology and Management of Grape Phylloxera. *Annu Rev Entomol* **46**:387–412.

Gray SM, Smith DM, Barbierri L, Burd J (2002) Virus Transmission Phenotype Is Correlated with Host Adaptation Among Genetically Diverse Populations of the Aphid *Schizaphis graminum*. Phytopathology® 92:970–975.

Grayson J, Gardner S, Stephens M (2015) *Building Better Models with JMP Pro*. SAS Institute.

Grover S, Puri H, Xin Z, Sattler SE, Louis J (2022) Dichotomous Role of Jasmonic Acid in Modulating Sorghum Defense Against Aphids. *MPMI* **35**:755–767.

Guerrieri E, Digilio MC (2008) Aphid-plant interactions: a review. J of Plant Interactions 3:223–232.

Gupta GS, Miles P (1975) Studies on the susceptibility of varieties of apple to the feeding of two strains of woolly aphis (Homoptera) and relation to the chemical content of the tissues of the host. *Australian Journal of Agricultural Research* **26**:157–168.

Haley SD, Peairs FB, Walker CB, Rudolph JB, Randolph TL (2004) Occurrence of a new Russian wheat aphid biotype in Colorado. *Crop science* **44**:1589–1592.

Hall R (1977) The potential of the fungus, *Verticillium lecanii* as a control agent of glasshouse aphid pests.

Hall D (1987) The sugarcane aphid, *Melanaphis sacchari*. Florida sugarcane J Am Soc Sugar Cane Technol 7:26–29.

Harrington CD (1943) The Occurrence of Physiological Races of the Pea Aphid. *Journal of Economic Entomology* **36**:118–119.

Harris-Shultz K, Armstrong J, Jacobson A (2020) Invasive cereal aphids of North America: Biotypes, genetic variation, management, and lessons learned.

Harris-Shultz K, Ni X, Wadl PA, et al. (2017) Microsatellite Markers Reveal a Predominant Sugarcane Aphid (Homoptera: Aphididae) Clone is Found on Sorghum in Seven States and One Territory of the USA. Crop Science 57:2064–2072.

Harvey T, Hackerott H (1969a) Recognition of a greenbug biotype injurious to sorghum. *Journal of Economic Entomology* **62**:776–779.

Harvey TL, Hackerott HL (1969b) Plant Resistance to a Greenbug Biotype Injurious to Sorghum12. *Journal of Economic Entomology* **62**:1271–1274.

Hatchett J, Gallun R (1967) Genetic control of the Hessian fly. 100–1.

Hentz M, Nuessly G (2004) Development, Longevity, and Fecundity of *Sipha flava* (Homoptera: Aphididae) Feeding on Sorghum bicolor. *Environmental Entomology* **33**:546–553.

Hill CB, Crull L, Herman TK, Voegtlin DJ, Hartman GL (2010) A New Soybean Aphid (Hemiptera: Aphididae) Biotype Identified. *ec* **103**:509–515.

Hogenhout SA, Ammar E-D, Whitfield AE, Redinbaugh MG (2008) Insect Vector Interactions with Persistently Transmitted Viruses. Annu Rev Phytopathol 46:327–359.

Howe GA, Jander G (2008) Plant immunity to insect herbivores. *Annu Rev Plant Biol* **59**:41–66.

Hoy MA, McKelvey JJ (1979) Genetics in Relation to Insect Management: A Rockefeller Foundation Conference, March 31-April 5, 1978, Bellagio, Italy. Rockefeller Foundation.

Huang X, Liu D, Gao S, Chen H (2013) Differential Performance of (*Sitobion avenae*) Populations From Both Sides of the Qinling Mountains Under Common Garden Conditions. env entom 42:1174–1183.

Hussain S, Hazarika GC (2014) Educational data mining using jmp.

Huxley J (2010) Evolution: the modern synthesis. Definitive ed. Cambridge, Mass: MIT Press.

Inayatullah C, Webster JA, Fargo WS (1987) Morphometric Variation in the Alates of Greenbug (Homoptera: Aphididae) Biotypes. Annals of the Entomological Society of America 80:306–311.

Jankielsohn A (2011) Distribution and diversity of Russian wheat aphid (Hemiptera: Aphididae) biotypes in South Africa and Lesotho. *Journal of economic entomology* **104**:1736–1741.

Jean P, Jean-Christophe S (2010) The pea aphid complex as a model of ecological speciation. Ecological Entomology 35:119–130.

