5-2020

# Effects of Arbuscular Mycorrhizal Fungi (AMF) on Growth and Herbivore Defenses in Sorghum Sudangrass (Sorghum X drummondii)

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## Recommended Citation
Kaur, Jasleen, "Effects of Arbuscular Mycorrhizal Fungi (AMF) on Growth and Herbivore Defenses in Sorghum Sudangrass (Sorghum X drummondii)" (2020). *Theses and Dissertations*. 487.
https://scholarworks.utrgv.edu/etd/487

# EFFECTS OF ARBUSCULAR MYCORRHIZAL FUNGI (AMF) ON GROWTH AND HERBIVORE DEFENSES IN SORGHUM SUDANGRASS

# (SORGHUM X DRUMMONDII)

A Thesis

by

JASLEEN KAUR

Submitted to the Graduate College of
The University of Texas Rio Grande Valley
In partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

May 2020

Major Subject: Biology

# EFFECTS OF ARBUSCULAR MYCORRHIZAL FUNGI (AMF) ON GROWTH AND HERBIVORE DEFENSES IN SORGHUM SUDANGRASS

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May 2020

# ABSTRACT

Kaur, Jasleen., Effects of arbuscular mycorrhizal fungi (AMF) on growth and herbivore defenses in Sorghum sudangrass (*Sorghum x drummondii*). Master of Science (MS), May 2020, 95pp., 2tables, 12figures, 214 references.

Chapter 2: In this chapter we have examined the role of trichomes in plant stress biology, reviewed the studies on herbivore X trichome interactions, and their role in plant defences. Ultimately, we have proposed new areas of research for future work.

Chapter 3: In this chapter, we examined whether AMF has cascading effects on insect community dynamics through attraction/repulsion of beneficial and damaging insects using Sorghum-sudangrass (*Sorghum x drummondii*), either inoculated with commercial AMF mix or left as control in lab and field experiments. Our results suggest positive effects of AMF on plant growth, and a lower initial incidence of fall armyworm (*Spodoptera frugiperda*), a major herbivore on Sorghum in the region. Besides, AMF inoculated plants attracted significantly more beneficial insects (predators and parasitoids) and a lower number of damaging herbivores. Therefore, our data suggests that AMF can have implications for sustainable pest management strategies.

# DEDICATION

I dedicate my thesis work to my father S. Gurmail Singh, mother Ms. Parminder Kaur and my brother Jasmeet Singh for their shared wisdom, love and constant support.

# ACKNOWLEDGMENTS

I owe a big thanks to the following people whose support and help has been incredible:

Firstly, I am sincerely grateful to my major advisor, Dr. Rupesh Kariyat.  The reason I can pursue my dreams in the United States is because he believed in my potential and provided me opportunity to begin my career in his lab. His expertise and valuable insight of the subject matter has guided me constantly throughout my journey. This thesis would not have been possible without his constant support and encouragement. It is for his incredible time investment in shaping my personality as a budding research scientist that has helped me improve leaps and bounds in my presentation and writing skills. His enthusiasm for research and data is equally contagious and encouraging to any student learning and practicing science under his guidance (*Thanks for bearing with me, Dr Kariyat!).*

I also thank Dr Teresa P. Feria and Dr Matthew Terry for being on my committee and providing their valuable feedback. Dr Kristine Lowe, Dr Robert Dearth, Dr Bradley Christoffersen, Dr Alexis Racelis, and Dr Christopher Vitek have been very supportive throughout. Special thanks to my colleagues Jesus Chavana, Juan Raya, Mandeep Tayal, Sukhman Singh, Yanelly Hinojosa, Paloma Flores, Lili Martinez and other Kariyat lab members for their collaboration. Ultimately, my master's journey has prepared me to face challenges of the advanced doctoral program.

I am extremely grateful to Dr VK Dilawari, Dr Parwinder Grewal and Dr Sukhbir Grewal for their blessings, guidance and persistent support in this journey. I am also grateful to Dr Surinder Sandhu and Dr Vijay Kumar for preparing me to pursue a career abroad and writing me recommendation letters to make it a reality. My dear friends Flor Martinez and Lily Lozano have been extremely compassionate, generous, kind and supportive to me as a new student and helped me get comfortable in Edinburg. I extend my thanks to my family and friends in Punjab, India for their constant support, care and believing in me!

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# CHAPTER I

# INTRODUCTION

## Plants under different abiotic and biotic stresses

Plants are always under the constant pressure of biotic and abiotic stresses (Mithofer and Boland, 2012). Among these temperature, drought and salinity (Thakur *et al.*, 2010, Ashraf *et al.*, 2018) are known as abiotic stresses while pathogenic microbes and insect pests are the biotic stressers (Atkinson and Urwin, 2012).

## Overview of plant defenses

Plants have evolved a wide range of defense strategies to ward-off the suite of herbivores attacking them. These include constitutive defenses or the previously existing defenses, and the induced defenses or the defenses induced upon attack by a pest (Howe and Jander, 2008; Kariyat *et al.,* 2012 and 2013). Both constitutive and induced defenses could also be direct or indirect defenses. (Howe and Jander, 2008; Kariyat *et al.*, 2012 and 2013), in their mode of action. Different morphological structures/physical structures like spines, trichomes and thorns, thicker epidermis, waxy cuticle, or certain biochemicals affecting the pest biology can be considered as direct defenses (Kariyat *et al.*, 2017; 2019).

Constitutively, most plants emit a range of volatile compounds that either repel or attract the herbivores, and or pollinators. However, when a plant is attacked upon by an herbivore, it usually emits herbivory induced plant volatiles (HIPVs) (Carroll *et al*., 2006; Ye *et al.*, 2018)which include semiochemicals that act as repellents for herbivorous pests and as attractantsfor organisms antagonistic to these pests, such as predators and parasitoids (De Moraes *et al.*, 1998). This is because HIPVs, as a part of its direct defense mechanism, act as signals to herbivores indicating that host plant, being already infested, is less suitable for feeding. On the other hand, these might also increase the recruitment of natural enemies (Pare´ and Tumilson, 1999). The former comprises of the direct defenses, while the latter consists of indirect defense mechanism in infested host plants (Pare´ and Tumilson, 1999, Heil, 2008).

Interestingly, it has been reported that natural enemy communities have been found to be responsible for reduced numbers of pests in 75 percent of all the cases investigated (Symondson *et al*., 2002). Although, most studies on tri-trophic interactions have been documented in natural and non-domesticated systems; we currently lack a comprehensive understanding of these interactions in organic farming, and sustainable agricultural cropping systems.

## Importance of cover crops

Cover crops, a critical component of sustainable cropping systems, are mainly grown to boost and protect soil properties. These crops aid in organic and traditional agriculture by directly competing with weeds for resources such as light, nutrients, water, and space and indirectly by allelopathy, thereby leading to weed suppression (Gfeller *et al.,* 2018). Besides, cover crops also make available essential elements like nitrogen to cash crops, pile up organic matter in the soil, and boost the physical properties of soil. In addition, they have also been documented to suppress herbivore populations (Wang *et al.,* 2008). Sudangrass, a common cover crop- is a forage species from Sudan and southern Egypt, adapted to dry and hot climates (Hariprasanna *et al.,* 2015;

Venkateswaran *et al.*, 2019). Sudan grass is a fodder crop grown majorly in the summer season and has been found to act as a common natural weed suppressant owing to its dense canopy (Wang *et al.*,2008), thereby eliminating the need of weedicides in sustainable and organic agriculture. However, we have limited understanding on the insect community dynamics mediated by Sudan grass, an area that forms the major part of this project.

## Major herbivore of interest

The fall armyworm (*Spodoptera frugiperda)* is a tropical and sub-tropical polyphagous pest (Chapman *et al.*, 1999; Lange *et al.*, 2018) that feed on grasses (Gramineae); most preferred hosts include corn, peanuts, sorghum, and Bermuda grass (Sparks, 1979). *S. frugiperda* is also an active forager (Buntin, 1986) and has been considered as one of the most destructive pests attacking *Zea mays* (Degen, 2012), having great economic consequences in the United States. The adults are known to feed and mate nocturnally. Their larvae pass through six instars before pupating in the soil. As a part of their biology, mild climates of South Florida and Texas are favorable for their overwintering stage because of the absence of diapause mechanism in them. However, most of the continental United States and Southern regions of Canada are reinvaded by fall armyworm on an annual basis (Sparks, 1979). Previous studies reveal that *S. frugiperda* is also vulnerable to considerably large predation rates, which can be a result of attraction by HIPVs from infested crop plants (Carroll *et al*., 2006). We are interested in *S. frugiperda* since it has been considered as a major herbivore in the area and can dramatically reduce yield in both cover and cash crops

## Arbuscular mycorrhizal fungi as beneficial microbes

Soil microbes are an integral component of soil rhizosphere. Among these, arbuscular mycorrhizal fungi (AMF) are an important group known for a multitude of benefits to the plants. AMF, especially from the phylum Glomeromycota, are considered highly influential in rhizosphere dynamics. These fungi have been found to aid the host plants in nutrient exchange through the intercellular structures called arbuscules that colonize the root cortex of plants, thereby establishing a mutualistic relationship (Bonfante *et al.*, 2009; Fiorilli *et al.*, 2009; Vannette and Hunter, 2009). For example, the supply of inorganic nutrients to the plant, especially nitrates and phosphates, are enhanced by the extracellular hyphal threads of the AM fungus (Tobar *et al.*, 1994; Pfeffer *et al*., 1999; Goverde *et al.*, 2000; Smith *et al*., 2011). Moreover, plants incorporated with AMF are also found to have increased tolerance to the abiotic stresses like drought, salinity and heavy metals in the rhizosphere (Aroca *et al*., 2008; Fiorilli *et al.*, 2009; Miransari, 2010; Smith *et al*., 2010; Jung *et al.*, 2012). More recent studies have documented that AMF can also play a role in mediating plant interactions with their attackers (herbivores), natural enemies of herbivores and even pollinators (Pineda *et al*., 2010). Therefore, AMF plays a key role in shaping the organization and composition of plant communities that they associate with (Putten *et al*., 2001; Hartley and Gange, 2009). However, we currently lack a clear understanding on the intricacies of how AMF can impact insect community dynamics in cropping systems, an area this project directly addresses.

## Significance of this project

Although, AMF improves plant fitness through the establishment of mycorrhizal symbioses and has been successfully established in various natural and artificial study-systems, relatively little is known on whether organic farming techniques such as cover cropping can be used to harvest the benefits of AMF, and whether AMF has cascading effects on plant defense traits. Using Sudan grass as a summer cover crop, we tested whether AMF can provide growth and developmental benefits to Sudan grass to be used as an effective cover crop in organic farming and whether AMF has any cascading effects on plant defense and insect community dynamics in Sudan grass. In the first part (Chapter 2), I have reviewed the literature about trichomes as first line of plant defenses against abiotic and biotic stressors. Major emphasis has been laid on the role of trichomes in plant stress biology, role against major abiotic stress factors, their interactions with different insect herbivores and their classification and synthesis. In addition, new avenues of research have been proposed for a better and comprehensive understanding of the interaction of trichomes with herbivores. In second part (chapter 3), the different growth and defense benefits of AMF have been tested using Sorghum-sudangrass (*Sorghum x drummondii*), in a local and natural agricultural setting. We have also examined whether AMF can mediate tritrophic interactions in the treated plants. Our results suggest that AMF can help in both growth and defense benefits against biotic stressors.

Note: <u>Chapter II has been accepted to be published</u> as "Kaur J and Kariyat RR. Role of trichomes in plant-stress biology. Book chapter edited by JuanNunez-Farfan. Evolutionary ecology of plant-herbivore interactions. Springer (2020).

Note: <u>Chapter III is published as</u> "**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(3), 301–315. doi: 10.1007/s11829-020-09747-8.

**Online access: https://link.springer.com/article/10.1007%2Fs11829-020-09747-8**

# CHAPTER **I**I

# ROLE OF TRICHOMES IN PLANT STRESS BIOLOGY

## Abstract

In this chapter we will examine the role of trichomes in plant stress biology. Trichomes have been long studied as the first line of defense against herbivores. We review the studies that have examined the structure, classification and diversity of trichomes in flowering plants, followed by their modes of action against abiotic and biotic stressors. We also review the studies on herbivore X trichome interactions, and how trichomes play a role in both direct and indirect plant defences. Next, we use results from our research to highlight the importance of trichomes as an effective measure of plant defense, and how genetic variation affects trichome mediated defences. And, finally we propose some exciting new areas of research on trichomes and trichome mediated defenses for future work.

## Introduction

Plants, being sessile living organisms, are constantly exposed to unfavorable abiotic and biotic factors that exert significant selection pressures on them (Mithofer and Boland, 2012). Various abiotic stressors include extreme temperature, drought and salinity (Kaur *et al.,* 2008; Thakur *et al.,* 2010) whereas biotic stressors mainly include pathogen and insect herbivory

(Atkinson and Urwin, 2012). While the biotic stressors directly attack the host plant, the abiotic stressors, in addition to affecting their growth and development, also render them weak and predispose them to attack by biotic stressors. However, despite the absence of a brain or a central nervous system, plants have evolved a suite of coordinated defenses to tolerate/ protect themselves against both biotic and abiotic stressors (Singh *et al.,* 2016). They employ a wide gamut of constitutive (preformed) and induced defenses (induced upon herbivory/ pathogen infection) in response to these stressors (Howe and Jander, 2008; Kariyat *et al.*, 2012 and 2013). More specifically, both constitutive and induced defenses can either be direct or indirect (Howe and Jander, 2008; Kariyat *et al.*, 2012 and 2013), in their mode of action. Direct defenses, include the innate ability of the plants to counter/ offset herbivory using morphological structures like spines, trichomes, thorns, thicker epidermis, waxy cuticle, or by the production of defensive biochemicals like alkaloids, tannins, phenols, anthocyanins etc., that affect herbivore growth and development (Hanley, 2007; Hauser, 2014; Kariyat *et al.*, 2017; 2019). On the other hand, indirect defenses include ability of plants to attract natural enemies like predators and parasitoids by the emission of unique blend of volatiles known as herbivore-induced plant volatiles (HIPVs) (De Moraes *et al*., 1998; Pichersky *et al*., 2006; McCormic *et al*., 2012; Aljbory and Chen, 2017), or through extrafloral nectaries (Heil *et al*., 2001). Among these different types of defenses, trichomes, commonly considered as the first line of defense- can protect the plants by both physical and chemical means (Shanower, 2008). Trichomes are the epidermal cells that differentiate into hair-like protuberances on the aerial plant organs (Marks,1997; Glas *et al.,* 2012; Kariyat *et al.,* 2013), and armor the immobile plants against biotic stressors like herbivores and their feeding and oviposition and, also against other abiotic stressors like extreme low and high temperatures, excessive light intensity, drought and even harmful ultraviolet (UV) radiation (Kaur *et al.*, 2008; Shanower, 2008; Thakur *et al.*, 2010; Burrows *et al.*, 2013; Ashraf *et al.*, 2018).

## Classification and synthesis

Trichomes are found in a diverse array of shapes and structures. (Xiao *et al.*, 2017). They can be broadly classified into non-glandular (Figure 2.1) and glandular trichomes (Figure 2.2) and could be either unicellular or multicellular (Wagner *et al.*, 2004). Non-glandular trichomes are usually unicellular and are found on the majority of the angiosperms, few bryophytes, and gymnosperms (Werker, 2000). For example., both Thale cress (*Arabidopsis thaliana*, Family: *Brassicaceae*) and cotton (*Gossypium spp.*, Family: *Malvaceae*) possess only simple non-glandular trichomes (Mathur and Chua, 2000; Glas *et al.*, 2012). However, trichomes in cotton are unbranched (Figure 2.1a) and more pronounced on seed surface, while, the trichomes in *Arabidopsis sp.*, could be either unbranched (Figure 2.1a) or branched (also known as stellate, and multicellular) (Figure 2.1b) and can be produced on any aerial plant part (Kim and Triplett, 2001; Wang, 2004.).

Glandular trichomes (Figure 2.2), on the other hand, are usually multicellular and have well-differentiated basal, stalk and apical cells. Approximately 30% of the vascular plants possess glandular trichomes. For example, plants of plant families *Lamiaceae* (Salvia- *Salvia divinorum*, Mint- *Mentha x piperita.* etc.) and *Solanaceae (Solanum lycopersicum, S. tuberosum, Nicotiana tabacum* etc.) have dense glandular trichomes (Fahn, 2000; Glas *et al.*, 2012). Furthermore, glandular trichomes can be characterized into two main classes- peltate and capitate trichomes. As an example, capitate trichomes are encountered in plants of the families *Solanaceae* and some *Lamiaceae* (e.g. *Salvia sp.*), while the peltate trichomes can be found in some other members of

*Lamiaceae* family like Peppermint (*M. piperita*) and Basil *(Ocimum basilicum)*. The major difference between these two trichome types is in the production and storage location of the secondary metabolites in the trichome structure. Peltate trichomes utilize the subcuticular cavity to produce and synthesize volatile secondary metabolites. Whereas, the capitate trichomes usually synthesize and store their secondary metabolites in the terminal cells/or at the stalk tip (Gerd, 2014).

Various histochemical studies suggest that secretions of peltate trichomes mainly comprise of terpenoids (e.g., Wild dagga- *Leonotis leonurus*, Peppermint- *M. piperita*) and those of capitate trichomes consist of mostly polysaccharides, proteins and lipids (Werker *et al.,* 1985; Ascensão *et al.*, 1997; Ascensão and Pais, 1998). However, small amounts of terpenoids can also be found in capitate trichomes of some plants. For instance, *M. piperita* capitate trichomes can contain monoterpene traces in their essential oil secretions (Amelunxen *et al.,* 1969; Ascensão *et al.,* 1997). and *L. leonurus* trichomes can also contain meager quantities of flavonoids (Ascensão and Pais, 1998). However, it had been emphasized that peltate glands are responsible for a major portion of terpenes production in peppermint (Turner *et al.,* 2000). Also, the secretions of capitate trichomes are mostly nonvolatile compounds while those of peltate trichomes are mostly volatile compounds (Tissier, 2012). For example., geraniol is contained in the peltate trichomes of lemon basil, which after oxidization produces the aldehyde geranial. Further, geranial undergoes keto-enol tautomerization to form neral. Together, the mixture of the new compound neral and the aldehyde geranial is known as citral, responsible for the lemony flavor of the plant. Another member of *Solanaceae*, Tobacco (*Nicotiana tabacum*, Family: *Solanaceae*) trichomes have been found to contain defense compounds such as cembratrieneols and cembratrienediols. Furthermore, a hallucinogen terpene, namely Salvinorin is contained in *Salvia divinorum.* Recently, antimalarial properties have been studied for the sesquiterpene secretion called artemisinin in the trichomes of *Artemisia annua* (Family: *Asteraceae*) (Schilmiller *et al.*, 2008).