Johnson Z, Kaur I, Castillo F, Kariyat R, Bandyopadhyay D (2023) *Aloe barbadensis* rinds employ physical and chemical defense mechanisms against insect herbivores with varying success. *Industrial Crops and Products* **194**:116347.

Kaplan I (2007) Inducible plant responses linking above- and below-ground herbivory: Ecological significance and underlying mechanisms. Ph.D. University of Maryland, College Park.

Kariyat RR, Gaffoor I, Sattar S, *et al.* (2019) Sorghum 3-Deoxyanthocyanidin Flavonoids Confer Resistance against Corn Leaf Aphid. *J Chem Ecol* **45**:502–514.

Kariyat RR, Hardison SB, De Moraes CM, Mescher MC (2017) Plant spines deter herbivory by restricting caterpillar movement. *Biology Letters* **13**:20170176.

Kaur J, Chavana J, Soti P, Racelis A, Kariyat R (2020) Arbuscular mycorrhizal fungi (AMF) influences growth and insect community dynamics in Sorghum-sudangrass (*Sorghum x drummondii*). *Arthropod-Plant Interactions* **14**:301–315.

Kaur J, Kariyat R (2020) Role of Trichomes in Plant Stress Biology. In J Núñez-Farfán and PL Valverde (eds). *Evolutionary Ecology of Plant-Herbivore Interaction*. Cham: Springer International Publishing, 15–35.

Kaur I, Kariyat R (2023) Trichomes mediate plant–herbivore interactions in two Cucurbitaceae species through pre- and post-ingestive ways. *J Pest Sci*, doi:10.1007/s10340-023-01611-x.

Kaur I, Watts S, Raya C, Raya J, Kariyat R (2022) Surface Warfare: Plant Structural Defenses Challenge Caterpillar Feeding. In RJ Marquis and S Koptur (eds). *Caterpillars in the Middle: Tritrophic Interactions in a Changing World*. Cham: Springer International Publishing, 65–92.

Keep E, Knight RL (1967) A new gene from *Rubus occidentalis* L. For resistance to strains 1, 2, and 3 of the rubus aphid, *Amphorophora rubi* Kalt. *Euphytica* **16**:209–214.

Keep E, Knight R, Parker J (1970) Further data on resistance to the rubus aphid *Amphorophora rubi* Kltb. *Further data on resistance to the rubus aphid Amphorophora rubi Kltb* **199**.

Kennedy G, McLean D, Kinsey M (1978) Probing behavior of *Aphis gossypii* on resistant and susceptible muskmelon. Journal of Economic Entomology 71:13–16.

Kerns D, Brown S, Beuzelin J, Guidry K (2015) Sugarcane aphid: a new invasive pest of sorghum. *Louisiana Agriculture* **58**:12–14.

Khanal N, Vitek C, Kariyat R (2023) The Known and Unknowns of Aphid Biotypes, and Their Role in Mediating Host Plant Defenses. *Diversity* **15**:186.

Kim K, Hill CB, Hartman GL, Mian MR, Diers BW (2008) Discovery of soybean aphid biotypes. *Crop Science* **48**:923–928.

Kindler SD, Harvey TL, Wilde GE, Shufran RA, Brooks HL, Sloderbeck PE (2001) Occurrence of greenbug biotype K in the field. *J Agric Urban Entomol* **18**:23–34.

Kindler S, Hays D (1999) Susceptibility of cool-season grasses to greenbug biotypes. *J Agric Urban Entomol* **16**:235–243.

Kindler S, Spomer S (1986) Biotypic status of six greenbug (Homoptera: Aphididae) isolates. *Environmental entomology* **15**:567–572.

Kiriac I, Gruber F, Poprawski T, Halbert S, Elberson L (1990) Occurrence of sexual morphs of Russian wheat aphid, *Diuraphis noxia* (Homoptera: Aphididae), in several locations in the Soviet Union and the northwestern United States. *Proceedings of the Entomological Society of Washington* **92**:544–547.

Knight R, Briggs J, Keep E (1960) Genetics of resistance to *Amphorophora rubi* (Kalt.) in the raspberry II. The genes A2–A7 from the American variety, Chief. *Genetics Research* **1**:319–331.

Kowalski SP, Eannetta NT, Hirzel AT, Steffens JC (1992) Purification and characterization of polyphenol oxidase from glandular trichomes of *Solanum berthaultii*. *Plant Physiology* **100**:677–684.

Kring JB (1959) The life cycle of the melon aphid, *Aphis gossypii* Glover, an example of facultative migration. Annals of the Entomological Society of America 52:284–286.