Structurally, stalk length in capitate trichomes surpasses half the height of the secretory head, while the peltate trichomes are shorter (Abu-Asab and Cantino, 1987; Ascensao and Pais 1998). However, the capitate trichomes exist in varied morphologies with respect to stalk length and their secretory head, and further classified into different types (Werker *et al.*, 1985; Ascensao and Pais 1998). For instance, by the virtue of electron microscopy type 1 and type 2 capitate trichomes were identified in *L. leonurus* - such that former referred to the trichomes with short cylindrical stalk while the latter possessed long conical stalk *(*Ascensao and Pais 1998).

Also, peltate trichomes had been described as having more variability than the capitate trichomes with respect to form of the stalk cells and the number of cells on the secretory head and surrounding cells. Studies have described peltate trichomes to possess distinct subcuticular storage cavity resting on a pedestal made up of vacuolated basal cell, stalk cell and eight-celled apical glandular disc (Fahn, 1979; Maffei *et al.,* 1989; Brun *et al*., 1991, Ascensão *et al.*, 1997, Turner *et al.,* 2000, Camina *et al.,* 2018). Peltate trichomes also have a globular shape owing to the acquisition of secretions in the apical cavity. On the contrary, capitate trichomes are usually four celled, but with varied morphologies (as described previously). For instance, *M. piperita* has both capitate and peltate structures where capitate hair consists of the circular head having four cells and a stalk with various morphologies (Werker *et al.*, 1985; Gonçalves *et al.,* 2010).

Trichome rich species of wild (*Solanum pennellii, S. carolienense, S. habrochaites, and S. peruvianum*) and cultivated tomatoes (*S. lycopersicum)* have been extensively studied in the past owing to their importance in plant resistance against herbivory, and also for the presence of non-glandular, glandular and even both in some cases (Kariyat *et al.,* 2013; 2017; 2018; 2019). Glas *et* *al.*, in 2012 have re-described the trichome morphology in wild and cultivated *Solanum spp.* Out of the total eight trichome types previously described by Luckwill (1943) and revised by Channarayappa *et al.* (1992), they have clarified the classification as follows: type I, IV, VI and VII are glandular trichomes and type II, III, V and VIII are simple non-glandular trichome types (Luckwill, 1943; Channarayappa *et al.*, 1992; Glas *et al.*, 2012). Of the glandular trichomes, Type I and IV are both described as capitate with differences in their length. Type I trichomes are significantly longer when compared to type IV. Both types I and IV trichomes are predominant in wild *Solanum* species like *S. habrochaites.* However, in the cultivated tomato species, type I is rarely found and type IV is completely absent. Interestingly, type VI is predominant in both wild and cultivated tomato species (Glas *et al.*, 2012). The differences among these types are mainly due to the variation in the number of stalk cells and secretory cells. Also, these trichome types differ in genes and transcripts responsible for the synthesis of different biochemicals that they harbor (Glas *et al.*, 2012).

At the functional level, non-glandular trichomes (Figure 2.1) act as structural defenses, physically affecting insect herbivores by restricting their access to leaf epidermis, causing physical entrapment or inflicting injury to them (Dalin *et al.*, 2008; Peiffer *et al.*, 2009; Weinhold and Baldwin, 2011; Szyndler *et al.*, 2013; Weigend *et al.*, 2018). On the other hand, glandular trichomes (Figure 2.2) assist in herbivore defense as a chemical means or as a combination of both physical and chemical defense. This is primarily accomplished by storing and injecting toxins and /or signaling molecules that either directly reduce herbivore feeding or in some cases lead to multi-trophic interactions (Peiffer *et al.*, 2009; Weinhold and Baldwin, 2011). Usually, they contribute to direct toxicity in insects, entrapping them by the production of sticky exudates, anti-nutrition and inducing anti-herbivore defense signaling in the host plant–primarily mediated through the phytohormone jasmonic acid (Peiffer *et al.*, 2009; Kariyat *et al.*, 2017).

Although a significant amount of research has been dedicated to understanding trichome mediated effects in various systems, we still lack a detailed understanding of developmental variation in trichomes. Studies in the model plant *A. thaliana*, that possesses only non-glandular trichomes (Schnittger and Hulskamp, 2002), suggest that unicellular trichomes in this plant emerge from the primary meristem of the plant epidermis (Figure 2.3) by dividing mitotically. In the process, several epidermal cells surrounding the germinating trichomes divide continuously and simultaneously. Consequently, trichome cells enter the endoreduplication cycle, where DNA replication takes place without any nuclear and cellular divisions. Usually, this cycle is accomplished before trichome outgrowth on the surface. And, trichome cell alters its polarity and starts emerging as an outgrowth (first branching event). Simultaneously, the second endoreduplication cycle is initiated, followed by the second branching event. The third endoreduplication and the second branching events take place concomitantly. While the fourth (last) endoreduplication cycle is ongoing, trichome cell undergoes expansion, yielding approximately 0.5 mm long mature tri-branched/ unbranched trichomes (Marks and Feldmann, 1989; Schnittger and Hulskamp, 2002) perpendicular to plant surface (Marks and Feldmann, 1989). Despite elaborative studies of the trichome types and their characterization we also have limited knowledge of the synthesis of glandular trichomes.

Trichome formation is a complex process and one simple model is not enough to explain their formation, patterning and development through the different growth stages. Different phytohormones pathways have been found to be responsible for the regulation of trichome development (An *et al*., 2011). For example, gibberellins, cytokinins, jasmonic acid and salicylic acid have been shown to regulate trichome initiation (An *et al*., 2011). However, their interplay and potential feedbacks largely remain unclear. Among these, jasmonic acid (JA) is considered as the key signaling molecule responsible for trichome induction. For instance, JA and JA-Ile have been found to induce resistance to herbivory by inducing trichomes in *Arabidopsis spp.* ( Traw and Bergelson, 2003; Li *et al*., 2004; Boughton *et al.*, 2005, Peiffer *et al.*, 2009; An *et al*., 2011). It has been recently demonstrated that MYB-bHLH-WDR (MBW) complex can modulate the gene expression of *GL1* and *GL3,* successfully resulting in the trichome induction in wild type of *Arabidopsis spp.* but compromised in the JA mutant *coi1-2*. In the absence of JA, JA-ZIM domain (JAZ) proteins (present in plant cells) can physically bind to *GL1* and *GL3*, which inhibits the formation of MYB complex, clearly showing that JAZ proteins can attenuate JA signaling. In the presence of JA, 26S proteasome system along with the SCF$^{COI}$ complex (containing the F-box protein CORONATINE INSENTIVE1 (COI1), Cullin1 (CUL1), ASK1/ASK2 and Rbx1), acts upon the JAZ proteins to degrade them, thereby releasing the genes *GL1 and GL3* (Thines *et al.*, 2007, Pattanaik *et al.*, 2014). And, these genes form a complex with TRANSPARENT TESTA GLABRA 1 (TTG1) (Yoshida *et al.*, 2009, Qi *et al.*, 2011; Pattanaik *et al.*, 2014) and positively regulate the expression of downstream targets, which ultimately leads to trichome induction (Qi *et al.*, 2011; Pattanaik *et al.*, 2014). Besides, JA has also been shown to specifically regulate the synthesis of type VI trichomes in some plants, besides mediating a wide range of other plant developmental processes. This has been confirmed by silencing the gene OPR3, a precursor of JA, which largely undermined the density of type VI trichomes in tomato (Bosch *et al.*, 2014; Hutchelmann *et al.*, 2017).

Using molecular genetic tools, studies have identified various transcription factors and genes controlling the development of various trichome types in different plants. For instance, in tomato, it has been suggested that the development of trichome type I is regulated by three genes- Cyclin B2 gene, Woolly gene (Wo) responsible for encoding homeodomain-Leu zipper (HD-ZIP) protein along with Hair gene encoding zinc finger protein (Cys2-His2) that interacts with the Wo gene product (Marks and Feldmann, 1989; Yang *et al*., 2011; Gao *et al*., 2017; Chang *et al*., 2018). Recent molecular studies on the hairless gene (*hl*) and its mutant from tomato clearly display its key importance in the trichome synthesis. This gene is responsible for the nucleation of actin, a protein responsible for the development of various trichome types (Kang *et al.,* 2010a, 2016). Also, the density of both glandular and non-glandular trichomes in tomato was found to be controlled by SlMIXTA1 with ectopic expression experiments, through an MYB transcription factor in tomato (Ewas *et al.,* 2016, 2017). Additionally, it has also been suggested that the helix-loop-helix transcription factor, SlMYC1 is directly responsible for the synthesis of glandular trichomes in cultivated tomato plants- type VI trichome production consequently decreased with reduction in the levels of S1MYC1, and completely failed to develop in its absence/when turned off (Xu *et al.*, 2018).