Kumari A, Goyal M, Mittal A, Kumar R (2022) Defensive capabilities of contrasting sorghum genotypes against *Atherigona soccata* (Rondani) infestation. *Protoplasma* **259**:809–822.

Kusi F, Obeng-Ofori D, Asante S, Padi F (2010) New Sources of Resistance in Cowpea to the Cowpea Aphid (*Aphis craccivora* Koch) (Homoptera: Aphididae). *Journal of the Ghana Science Association* **12**:95–104.

Lammerink J (1968) A new biotype of cabbage aphid (*Brevicoryne brassicae* (L.)) on Aphid Resistant rape (*Brassica napus* L.). *New Zealand Journal of Agricultural Research* **11**:341–344.

Lehman WF, Stanford EH, Nielson MW, *et al.* (1971) Registration of C937 Parental Clone of ALfalfa (Registration No. PL 3) [1]. *Crop Sci* **11**:142–142.

Lei W, Li P, Han Y, Gong S, Yang L, Hou M (2016) EPG Recordings Reveal Differential Feeding Behaviors in *Sogatella furcifera* in Response to Plant Virus Infection and Transmission Success. *Sci Rep* **6**:30240.

Limaje A, Hayes C, Armstrong JS, *et al.* (2018) Antibiosis and Tolerance Discovered in USDA-ARS Sorghums Resistant to the Sugarcane Aphid (Hemiptera: Aphididae)1. *ents* **53**:230–241.

Lopes-da-Silva M, Rocha DA (2014) Potential population growth of *Melanaphis sacchari* (Zethner) reared on sugarcane and sweet sorghum. *Current Agricultural Science and Technology* 5.

Louis J, Gobbato E, Mondal HA, Feys BJ, Parker JE, Shah J (2012) Discrimination of Arabidopsis PAD4 Activities in Defense against Green Peach Aphid and Pathogens. *Plant Physiology* **158**:1860–1872.

Lowe H (1981) Resistance and susceptibility to colour forms of the aphid *Sitobion avenue* in spring and winter wheats (*Triticum aestivum*). *Annals of Applied Biology* **99**:87–98.

Markkula M, Roukka K (1970) Resistance of plants to the pea aphid *Acyrthosiphon pisum* Harris (Hom., Aphididae). I. Fecundity of the biotypes on different host plants. undefined.

Martinez LM (2020) Examining the Efficacy of Cover Crops as an Integrated Pest Management Tool in Organic Farms in the Lower Rio Grande Valley.

Maxwell FG, Jennings PR (eds) (1980) Breeding plants resistant to insects. New York: Wiley.

Mayr E (1999) Systematics and the origin of species, from the viewpoint of a zoologist. 1st Harvard University Press pbk. ed. Cambridge, Mass: Harvard University Press.

McLEAN DL, Kinsey MG (1964) A Technique for Electronically Recording Aphid Feeding and Salivation. *Nature* **202**:1358–1359.

Mclean DL, Kinsey MG (1965) Identification of Electrically Recorded Curve Patterns associated with Aphid Salivation and Ingestion. *Nature* **205**:1130–1131.

Mead F (1978) Sugarcane aphid, *Melanaphis sacchari* (Zehntner)-Florida-new continental United States record. Coop Plant Pest Rep 3:475.

Meier W (1958) Beiträge zur Kenntnis der auf Papilionaceen lebenden *Acyrthosiphon*-Arten (Hemipt. Aphid.). Impimerie la Concorde.

Merrill SC, Peairs FB, Miller HR, Randolph TL, Rudolph JB, Talmich EE (2014) Reproduction and development of Russian wheat aphid Biotype 2 on crested wheatgrass, intermediate wheatgrass, and susceptible and resistant wheat. *Journal of economic entomology* **101**:541–545.

Michel AP, Mittapalli O, Mian MR, Sudaric A (2011) Evolution of soybean aphid biotypes: understanding and managing virulence to host-plant resistance. *Soybean-molecular aspects of breeding InTech, New York* 355–372.

Miller GL, Favret C, Carmichael A, Voegtlin DJ (2009) Is There a Cryptic Species Within (*Aulacorthum solani*) (Hemiptera: Aphididae)? *ec* **102**:398–400.

Milne WM (1998a) Suitability of clovers (Trifolium species and cultivars) as hosts of spotted clover aphid, a biotype of *Therioaphis trifolii* (Monell) (Hemiptera : Aphididae). *Aust J Exp Agric* **38**:241–245.