## Role of trichomes against abiotic stresses

Drought, temperature extremities, intensive light intensities, salinity, heavy metal accumulation in the soil, soil salinity, water scarcity etc. are the most common abiotic stressors on plants (Cakmak, 2005). It is estimated that around 60% of soils pose growth restriction to the plants due to poor soil health and nutrition (Cakmak, 2005; You and Chan, 2015; Martinez *et al.,* 2018). These abiotic aggressors lower the plant yield between 50 to 80% (Martinez *et al.,* 2018) such that, heat stress and droughts coupled together led to $200 billion losses in the United States (Suzuki, 2014). However, various investigations on plant response to abiotic stress have also demonstrated the benefits of trichomes. Their effectiveness against abiotic stress largely relies on traits such as length, density, branching, and orientation; under the common umbrella ‘pubescence’ (Hanley *et al.*, 2007; Shanower, 2008).

Trichomes have been found to physically defend the plants against water loss and heat stress (Gutschick, 1999). Heat loss in pubescent leaves is accelerated through the process of convection leading to increased thermal conductivity of leaf surface (Dahlin *et al.*,1992). Consequently, plants undergo lower transpiration rates at higher trichome densities (Choinski and Wise 1999; Benz and Martin, 2006; Burrows *et al.*, 2013; Hauser, 2014) and thus regulate their water balance better (Hauser, 2014). Clawson *et al.* 1986 showed that pubescent soybean varieties had 50% higher water use efficiency owing to the reduced evapotranspiration (Clawson *et al.*, 1986; Dahlin *et al.*,1992). In addition, the presence of trichomes on the leaf surface also improves sunlight reflectance, and therefore lowers the heat load on the plants and consequently modulate and tolerates temperature variation and its impact on growth and development. Also, increased density of glandular trichomes (e.g., *Betula pendula,* Family: *Betulaceae*) was found to improve tolerance against frost by using trichomes as a structural adaptation under low-temperature regime (Prozherina *et al.,* 2003). In addition, trichomes have also been implicated to play a role in the regulation of photosynthesis, by decreasing photoinhibition (Hauser, 2014), and they can act as storehouses of UV absorbing compounds like flavonoids (Hanley *et al.*,2007), thereby protecting the underlying photosynthetic tissues against harmful UV-A and UV-B radiations (Morales *et al.*, 2002; Yan *et al.,* 2012).

A large body of scientific studies have clearly documented that certain abiotic stresses like heavy metal contamination of soil and environment, have been aggravated by anthropogenic activities (Straalen *et al.*, 1994; Yadav, 2010; Wang *et al.,* 2014; Wierzbicka, 2014). Rapid industrialization and current farming practices like heavy use of synthetic chemicals- pesticides and fertilizers have been deteriorating the soil conditions to a great extent. Specifically, d-block elements on the periodic table with density more than 5g per cu cm have been classified as heavy metals (Singh *et al.,* 2016). Interestingly, trichomes can sequester these elements and assist in detoxification of the plants (Choi *et al*., 2001, 2004; Quinn *et al.,* 2010). *Leontodon hispidus* (Rough Hawkbit- Family: *Asteraceae*), for instance, is known to accumulate calcium in its trichomes, whereas *N. tabacum,* on the other hand, has been shown to secrete Zinc and Cadmium through its trichomes (Glas *et al.,* 2012), acquired from the soil. Besides, Mustafa *et al* in 2017 and 2018 have proved the vitality of the biomineralization of the trichomes. They have suggested that mineralization of the trichomes majorly by calcium phosphate, calcium carbonate and silica compounds calcify them and thus, hardens them. This, in turn, strengthens their role as physical defenses by acting as stinging hair to the attacking herbivores (Mustafa *et al.,* 2018). More interestingly, possessing trichomes is also a beneficial trait to the plant in outperforming competitors and thriving against unfavorable climatic conditions. For example., Han *et al.,* 2019 showed that plants with trichomes, coupled with some other traits like upright leaves, a multilayered epidermis, or strong cuticles, have allowed them to outperform competitors in a forest succession (Han *et al.,* 2019).

Collectively, having trichomes has top-down effects as they effectively defend the plants against various abiotic stressors by reducing the heat load, lowering the evapotranspiration rates, and thus helping in maintaining water balance levels. Additionally, trichomes help protect photosynthetic tissues by absorbing damaging UV rays and accumulates harmful heavy metals.

## Role of trichomes against biotic stresses

Besides abiotic stresses, plants also experience several and severe biotic stresses. These include insect-pests, pathogens, weeds, and other animals which together cause about a 40 % decline in crop productivity (Oerke and Dehne, 2004). Among these, arthropod pests (mainly insects) cause ~ 15 % crop losses per annum (Mitchell *et al.*, 2016). Trichomes have been well studied in defending plants against biotic stressors. It has been found that non-glandular trichomes mainly physically defend plants against herbivory. Like with the abiotic stresses, their pubescence plays an important role in defending against biotic aggressors - denser, prolonged and more upright trichomes better defend against the insect attack (Shanower, 2008). However, selective breeding for fitness traits can reduce the effectiveness of trichome based plant defenses (Mitchell *et al.*, 2016), consequently, cultivated species are found to possess lower density of trichomes as compared to their wild relatives, (another line of reasoning on why cultivated crops are more prone to damage by insects than their wild relatives). For instance, 85% of neonate larval mortality in cotton bollworm *Helicoverpa armigera,* Family: *Noctuidae*) was observed in wild pigeonpea (*Cajanus spp.,* Family: *Fabaceae*) than the cultivated pigeonpea (*Cajanus cajan,* Family: *Fabaceae*). Interestingly, among other differences, wild pigeonpea pods possess twice the density of non-glandular trichomes when compared to the cultivated pigeonpea pods (Romeis *et al.,* 1999). Higher density of trichomes successfully prevented *H. armigera* from reaching the pod surface, thus having them starve or desiccate to death, prior to their feeding initiation (Shanower, 2008), very similar to multiple *Solanum* species (Kariyat *et al.*, 2018). Similarly, low infestation and sustenance of different caterpillars and beetles were observed in soybean varieties with denser trichomes than smooth leaved soybean. In addition to protecting against chewing herbivores, it has also been shown that denser trichomes in wheat (*Triticum aestivum,* Family: *Gramineae*) restricted the movement of yellow sugarcane aphid (*Melanaphis sacchari,* Family: *Aphididae*) (Webster *et al.,* 1994). Also, Chilean strawberry (*Fragaria chiloensis,* Family: *Rosaceae)* plants with dense trichomes were avoided by the black vine weevil (*Otiorhynchus sulcatu*s, Family: *Curculionidae*) (Doss *et al.,* 1987) and cabbage white butterfly larvae (*Pieris rapae,* Family: *Pieridae*) inflicted greater damage to field mustard (*Brassica rapa,* Family: *Brassicaceae*) with less dense pubescence (Agren and Schemske, 1993; Hanley *et al.,* 2007). Szyndler *et al.,* in 2013, showed a differed mode of herbivore defense in trichomes. Non-glandular trichomes present on the leaf surface of the bean plants *(Phaseolus vulgaris,* Family: *Fabaceae*) was found to mechanically entrapped bedbugs (*Cimex lectularius*) and kill them by the hooked sharp needle-like trichomes (Figure 2.1c)- an interesting strategy for controlling bed bug infestations (Szyndler *et al.,* 2013). However, adaptive features have been observed in the larvae of specialist herbivore *Heliconius charithonia* (Family: *Nymphalidae*) on the plant *Passiflora lobata* (Family: *Passifloraceae*) against the hooked trichomes borne on it. The insect was found to weave silken mats on the terminal of the hooked trichomes on the plant, speculated to facilitate the herbivore movement in their presence. Eventually, the presence of tips of the hooked trichomes in the feces of the insect suggested that they were successful in handling the mechanical defenses of the plant. While, generalist insects (*Heliconius pachinus,* Family: *Nymphalidae*) were found struggling with the defenses and had a higher mortality rate. (Cardoso, 2008).

Additionally, non-glandular trichomes have been found to have a varied mode of action against insects at different life stages. Plant surface bearing trichomes is an unfriendly landscape for majorly the soft-bodied chewing insects like the lepidopteran larvae. This is more evident in the case of neonate caterpillars, who tend to feed on the leaf epidermis upon emergence. As previously shown by Kariyat *et al.* in 2017 and 2018, trichome bearing *Solanum* leaves pose difficulties to the first instar larvae of *M. sexta* to initiate feeding. They struggle to reach the leaf epidermis in the presence of spine-like hair extensions (trichomes) which causes them to starve, desiccate and ultimately die. However, both glandular and non-glandular trichomes are ineffective against the late instar larvae in disrupting their feeding. Interestingly, stellate trichomes (non-glandular) were found to poke through the late instar larvae, rupturing their tender epidermis to leak hemolymph, and ultimately leading them to death. Therefore, non-glandular trichomes are found to be effective against both early and late instar larvae with different modes of action under direct defenses.

Glandular trichomes are more commonly found to produce defense chemicals in plants. These may constitute up to 30 % of the plant's dry leaf weight. Depending on the plant family and species, the glandular trichomes have been found to possess a diverse range of biologically active defense compounds such as alkaloids, glucosinolates, cyanogenic glycosides, terpenoids besides latex and protein inhibitors (Glas *et al.,* 2012; Tissier, 2012; Kariyat *et al.,* 2018). These compounds affect herbivory by affecting insect metabolism, membrane disruption, inhibition of signal transduction processes, nutrient and ion transport or other physiological processes, collectively leading to reduced feeding, growth, development and even mortality in the affected herbivore species (Shanower, 2008; Glas *et al.*, 2012; Kariyat *et al.,* 2019). Besides, these glandular trichomes are also reservoirs for terpenes, aromatic compounds and fatty acid derivatives. These compounds, collectively known as volatile organic compounds (VOCs), defend the plants indirectly by the attraction of natural enemies of the herbivores feeding the host plant (Weinhold and Baldwin, 2011).

Besides, studies have been conducted on the post-feeding impacts of trichomes on insect behavior and physiology. They have been found to interfere with the insect digestive system.

(Shanower, 2008). Consumption of trichomes is a commonly observed behavior in chewing caterpillars. To gain access to the underlying leaf tissue for infestation/feeding, caterpillars tend to feed on the trichomes, eventually consuming them. Kariyat *et al.* (2017) showed that consumption of non-glandular trichomes isolated from the weed Carolina horsenettle (*S. carolinense* L., Family: *Solanaceae*) led to breaching of the peritrophic matrix – the gut membrane, which plays a key role in defense, in Tobacco hornworm (*M. sexta*, Family: *Sphingidae*) larvae post-ingestion. This is a vital study that implies trichomes to be major plant defenses by themselves, without any coupled effects of other structural and non-structural defenses (Howe and Jander, 2008; Kariyat *et al.*, 2013). Manipulative studies de coupling trichomes from other defenses have shown that trichomes negatively impact both the growth and development of caterpillars (Kariyat *et al.,* 2019). Therefore, trichomes can resist herbivory both before and after the insect feeding is initiated-leading to pre and post ingestive effects (Kariyat *et al.*, 2017).

In addition, genetic variation has been found to play an important role in trichome mediated defenses against herbivory. Kariyat *et al.,* (2013) demonstrated that both abaxial and adaxial trichomes are found to significantly vary among maternal families in Carolina horsenettle, and even more importantly inbreeding affected both constitutive and induced trichome production. In a recent study from the same group (Nihranz *et al.,* 2019) showed that not only does inbreeding lower the plant defenses by reducing the trichome production (Kariyat *et al.*, 2019; Kariyat and Stephenson, 2019) in *S. carolinense* upon damage by *M. sexta,* but also compromises their induction over the generations. Not surprisingly, they also reinforced the fact that higher trichome density post damage (induced) were found on plants under herbivory as compared to the undamaged control plants.

Hence, the consensus is that higher trichome density positively correlates with resistance to herbivory (Glas *et al.,* 2012). This could be achieved by restricting herbivore access to the epidermis (Kariyat *et al.*, 2018) thereby delaying feeding (Kariyat *et al.,* 2017) or impeding growth (Kariyat *et al.*, 2018), and development (Kariyat *et al.,* 2019) upon consumption of trichomes. And, while non-glandular trichomes are more effective as a structural defense (Lanning and Eleuterius, 1985), it is also clear that they are more effective against neonates and early instars, as seen in the plant family *Solanaceae* (Kariyat *et al.,* 2018). Since the first cue for imminent herbivory on plants is oviposition, studies have also examined whether trichomes can affect oviposition. For example., the herbivore Chinese bruchid (*Callosobruchus chinensis,* Family: *Chrysomelidae*) prefers oviposition on glabrous pigeon pea pods over the hairy type, cereal leaf beetle (*Oulema melanopus,* Family: *Chrysomelidae*) prefers to oviposit on wheat cultivars with lower trichome density. On the contrary, oviposition and trichome density have been found to positively correlate in soybean and cotton cultivars. For instance, *Helicoverpa spp.* (Family: *Noctuidae*), Western tarnished plant bug *(Lygus Hesperus,* Family*: Miridae),* Spiny bollworm (*Earias vitelli,* Family: *Noctuidae)* and cotton spotted bollworm (*E. fabia,* Family: *Noctuidae*) lay more eggs on cotton with higher trichome density. Similarly, bean fly (*Ophiomyia phaseoli*, Family: *Agromyzidae*) and soybean pod borer (*Laspeyresia glycinivorella,* Family: *Olethreutidae)* prefer soybean pods with higher trichome density than smooth pods (Shanower, 2008). While there are contrasting examples, in general, both oviposition and neonate larvae survival are found lower on plants with more trichomes (Kariyat *et al.*, 2017; 2018). It is plausible to speculate that the presence of trichomes and any associated cues (tactile or volatile) might be used by the ovipositing females as an honest signal for host location, in these few cases- an area that must be explored in detail.

Besides density, other factors of pubescence such as length and morphology of trichomes can effectively restrict herbivory, especially by the small-bodied insects. For instance, prolonged glandular trichomes were found to successfully defend the leaves of the plant field beans, (*P. vulgaris*) against potato leafhopper (*Empoasca fabae,* Family: *Cicadellidae*), irrespective of trichome density (Shanower, 2008). Potato leaf hopper is small bodied insect pest mainly responsible for causing devastating hopper burn symptoms in crops. And, pink bollworm larvae (*Pectinophora gossypiella,* Family: *Gelechiidae*) on cotton cultivars were eventually found dead of starvation and desiccation after their failed struggle to move around on the leaves of the plant to initiate feeding (Shanower, 2008).

The efficiency of metabolites allocation and energy administered to the defensive activities in plants is based on the adaptive modulation of plant metabolism, recognition of the herbivore and precision in host plant's activity with intercellular signaling and accurate biochemical, physiological and cellular responses (Maffei *et al.,* 2007, 2007; Mithofer and Boland, 2012). And, due to the dynamic nature of herbivore infestation, and host-herbivore species specificity (e.g., specialists vs generalists), studies have also found that the structure, density and distribution of trichomes varies at both inter and intraspecific levels, with implications for trade-offs in the plants for resource allocation towards nutrition and/or defenses (Kariyat *et al.,* 2013; Hauser, 2014).

## Trichomes and their role in direct and indirect defenses

As a part of the herbivore-resistance mechanism, glandular trichomes act by releasing specialized metabolites that are directly toxic, feeding deterrents or discourages insect oviposition. Their appendages mediate the toxin delivery to the herbivores that discourages their feeding activity (Agren and Schemske, 1993; Agrawal *et al.*, 2004; Kariyat *et al.,* 2019). Since trichomes are the storehouses for various toxins, it gives rise to the potential risk of self-intoxication. To prevent this, trichomes usually store these phytochemicals in their vacuoles or apoplasm, thereby minimizing the risk (Mithofer and Boland, 2012). Upon attack by an herbivore, the glandular trichomes rupture, causing the toxic exudates to be released to the plant surface. Upon oxidation of released compounds (For example, polyphenols and O-acyl sugars), the exudates become sticky leading to the entrapment of the arthropods on the surface. Ultimately, the insects die of starvation or toxicity due to consumed plant metabolites or suffocation, or they activate jasmonic acid-mediated defense gene expression, leading to both site and systemic defenses (Peiffer *et al.*, 2009; Tian *et al.*, 2013).

In addition, the toxic metabolites produced by the glandular trichomes also aid in systemic immunization of the attacked plant. These defense compounds are found to be transported to distal plant parts, thereby priming the plant against herbivory. Pyrethrins, found in *Tanacetum cinerariifolium (*Family: *Asteraceae)* are produced in the glandular trichomes present on the fruits. Eventually, the pyrethrins from the fruits are transmitted to the seedlings via the seeds. Astonishingly, these seedlings which are trichome free themselves are found to be insect as well as pathogen-resistant/tolerant (Glas *et al.,* 2012; Mithofer and Boland, 2012). Besides, various studies have shown anti herbivore activity of sesquiterpene lactones found in the trichome extracts of *Tanacetum spp.* For instance, *Spodoptera littoralis* (Mediterranean Brocade, Family: *Noctuidae*), feeds poorly on *T. cadmium* ssp. c*admium* and *T. corymbosum ssp. cinereum* flowers as they have sesquiterpene lactones with antifeedant activity (Goren *et al.,*1994).

To defend indirectly, glandular trichomes have been found to release volatile organic compounds (VOCs) leading to the attraction of natural enemies of the infesting insect pest species (De Moraes *et al.,* 1998; Walling 2000; Heil *et al.,* 2001; Mithofer and Boland, 2012). VOCs vary in their composition (Mithofer and Boland, 2012) and mostly comprise of terpenes, phenylpropanoids and glycerides etc. (Kariyat *et al.*, 2012; Tissier *et al.*, 2017). Their composition largely varies with the type of insect, feeding damage, mode of damage, insect oviposition or injuries inflicted (Mithofer and Boland, 2012). Predatory insects are attracted by the phytochemicals produced by the glandular trichomes. For example., O-acyl sugars produced by the glandular trichomes of *N. tabacum*, upon consumption by the neonate caterpillars of *M. sexta* renders them attractive to the predatory ants (Weinhold and Baldwin, 2011). On the contrary, few studies have also shown that trichomes can aid herbivores to escape predation or parasitization by their natural enemies. In the presence of trichomes, natural enemies usually have to spend more time searching for their prey. Additionally, they are more prone to physical entrapment in the toxic sticky exudates and are affected by chemical repellants emitted from the secondary metabolites of trichomes. For instance, *Trichogramma spp.* (egg parasitoid) (Family: *Trichogrammatidae*) has been found to have faster locomotion in search of prey on the glabrous leaves than the trichome bearing leaves of cotton and pods of pigeon pea (Shanower, 2008). In another study, locomotion of the predatory green lacewing (*Chrysoperla carnes,* Family: *Chrysopidae)* was found to be hampered on trichome bearing plant California pipevine (*Aristolochia californica*, Family: *Aristolochiaceae*) eventually protecting the larvae of Pipevine Swallowtail (*Battus philenor*, Family: *Papilionidae)* against predation (Fordyce and Agrawal, 2001). However, the feeding damage by the caterpillars was restricted to less than 30 % on the same plant, owing to the trichomes (Mithofer and Boland, 2012).