Milne WM (1998b) Comparative performance of two biotypes of *Therioaphis trifolii* (Monell) (Hemiptera: Aphididae) on clovers (Trifolium) and medics (Medicago). *Australian Journal of Entomology* **37**:350–355.

Miura T, Braendle C, Shingleton A, Sisk G, Kambhampati S, Stern DL (2003) A comparison of parthenogenetic and sexual embryogenesis of the pea aphid *Acyrthosiphon pisum* (Hemiptera: Aphidoidea). J Exp Zool 295B:59–81.

Mondor EB, Rosenheim JA, Addicott JF (2005) Predator-induced transgenerational phenotypic plasticity in the cotton aphid. Oecologia 142:104–108.

Montllor CB, Campbell BC, Mittler T (1983) Natural and induced differences in probing behavior of two biotypes of the greenbug, *Schizaphis graminum*, in relation to resistance in sorghum. *Entomologia experimentalis et applicata* **34**:99–106.

Morais Cardoso L de, Pinheiro SS, Martino HSD, Pinheiro-Sant'Ana HM (2017) Sorghum (*Sorghum bicolor* L.): Nutrients, bioactive compounds, and potential impact on human health. Critical reviews in food science and nutrition 57:372–390.

Moran NA (1992) The Evolution of Aphid Life Cycles. Annu Rev Entomol 37:321–348.

Moran NA, McCutcheon JP, Nakabachi A (2008) Genomics and Evolution of Heritable Bacterial Symbionts. Annu Rev Genet 42:165–190.

Musetti L, Neal JJ (1997) Toxicological effects of *Lycopersicon hirsutum* f. *glabratum* and behavioral response of *Macrosiphum euphorbiae*. *Journal of Chemical Ecology* **23**:1321–1332.

Mutti NS, Louis J, Pappan LK, *et al.* (2008) A protein from the salivary glands of the pea aphid, *Acyrthosiphon pisum*, is essential in feeding on a host plant. *Proceedings of the National Academy of Sciences* **105**:9965–9969.

Neupane SB, Kerns DL, Szczepaniec A (2020) The impact of sorghum growth stage and resistance on life history of sugarcane aphids (Hemiptera: Aphididae). *Journal of economic entomology* **113**:787–792.

Nibouche S, Costet L, Holt JR, *et al.* (2018) Invasion of sorghum in the Americas by a new sugarcane aphid (*Melanaphis sacchari*) superclone. B-S Yue (ed). *PLoS ONE* **13**:e0196124.

Nibouche S, Costet L, Medina RF, *et al.* (2021) Morphometric and molecular discrimination of the sugarcane aphid, *Melanaphis sacchari*, (Zehntner, 1897) and the sorghum aphid *Melanaphis sorghi* (Theobald, 1904). *PLOS ONE* **16**:e0241881.

Nibouche S, Mississipi S, Fartek B, Delatte H, Reynaud B, Costet L (2015) Host Plant Specialization in the Sugarcane Aphid *Melanaphis sacchari*. OR Edwards (ed). *PLoS ONE* **10**:e0143704.

Nielson MW, Don H (1974) A New Virulent Biotype of the Spotted Alfalfa Aphid in Arizona12. Journal of Economic Entomology 67:64–66.

Nielson MW, Lehman WF, Marble VL (1970) A New Severe Strain of the Spotted Alfalfa Aphid in California12. *Journal of Economic Entomology* **63**:1489–1491.

Nielson MW, Schonhorst MH, Don H, Lfhman WF, Marble VL (1971) Resistance in Alfalfa to Four Biotypes of the Spotted Alfalfa Aphid1. *Journal of Economic Entomology* **64**:506–510.

Nosil P (2004) Reproductive isolation caused by visual predation on migrants between divergent environments. Proc R Soc Lond B 271:1521–1528.

Nosil P (2012) Ecological speciation. Oxford ; New York: Oxford University Press.

Oliver KM, Degnan PH, Burke GR, Moran NA (2010) Facultative Symbionts in Aphids and the Horizontal Transfer of Ecologically Important Traits. Annu Rev Entomol 55:247–266.

Padmaja PG, Shwetha BL, Swetha G, Patil JV (2014) Oxidative Enzyme Changes in Sorghum Infested by Shoot Fly. *Journal of Insect Science* **14**:193.

Painter RH (1951) Insect Resistance in Crop Plants: Soil Science 72:481.

Painter R, Pathak M (1962) The distinguishing features and significance of the four biotypes of the corn leaf aphid, *Rhopalosiphum maidis* (Fitch). *Verhandlungen 11th int Kongr Ent* **2**.