Although a significant amount of research has been conducted to examine the chemical composition of trichomes, a common bottleneck is the lack of easy and precise methodology to isolate the trichomes without contamination from extraneous plant matter. Various traditional isolation techniques involved methods like shaving the trichomes off the leaf blade with a scalpel blade (Croteau, 1977; Kariyat *et al.,* 2017), abrasion of the leaf surface with a microscope coverslip (Keene and Wagner, 1985; Yerger *et al.*, 1992) / a glass slide (Keene and Wagner, 1985; Tissier 2012)/ a brush (Yerger *et al*., 1992), and the use of liquid nitrogen-based extraction (Kariyat *et al*., 2019) to name a few. The leaf samples are usually placed in a buffer before brushing the trichomes off the surface. Additionally, some studies also use an adhesive tape (Leonardo *et al.*, 2018) or double-sided tape attached to a glass slide (Gopfert *et al.*, 2006; Tissier 2012) to isolate the trichomes. This method involves detaching the trichomes from the sticky surface by either submerging the tape in water or by placing it in different kinds of buffer solutions and vortexing the container or removal of trichomes using paint brush. Then, the trichomes are filtered by passing the solvent containing trichomes through a fine metal meshes sized 100µm and 80µm, consecutively (Leonardo *et al.,* 2018). Methods using swabbing the leaf surface with cotton submerged in antioxidant (Kowalski *et al.,* 1990), using Percoll density gradient centrifugation (Yerger *et al.,* 1992; Tissier 2012), using glass beads and mesh technique for cryopreserved plant tissues (Ranger *et al.*, 2004; Tissier 2012), abrading the leaf surface with dry ice (Yerger *et al.*, 1992; Tissier 2012) have also been used with different levels of success. Recently, it has been shown that a new generation technique namely, pressurized cyclic solid-liquid extraction using Naviglio extractor is about 15 times more efficient than the traditional methodologies in harvesting the trichomes, as found in *Artemisia annua*. This technique involves compressions and immediate decompressions on the plant tissue, which subsequently creates a pressure gradient that ultimately leads to the extraction of trichomes without any actual maceration (Zarrelli *et al.,* 2019).

## Future directions

As outlined above, significant research has been conducted to understand the role of trichomes in plant–herbivore interactions, in both non-domesticated and domesticated species. However, we still lack a comprehensive understanding of certain aspects of trichomes.

The majority of the studies conducted in the past have mainly focused on trichomes contained in the cultivated crops or their wild relatives (Glas *et al*., 2012; Livingston *et al*., 2019). However, few have targeted flora related to forest ecosystems and horticultural fruits, and vegetable crops (Li *et al.*, 2015; Liu *et al*., 2016, Wang *et al.*, 2016, Lujan *et al.*, 2018). Therefore, we need to study and characterize the trichomes in these systems as well. While various studies have described the role of trichomes against different abiotic stressors to the plants. However, we lack the understanding of how these key abiotic factors influence the trichome development and induction, and their effects in growth-defense tradeoffs (Kariyat *et al.*, 2013)

Recent developments in the use of molecular genetics in trichome research have led to the creation of cDNA libraries from various plants – *Solanum sp., M. piperita, O. basilicum* etc. However, more elaborative studies aiming at the expression and quantification of genes associated with trichome biosynthesis and biochemistry in different plants are required- with a possible target of genetically engineered plants with efficient defense allocation in trichomes without compromising the yield. Also, such studies can be used to understand and regulate the mechanisms responsible for the synthesis of secondary metabolites and their transport throughout the plant organs.

We also speculate that non-glandular trichomes can possibly do more harm than understood so far. Since these trichomes physically breach the young larvae (pierce through their skin), they inflict mechanical injury to them (Kariyat *et al.*, 2017, 2018, 2019). Besides, it can be speculated that microbes harbored on trichome surface are also potentially injected and translocated during the process. Hence, the aftereffects of these microorganisms post their transfer into the insect body needs a detailed investigation- role in mediating multi-trophic interactions, effects on insect metabolism etc. Few studies have addressed these questions so far (Lahlali and Hijri, 2010; Kim *et al.*, 2019). And, finally, we need to assess convenient and efficient methodologies/protocols to extract intact trichomes, without harming the leaf epidermis. This would expand horizons for conducting more elaborative and comprehensive research in various aspects like histochemical and ultrastructural microscopy-based studies.

**Acknowledgements**: The authors thank Dr. Juan Nunez-Farfan for the invitation to submit a chapter to the book. We also thank the reviewers, and this chapter was written with Start up, and RISING star funds awarded to Dr. Rupesh Kariyat from University of Texas Rio Grande Valley.

## Figures

Figure 2.1: Schematic representation of the structure of non-glandular trichomes a) simple unbranched: sharp and pointed trichomes with either long or short length. b) stellate: branched trichomes with arms arranged into a characteristic star-like shape surrounded by few epidermal cells on a vertical spike (Kim and Triplett, 2001; Wang, 2004). c) hooked type: pointed trichomes have a prominent curve at their tip, usually aligned at an angle between 20° and 90° to the leaf epidermis (Szyndler *et al.*, 2013). Credits: Annette Diaz

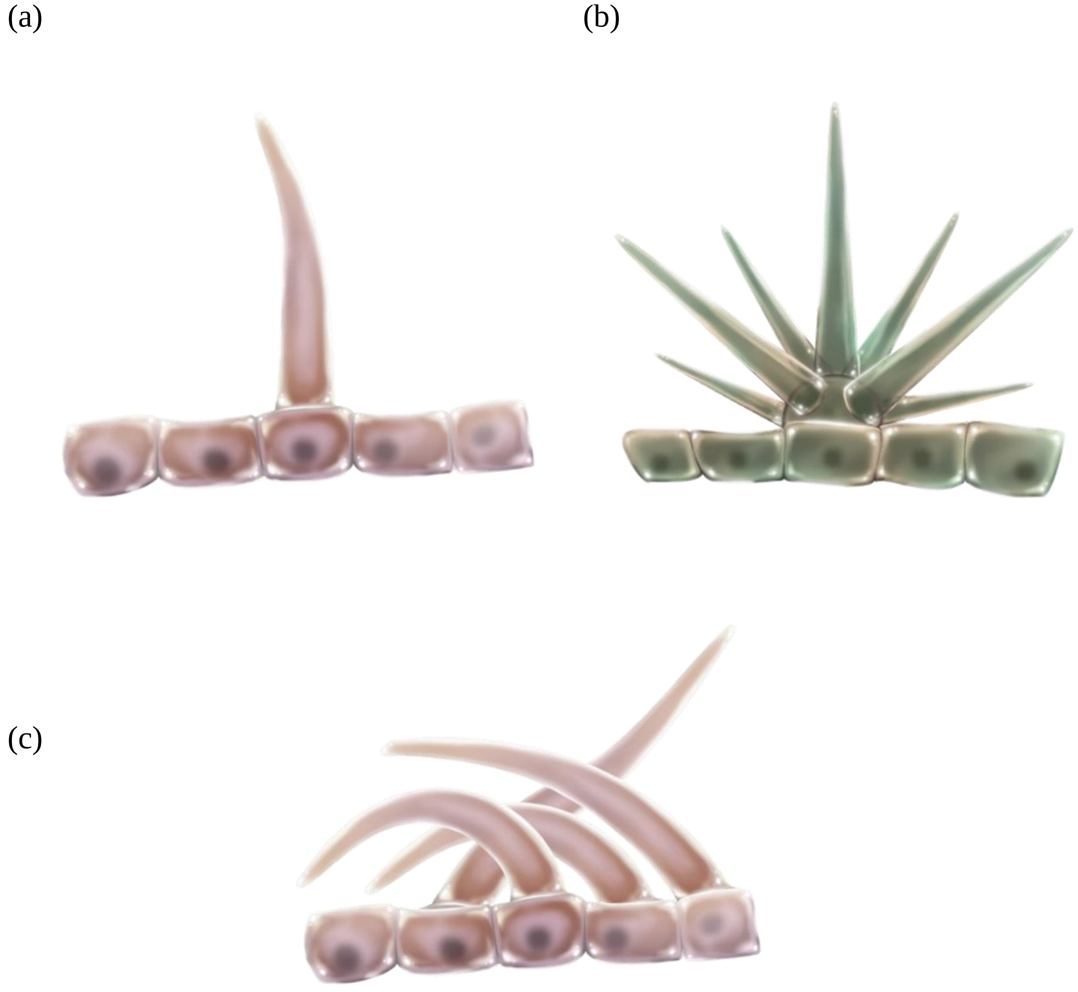


Figure 2.2 Schematic representation of structure of glandular trichomes: multicellular trichomes consisting of secretary head supported on pedestal made of vacuolated basal cell and stalk cell. The secretary head contains secondary metabolites (Wagner *et al.,* 2004). Credits: Annette Diaz

Figure 2.3 Schematic representation of initiation of trichome formation: Unicellular cell germinating from the leaf epidermis is illustrated. Consequently, this small protuberance enters mitosis and then endoreduplication cycle (Marks and Feldmann, 1989; Schnittger and Hulskamp, 2002). Credits: Annette Diaz

# CHAPTER II

# ARBUSCULAR MYCORRHIZAL FUNGI (AMF) INFLUENCES GROWTH AND INSECT COMMUNITY DYNAMICS IN SORGHUM-SUDANGRASS (SORGHUM X DRUMMONDII)

## Abstract

Beneficial plant–microbe interactions in the rhizosphere have been found to enhance plant growth and development. Arbuscular Mycorrhizal Fungi (AMF), a major group among these microbes, have been found to improve plant fitness through mycorrhizal symbiosis. Despite being well documented in various natural and domesticated study systems, few studies have examined whether AMF also has cascading effects on other traits, such as influencing insect community dynamics through attraction/repulsion of beneficial and damaging insects. To test this, we planted Sorghum-sudangrass (*Sorghum x drummondii*), a fast-growing annual grain/ forage crop, either inoculated with commercial AMF mix or left as control in lab and field experiments. We hypothesized that AMF would enhance plant growth and influence the recruitment of insect herbivores and their natural enemies due to possible alterations in plant defense pathways. Our results suggest that while AMF inoculated plants had significantly better germination, growth and establishment; they also experienced a lower initial incidence of fall armyworm (*Spodoptera frugiperda*), a major herbivore on Sorghum in the region. In addition, our insect community trapping experiment revealed that AMF inoculated plants attracted significantly more beneficial

insects (predators and parasitoids) and a lower number of damaging herbivores. Taken together, our field and lab data show that AMF can not only positively influence plant growth traits but can also provide defenses against herbivores by selectively attracting beneficial insects and repelling herbivores, with implications for sustainable pest management strategies.

## Introduction

Soil microorganisms are a critical component of the rhizosphere. Associations of beneficial microbes such as arbuscular mycorrhizal fungi (AMF) and plants date back millions of years (Reynolds et al. 2003). AMF are highly cosmopolitan and associate with nearly 80% of terrestrial autotrophs (Smith 2008; Fontana et al. 2009; Vannette and Hunter 2009). Mutualistic associations of AMF with their host plants has been found to influence plant growth and fitness by the exchange of resources (Goverde et al. 2000; Smith 2008; Fontana et al. 2009; Vannette and Hunter 2009; Kempel et al. 2010; Smith et al. 2011) which has been associated with increased yield (Anderson 1988) in various crops like maize (*Zea mays*), potato (*Solanum tuberosum*), yam (*Dioscorea alata*; Begum et al. 2019; Posta and Duc 2019) cowpea (*Vigna unguiculata*) , flax (*linum usitatissimum*; Posta and Duc 2019) and pepper (*Capsicum annuum*; Kaya et al. 2009). Moreover, they improve host plants' tolerance to abiotic stresses like drought, salinity and heavy metals, and can modify plant defenses (Bennett et al. 2006; Fiorilli et al. 2009; Kempel et al. 2010; Jung et al. 2012). Few studies have also documented that AMF can modulate plant interactions with herbivores, (Kempel et al. 2010) their natural enemies and pollinators (Pineda et al. 2010; Willis et al. 2013). Therefore, it is possible that AMF can have cascading effects on plant-insect interactions (Gehring and Bennett 2009; Khaitov et al. 2015), an area of research that warrants more attention.

As the major biotic stress, herbivorous insects either damage plant tissues and /or act as vectors for pathogens. While plants inoculated with mycorrhizae can defend better against root herbivores (Gange 2007; Gehring and Bennett 2009), few studies also suggest that they usually harbor higher proportions of sucking insects (e.g., aphids) and lower proportions of chewing insects (Gange 2002). Previous findings also report that generalist herbivores may perform poorly on the mycorrhizae inoculated plants (Rabin and Pacovsky 1985, Gange and West 1994; Kempel et al. 2010), while the specialists can overcome such changes, and even gain from the association (Gehring and Whitham 2002; Gehring and Bennett 2009; Kempel et al. 2010). Fall armyworm (*Spodoptera frugiperda;* FAW*)* (J.E. Smith) (Lepidoptera: Noctuidae) is one such leaf chewing generalist herbivore that can potentially be impacted by mutualism between AMF and the host plants (Mukherjee 2017). FAW is a polyphagous insect pest (Chapman 1999; Lange et al. 2018) that mainly feeds on grasses (*Gramineae*) and is an active forager (Buntin 1986), distributed worldwide, and has been considered as one of the most destructive crop pests (Degen et al. 2012; Padhee and Prasanna 2019).

AMF possibly alters the host plant defense chemistry by changing its nutritional status (Gange and West 1994; West 1995; Gosling et al. 2006), thereby mediating a wide range of species interactions. Consequently, AMF has been speculated to play a key role in shaping the organization and composition of ecological communities (Putten 2008; Hartley and Gange 2009). As one of the key mediators of insect plant interactions, plants emit a range of constitutive volatile compounds that either repel or attract herbivores, pollinators, and predators/parasitoids (Moraes et al. 1998; Chen et al. 2019). However, under herbivory they emit herbivore induced plant volatiles (HIPVs) (Pare and Tumlinson 1999; Kariyat et al. 2012; Ye et al. 2018) that can vary in quality and quantity from constitutive volatiles (Rowan 2011). Consequently, HIPVs have been found to act as signals to herbivores indicating that the host plant is already infested and is less suitable for feeding (Kariyat et al. 2014). More importantly, these volatiles can also increase the recruitment of natural enemies that either parasitize or predate the herbivores (Pare and Tumlinson, 1999; Dicke and Baldwin 2010), altering the tri-trophic interactions - a sustainable pest management approach that has gained momentum recently (Hill et al. 2018). Due to the ability of AMF to modify plant chemistry (Laird and Addicott 2007; Pozo and Azcón-Aguilar 2007; Hill et al. 2018), it is possible to expect that AMF can also alter multi-trophic interactions through plant-herbivore-natural enemy community dynamics.

Sorghum-sudangrass (*Sorghum x drummondii)* is a forage species from Sudan and southern Egypt that is well adapted to dry and hot climates. It is a common cover crop grown in the summer season in various agricultural ecosystems worldwide (Hariprasanna and Patil 2015; Venkateswaran et al. 2019), including the United States. The species can act as a natural weed suppressant due to its dense canopy (Wang et al. 2019). However, the plant along with its congener *Sorghum bicolor*, is also a host to a wide range of insect herbivores (Kariyat et al. 2019). Interestingly, there is limited understanding on the dynamics of insect community associated with this species, and more importantly, whether mycorrhizal association can potentially alter these interactions. Since the few studies on AMF-insect-plant interactions have reported varied results with different study systems (Johnson et al. 1997; Reynolds et al. 2005; Fonatana et al. 2009), using Sorghum-sudangrass as our host plant species, we examined whether the commercial AMF has cascading effects on plant growth and development, herbivory, and insect community dynamics, in an organic cropping system in LRGV in south Texas. We employed a combination of field and lab experiments to answer these questions. We hypothesized that: AMF inoculated plants will have better growth traits than the non-inoculated control plants, herbivore will be lower on the AMF inoculated plants as compared to the control and that AMF inoculated plants will attract more beneficial insects and lower number of herbivores.

## Materials and Methods

The field experiment was conducted at a 21-acre organic farm owned and operated by PPC farms in Mission, Texas, United States, 78572 (26.168425, -98.313547). The field was sown with seeds of two treatments - Sorghum-sudangrass seeds (Super sugar sudex variety, Green Cover Seed company, USA) inoculated with AMF (Wildroot® Organic Mighty Mycorrhizal Concentrate USA), and seeds without AMF inoculation (control), separated in the middle by a fallow land area, such that area under cover for each treatment is 4.5, 4.5 and 2 acres, respectively. Details of mycorrhizal species included in the commercial mix are included in the supplementary file (Data S1). Each seed lot of 22.67kg was inoculated with 200g of AMF (as recommended by the manufacturer) along with 60 ml water to make the powdered AMF formulation adhere to the seeds. The seeds were sown on ridges maintaining the seed rate of 22.67 kg/acre, during early summer of 2018. AMF inoculated seeds, as well as control seeds, were each sown on 106 ridges separately (9.5 acres per treatment), separated in the middle by 22 ridges (2 acres) of fallow; however, the experiments and observations were based on 4.5, 4.5 and 2 acres for seeds with AMF inoculation, without inoculation and fallow land area, respectively. Seeds were covered with 0.5 cm of soil after broadcasting and the field was flood irrigated immediately after sowing.

### Plant growth traits (field)

Various growth traits were recorded at early season (30-40 Days After Planting-DAP), mid-season (45-55 DAP) and late season (60 DAP and after), as described below:

**Plant height**. We recorded the height of 60 plants per treatment using the measuring tape from the base of the plant to the tip of the youngest leaf, during the mid-season. For this, we selected 6 rows randomly from each treatment, out of which 10 plants per row were further randomly selected. Additional height measurements were taken in the late season and recorded from another 100 plants per treatment using the same method.