Panda N, Khush GS (1995) *Host plant resistance to insects.* CAB international.

Pathak RS (1991) Plant genetics in pest management. *International Journal of Tropical Insect Science* **12**:553–564.

Paudel S, Lin P-A, Foolad MR, Ali JG, Rajotte EG, Felton GW (2019) Induced Plant Defenses Against Herbivory in Cultivated and Wild Tomato. J Chem Ecol 45:693–707.

Paudyal S, Armstrong JS, Giles KL, Hoback W, Aiken R, Payton ME (2020) Differential responses of sorghum genotypes to sugarcane aphid feeding. *Planta* **252**:14.

Paudyal S, Armstrong JS, Giles KL, Payton ME, Opit GP, Limaje A (2019) Categories of Resistance to Sugarcane Aphid (Hemiptera: Aphididae) Among Sorghum Genotypes. *Journal of Economic Entomology* **112**:1932–1940.

Paudyal S, Armstrong JS, Harris-Shultz KR, *et al.* (2019) Evidence of host plant specialization among the U.S. sugarcane aphid (Hemiptera: Aphididae) genotypes. 12.

Peccoud J, Mahéo F, De La Huerta M, Laurence C, Simon J (2015) Genetic characterisation of new host-specialised biotypes and novel associations with bacterial symbionts in the pea aphid complex. *Insect Conservation and Diversity* **8**:484–492.

Pekarcik AJ, Jacobson AL (2021) Evaluating Sugarcane Aphid, *Melanaphis sacchari* (Hemiptera: Aphididae), Population Dynamics, Feeding Injury, and Grain Yield Among Commercial Sorghum Varieties in Alabama. *Journal of Economic Entomology* **114**:757–768.

Peña-Martínez R, Muñoz-Viveros AL, Bujanos-Muñiz R, Luévano-Borroel J, Tamayo-Mejia F, Cortez-Mondaca E (2016) Formas Sexuales del Complejo Pulgón Amarillo del Sorgo, *Melanaphis sacchari*/*sorghi*1 en México. *swen* **41**:127–132.

Peña-Martínez R, Muñoz-Viveros AL, Marín-Jarillo A, *et al.* (2018) Spontaneously Aborted Embryos in the Sugarcane Aphid (Hemiptera: Aphididae). *Annals of the Entomological Society of America* **111**:312–318.

Pollard DG (1973) Plant penetration by feeding aphids (Hemiptera, Aphidoidea): a review. Bull Entomol Res 62:631–714.

Porter DR, Burd JD, Shufran KA, Webster JA, Teetes GL (1997) Greenbug (Homoptera: Aphididae) biotypes: selected by resistant cultivars or preadapted opportunists? *Journal of Economic Entomology* **90**:1055–1065.

Porter K, Peterson G, Vise O (1982) A New Greenbug Biotype 1. *Crop Science* **22**:847–850.

Powell G, Maniar SP, Pickett JA, Hardie J (1999) Aphid responses to non-host epicuticular lipids. In SJ Simpson, AJ Mordue, and J Hardie (eds). *Proceedings of the 10th International Symposium on Insect-Plant Relationships*. Dordrecht: Springer Netherlands, 115–123.

Powell G, Tosh CR, Hardie J (2006) Host plant selection by aphids: Behavioral, Evolutionary, and Applied Perspectives. Annu Rev Entomol 51:309–330.

Quisenberry SS, Ni X (2007) 13 Feeding Injury. *Aphids as crop pests* 331.

Rat Morris E, Crowther S, Guessoum M (1999) Resistance-breaking biotypes of rosy apple aphid, *Dysaphis plantaginea*, on the resistant cultivar 'Florina'. *IOBC WPRS Bulletin*.

Rausher MD (2001) Co-evolution and plant resistance to natural enemies. Nature 411:857–864.

Reddy KS (1988) Assessment of on-farm yield losses in sorghum due to insect pests. International Journal of Tropical Insect Science 9:679–685.

Reddy PS (2017) Sorghum, *Sorghum bicolor* (L.) Moench. Millets and Sorghum: Biology and Genetic Improvement 1–32.

Rosenheim JA, Wilhoit LR, Colfer RG (1994) Seasonal biology and polymorphism of the cotton aphid, *Aphis gossypii* in California. Beltwide Cotton Conferences (USA).

Rott P, Mirkov TE, Schenck S, Girard JC (2008) Recent advances in research on Sugarcane yellow leaf virus, the causal agent of sugarcane yellow leaf. Sugar Cane International 26:18–27.

Russell GE (ed) (1978) Studies in the Agricultural and Food Sciences. Plant Breeding for Pest and Disease Resistance. Butterworth-Heinemann, ii.

Russell A (2021) *AgriLife Today*. https://agrilifetoday.tamu.edu/2021/08/17/sugarcane-aphid-numbers-under-control-so-far/ (3 March 2023, date last accessed).

Sall J, Stephens ML, Lehman A, Loring S (2017) *JMP Start Statistics: A Guide to Statistics and Data Analysis Using JMP, Sixth Edition*. SAS Institute.

Saxena RC, Barrion AA (1987) Biotypes of insect pests of agricultural crops. *Int J Trop Insect Sci* **8**:453–458.

Saxena PX, Chada HL (1971) The Greenbug, *Schizaphis graminum*.1 1. Mouth Parts and Feeding Habits2. Annals of the Entomological Society of America 64:897–904.

Saxena RC, Rueda LM (1982) Morphological variations among three biotypes of the brown planthopper *Nilaparvata lugens* in the Philippines. *Int J Trop Insect Sci* **3**:193–210.

Schuster D, Starks K (1975) Preference of *Lysiphlebus testaceipes* for greenbug resistant and susceptible small grain species. Environmental Entomology 4:887–888.

Schwarzkopf A, Rosenberger D, Niebergall M, Gershenzon J, Kunert G (2013) To Feed or Not to Feed: Plant Factors Located in the Epidermis, Mesophyll, and Sieve Elements Influence Pea Aphid's Ability to Feed on Legume Species. *PLOS ONE* **8**:e75298.

Scott Armstrong J, Rooney WL, Peterson GC, Villenueva RT, Brewer MJ, Sekula-Ortiz D (2015) Sugarcane Aphid (Hemiptera: Aphididae): Host Range and Sorghum Resistance Including Cross-Resistance From Greenbug Sources. *Journal of Economic Entomology* **108**:576–582.

Sen Gupta GC (1969) The recognition of biotypes of the woolly aphid, *Erisoma lanigerum* (Hausmann), in South Australia by their differential ability to colonise varieties of apple rootstock, and an investigation of some possible factors in the susceptibility of varieties to these insects.

Shanks CH, Chase D (1976) Electrical Measurement of Feeding by the Strawberry Aphid on Susceptible and Resistant Strawberries and Nonhost Plants. *Ann Entomol Soc Am* **69**:784–786.

Sharma H (1993) Host-plant resistance to insects in sorghum and its role in integrated pest management. Crop protection 12:11–34.

Sharma HC, Ortiz R (2002) Host plant resistance to insects: an eco-friendly approach for pest management and environment conservation. *J Environ Biol* **23**:111–135.

Sharma S, Rajan N, Cui S, et al. (2017) Seasonal variability of evapotranspiration and carbon exchanges over a biomass sorghum field in the Southern US Great Plains. Biomass and Bioenergy 105:392–401.

Simon J-C, Rispe C, Sunnucks P (2002) Ecology and evolution of sex in aphids. Trends in Ecology & Evolution 17:34–39.

Singh S, Kaur I, Kariyat R (2021) The Multifunctional Roles of Polyphenols in Plant-Herbivore Interactions. *International Journal of Molecular Sciences* **22**:1442.

Singh B, Padmaja P, Seetharama N (2004) Biology and management of the sugarcane aphid, *Melanaphis sacchari* (Zehntner) (Homoptera: Aphididae), in sorghum: a review. *Crop Protection* **23**:739–755.

Singh SR, Painter RH (1964) Effect of Temperature and Host Plants on Progeny Production of Four Biotypes of Corn Leaf Aphid, *Rhopalosiphum maidis*1. *Journal of Economic Entomology* **57**:348–350.

Smith CF (1941) A New Species of Hymenopterous Parasite of the Pea Aphid (*Macrosiphum pisi* Kaltenbach)1. Annals of the Entomological Society of America 34:537–538.

Smith CM (2005) *Plant resistance to arthropods: molecular and conventional approaches*. Dordrecht, The Netherlands: Springer.

Smith CM, Belay T, Stauffer C, Stary P, Kubeckova I, Starkey S (2004) Identification of Russian wheat aphid (Homoptera: Aphididae) populations virulent to the Dn4 resistance gene. *Journal of Economic Entomology* **97**:1112–1117.

Smith CM, Chuang W-P (2014) Plant resistance to aphid feeding: behavioral, physiological, genetic, and molecular cues regulate aphid host selection and feeding: Plant resistance to aphid feeding. Pest Manag Sci 70:528–540.

Sohi SS, Swenson KG (1964) Pea aphid biotypes differing in bean yellow mosaic virus transmission1. *Entomologia Experimentalis et Applicata* **7**:9–14.

Soti P, Racelis A (2020) https://link.springer.com/article/10.1007/s13165-020-00285-4 (7 February 2023, date last accessed).

Souza MF, Davis JA (2021) Characterizing Host Plant Resistance to *Melanaphis sacchari* (Hemiptera: Aphididae) in Selected Sorghum Plant Introductions. L Hesler (ed). *Journal of Economic Entomology* **114**:959–969.

Srinivasan R, Alvarez JM (2011) Specialized Host Utilization of (*Macrosiphum euphorbiae*) on a Nonnative Weed Host, (*Solanum sarrachoides*), and Competition With (*Myzus persicae*). *env entom* **40**:350–356.

Srivastava P, Auclair J (1978) Differential responses of biotypes of the pea aphid, *Acyrthosiphon pisum* (Harris), to a chemically defined diet. Canadian Journal of Zoology 56:2481–2485.

Stark WS, Chen D-M, Johnson MA, Frayer KL (1983) The rdgB gene in drosophila: Retinal degeneration in different mutant alleles and inhibition of degeneration by norpA. Journal of Insect Physiology 29:123–131.

Stern VM, Smith RF, Bosch R van den, Hagen KS (1959) The integration of chemical and biological control of the spotted alfalfa aphid: The integrated control concept. Hilg 29:81–101.

Sunnucks P, Driver F, Brown WV, Carver M, Hales DF, Milne WM (1997) Biological and genetic characterization of morphologically similar *Therioaphis trifolii* (Hemiptera: Aphididae) with different host utilization. *Bulletin of Entomological Research* **87**:425–436.

Taggar GK, Arora R (2017) Insect Biotypes and Host Plant Resistance. In R Arora and S Sandhu (eds). *Breeding Insect Resistant Crops for Sustainable Agriculture*. Singapore: Springer Singapore, 387–421.

Tayal M, Somavat P, Rodriguez I, Thomas T, Christoffersen B, Kariyat R (2020) Polyphenol-Rich Purple Corn Pericarp Extract Adversely Impacts Herbivore Growth and Development. *Insects* **11**:98.

Taylor JR, Schober TJ, Bean SR (2006) Novel food and non-food uses for sorghum and millets. Journal of cereal science 44:252–271.

Teetes G, Schaefer C, Gipson J, McIntyre R, Latham E (1975) Greenbug resistance to organophosphorous insecticides on the Texas High Plains. *Journal of Economic Entomology* **68**:214–216.

Thompson JN (2009) The coevolutionary process. The Coevolutionary Process. University of Chicago press.

Thorpe WH (1930) Biological Races in Insects and Allied Groups. Biological Reviews 5:177–212.

Thottappilly G, Bath JE, French JV (1972) Aphid transmission characteristics of pea enation mosaic virus acquired from a membrane-feeding system. Virology 50:681–689.

Tjallingii WF (1978) Electronic recording of penetration behaviour by aphids. *Entomologia Experimentalis et Applicata* **24**:721–730.

Tolmay V, Lindeque R, Prinsloo G (2007) Preliminary evidence of a resistance-breaking biotype of the Russian wheat aphid, *Diuraphis noxia* (Kurdjumov)(Homoptera: Aphididae), in South Africa s. *African Entomology* **15**:228–230.

Urbano B, Gonzalez-Andres F, Ballesteros A (2006) Allelopathic potential of cover crops to control weeds in barley. *Allelopathy Journal* **17**:53.

van Rensburg (1973) https://journals.co.za/doi/epdf/10.10520/AJA00128789_3446 (21 October 2022, date last accessed).

Vanlerberghe-Masutti F, Chavigny P (1998) Host-based genetic differentiation in the aphid *Aphis gossypii* Glover, evidenced from RAPD fingerprints. *Molecular Ecology* **7**:905–914.

Villanueva RT, Sekula D (2014) A New Pest of Sorghum: the Sugarcane Aphid. 32.

Villanueva R, Brewer M, Way M, *et al.* (2014) Sugarcane aphid: a new pest of sorghum. *Texas A&M Agrilife Extension, Ento-035 URL: http://denton agrilife org/files/2013/08/ENTO-035-The-Sugarcane-Aphid-2014 pdf*.

Wall RE (1933) A Study of Color and Color-variation in *Aphis gossypii* Glover: A Thesis.

Walsh BD (1864) On Phytophagic Varieties and Phytophagic Species.

Wang D, Liu D, Zhai Y, Zhang R, Shi X (2019) Clonal Diversity and Genetic Differentiation of *Sitobion avenae* (Hemiptera: Aphididae) From Wheat and Barley in China. *Journal of Economic Entomology* **112**:1217–1226.

Wang D, Zhai Y, Liu D, Zhang N, Li C, Shi X (2020) Identification and Genetic Differentiation of *Sitobion avenae* (Hemiptera: Aphididae) Biotypes in China. J Econ Entomol 113:407–417.

Wang Y, Zhang P, Chen J (2004) Host--preference biotypes of the cotton aphid, (*Aphis gossypii*) Glover and the behavioral mechanism in their formatio. *Kun Chong Xue Bao* **47**:760–767.

Wang L, Zhang S, Luo J-Y, *et al.* (2016) Identification of *Aphis gossypii* Glover (Hemiptera: Aphididae) Biotypes from Different Host Plants in North China. N Desneux (ed). *PLoS ONE* **11**:e0146345.

Watson MA, Okusanya BAM (1967) Studies on the transmission of groundnut rosette virus by *Aphis craccivora* Koch. *Ann Applied Biology* **60**:199–208.

Watt M, Hales DF (1996) Dwarf phenotype of the cotton aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae). Australian Journal of Entomology 35:153–159.

Watts S, Kariyat R (2022) Are epicuticular waxes a surface defense comparable to trichomes? A test using two Solanum species and a specialist herbivore. *Botany* cjb-2021-0206.

Weibel D, Starks K, Wood Jr E, Morrison R (1972) Sorghum Cultivars and Progenies Rated for Resistance to Greenbugs 1. Crop Science 12:334–336.

White W, Reagan T, Hall D (2001) *Melanaphis sacchari* (Homoptera: Aphididae), a sugarcane pest new to Louisiana. Florida Entomologist 435–435.

Wilde G, Feese H (1973) A New Corn Leaf Aphid Biotype and Its Effect on Some Cereal and Small Grains12. *Journal of Economic Entomology* **66**:570–571.

Williams IS, Dixon AFG (2007) Life cycles and polymorphism. In HF van Emden and R Harrington (eds). *Aphids as crop pests*. Wallingford: CABI, 69–85.

Wood Jr E (1961) Biological studies of a new greenbug biotype. *Journal of Economic Entomology* **54**:1171–1173.

Xu T-T, Ma T-T, Liu X-D (2014) How does the host-specialized aphid deal with food deficiency?: Host use of host-specialized aphid. *Insect Science* **21**:334–341.

Young E, Rock G, Zeiger D, Cummins J (1982) Infestation of some malus cultivars by the north-carolina woolly apple aphid biotype. *HortScience* **17**:787–788.

Yu J-Z, Chi H, Chen B-H (2005) Life Table and Predation of *Lemnia biplagiata* (Coleoptera: Coccinellidae) Fed on Aphis gossypii (Homoptera: Aphididae) with a Proof on Relationship Among Gross Reproduction Rate, Net Reproduction Rate, and Preadult Survivorship. *Annals of the Entomological Society of America* **98**:475–482.

Zapata SD, Dudensing R, Sekula D, Esparza-Díaz G, Villanueva R (2018) Economic Impact of the Sugarcane Aphid Outbreak in South Texas. *J Agric Appl Econ* **50**:104–128.

Zarrabi A, Berberet R, Caddel J (1995) New biotype of *Acyrthosiphon kondoi* (Homoptera: Aphididae) on alfalfa in Oklahoma. *Journal of Economic Entomology* **88**:1461–1465.

BIOGRAPHICAL SKETCH

Neetu Khanal, born on October 16, 1997, in Nepal, received her bachelor's in science in Agriculture (Hons.) from Tribhuvan University, Nepal in 2019. She received the most prestigious “Presidential Graduate Research Assistantship” award from The University of Texas Rio Grande Valley and started her master’s degree in Fall 2021. She worked under Dr. Christopher Vitek and Dr. Rupesh Kariyat and gained experience and skills in entomology, plant biology, ecology, and plant disease vector. She earned Master of Science in Biology from The University of Texas Rio Grande Valley, Texas, USA in May 2023. In the future, she aspires to continue her education, acquire a PhD, and become an Entomologist to contribute to managing insects and promoting sustainable agriculture. After graduating from UTRGV in Spring 2023, Neetu will join Penn State Entomology department to pursue her PhD in plant-insect interactions.

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