**Number of fully opened leaves**. Total number of fully opened leaves per plant were recorded from 60 randomly selected plants per treatment (irrespective of any damage) during the mid-season. The same was recorded from 100 random plants per treatment twice during late season, using a 1m$^2$ quadrat made from PVC (Polyvinyl Chloride, Lowes Inc, Edinburg, Texas) pipes. The quadrat was randomly thrown 10 times into different directions per treatment. For each throw, data was recorded from 10 plants randomly selected from within the quadrat.

**Plant density**. During the mid-season, the quadrat was thrown 5 times and 8 times in the different directions within the AMF inoculated and control plots, respectively. The total number of plants contained in the quadrat was recorded. For the second density measurement, we doubled the sample size to 10 throws per treatment, and the number of plants in the quadrat was recorded during the late season. A third set of data was recorded again in late season.

**Plant girth**. To continue measuring the growth traits of the plant, girth of the plants from each treatment was recorded at the base of the plant. Data was recorded twice during the late season. The quadrat was thrown 10 times in the different directions within each treatment. Girth of 10 randomly selected plants contained in the quadrat was recorded using a digital Vernier caliper (Gyros® DIGI- SCIENCE™). It was calibrated before recording measurement from each plant. Similar data was also recorded for any throw even with less than 10 plants inside the quadrat.

### Plant defense traits

**Number of leaves damaged**. The field was surveyed for the number of insect-damaged leaves, more specifically, for the damage caused by the FAW. For this, 60 plants were randomly selected per treatment and carefully observed for any foliar damage done by FAW larvae such as ragged feeding on the foliage and the presence of small holes (Figure 3.S1) during the early season. Similarly, observations were recorded from 100 randomly selected plants per treatment during the late season.

**Presence of fall armyworm**. In addition to damage assessment, the treatments were also observed for the presence of FAW using two characteristic features- the actual presence of the FAW and/or the presence of caterpillar frass on the leaf whorls. During the early season, we examined 100 plants randomly selected per treatment and recorded the number of plants having presence of fall armyworms or its frass, or both. During the late season, the quadrat was randomly thrown 10 and 8 times within the AMF inoculated and control plots, respectively. The total number of plants contained in the quadrat was also recorded for signs of caterpillar incidence. The parameter was recorded again during the late season, again observing 100 randomly selected plants per treatment.

**Insect community**. To examine the insect community diversity associated with AMF inoculated and non-inoculated Sorghum-sudangrass, a trapping method comprising of three types of traps was employed (Figure 3.S2, B-S3). During the early season, six cages were set up diagonally (3-4 rows apart) in both treatments and fallow (n=18), covering an area of ~75m * 20 m in each plot. To build the cage, hardwire material (0.635 cm mesh size, 0.61 x 3.05 m - Lowe's, Blue Hawk, catalog number: 492388, model: 840147) was folded into a cylindrical shape (90 cm tall*76 cm diameter) and fastened with the zip ties. The top of the cage was fitted with the aluminum pie pan (22.2 cm dia. x 2.9 cm) fastened with two zip ties (28 cm) at the diametrically opposite ends. For sticky traps, white colored bridal veil nets (25 cm*30 cm) (Hobby Lobby, catalog number: 852640) covered with odorless tangle foot sticky glue (Tangle-Trap® Sticky Coating, catalog number: 300000676, Part No. LB8249) were placed uniformly opposite to each other on the cage, in the field. The sheets were secured with rubber bands placed at the top and bottom of the sheets, around the cage. For pitfall traps, two 266 ml clear plastic cups (Solo, Walmart, 554949033) were placed diametrically opposite to each other at the base of each cage in two holes dug (same size as the cup) around it, such that holes are situated on the ridges. The pitfall traps and aluminum pie pan traps were filled with water and Micro-90 odorless detergent (Cole-Parmer, catalog number:SK-18100-05) to trap insects. For details of the cage design, please see Kariyat et al. (2018), and the supplementary files (Figures B-S2, B-S3). The following day, pie pan traps were re-filled with soap water to replenish the water lost to evaporation. On the third day, the traps were removed and collected from the field (Kariyat et al. 2012; Kariyat et al. 2018). Each bridal veil was carefully removed and placed between two labeled sheets – an A4 size white sheet at the base and a clear acetate sheet at the top. Based on phylogeny and feeding guild, the insects trapped were identified in orders (and families when possible) (Kariyat et al. 2012) including predatory wasps and parasitoids (Hymenoptera), generalist and specialist herbivorous beetles (Coleoptera), caterpillars and adult moths and butterflies (Lepidoptera), true herbivorous bugs (Hemiptera), flies (Diptera) (Kariyat et al. 2012). The experiment was repeated later in the season following the same procedure.

### Seedling germination and establishment (lab)

In addition to the field experiments, we performed a series of controlled lab experiments to examine the germination and seedling establishment rates at different AMF concentrations: 0.1032g/500 seeds (recommended rate), two times the recommended rate (0.206g/500 seeds), half the recommended rate (0.052g/500 seeds) and a control (no AMF). Mean weight for 500 seeds (weighing balance-Accuris instruments, Bloodbankdepot®) was estimated (11.714g) to calculate the AMF required for each treatment. For each treatment, seeds were placed in separate vials containing a slurry made of required amount of AMF and 500µL of di-water. Control seeds were inoculated with di-water without AMF. Each vial containing inoculated seeds was vortexed for 30 seconds for uniform inoculation. Seeds were sown in trays (51.435cm*25.4cm) containing sterilized potting mixture (Berger- custom blend, Graco Fertilizer Company, Georgia, USA) and placed in an incubator (Sheldon Manufacturing, INC.) at a 25°C temperature and 16 hours day/ 8 hours night cycle. To ensure that each tray received an equal amount of light, they were rotated daily within the incubator. Number of emerged seedlings was recorded for three consecutive days, since the first day of emergence and final readings were recorded at 11th day after sowing. In addition, the length of seedlings (cm) was recorded at 10 days after emergence.

### Dry biomass measurements (field and lab)

To compare the shoots and roots biomass between both treatments, 30 plants per treatment from the field were uprooted along their roots near the time of crop termination. After separating the aerial parts and roots of each plant sample, the roots were washed to remove any attached soil from the field. Following this, the samples were allowed to dry in an oven (Quincy lab.INC, Fisher Scientific, USA) at 70°C for 2 days and weighed for dry biomass. Similar procedure was followed for the laboratory raised seedlings to analyze the difference between treatments for dry biomass, number of seedlings germinated and successfully established.

### Root staining and microscopy

A modified light microscopy based staining method (Mcgonigle et al. 1990) was followed to detect the colonization of plant roots by AMF. Five fine root fragments (1.5 cm) were collected from five random plants per treatment, uprooted from the field. The root cuttings were immersed in 10% KOH solution for 3-4 days to remove tannins in the roots and then gently rinsed with di-water twice. This was followed with immersing the roots in alkaline $H_2O_2$ bleach for 30 minutes and then rinsing with di- water twice. Next, the cuttings were drenched in 1% HCl for 30 minutes and gently rinsed with di-water. The processed roots were then stained overnight with a mixture of Trypan blue ink and acidified glycerol and later rinsed with di-water to clear the excess ink off the roots. To perform light microscopy, the stained root cuttings were mounted over slides and examined carefully for any vesicles, arbuscules or hyphal threads, under 100X to 400X magnification (Olympus BX53 upright microscope; Olympus camera adaptor U-TV1XC, C-mount; Software: LC micro 2.2, Olympus Soft Imaging Solutions, USA) (Figure 3.1).

### Statistical analysis

All analyses were performed using the statistical software JMP (Statistical Analysis Software Institute, NC, USA). The data for height parameter was analyzed with Mann-Whitney U test (non-parametric test) as it did not satisfy normality assumptions even after transformations. Two-tailed *t tests* were used to compare the mean number of fully opened leaves in AMF inoculated and non-inoculated treatments. Plant density was also analyzed using Mann-Whitney U test because the data did not meet normality assumptions. Two-tailed *t tests* were used to test compare the plant girth measurements between both treatments. Data for FAW damaged leaves in the mid-season was analyzed with Mann-Whitney U test (non-normal data) while two-tailed *t tests* were used for late season observations. For insect trapping experiments, pooled data from both collections was analyzed using univariate analyses. The counts for the orders Coleoptera and Diptera satisfied normality assumptions, so were analyzed with One-Way ANOVA, while

Hemiptera and Hymenoptera counts were analyzed with non-parametric Kruskal Wallis tests to compare insect diversity among both treatments and the fallow (Table 3.1). To reconfirm our univariate analyses (count data), we also ran a multivariate linear discriminant analysis to identify the separation of the main insect orders and the treatments from the insect diversity data. In our first analysis, we compared AMF and non-AMF treatments over the four insect orders of interest (Hymenoptera, Coleoptera, Diptera, and Hemiptera). A canonical plot was built with biplot axes using variables from the linear combination of covariates from the treatment groups and insect orders. We followed this by adding the fallow treatment and built additional canonical plot and used Wilks lambda to test the significance between the treatment groups. The logistical constraints of working in a farmer's field affected our ability to do replicated field trials, so detailed confirmation assays were carried out in lab. Data on seedling germination and establishment in the lab studies were analyzed using Chi-square tests for each pairwise comparisons among control seedlings without AMF and the three groups of seedlings with different AMF inoculations. Chi-square test was also used to examine if the data for FAW incidence/ presence at various stages are independent in both the treatments and whether there is significant difference in the presence of FAW between both the treatments. Lab studies data including shoot and root length and dry biomass were all normally distributed and were analyzed with One-Way ANOVA and, field dry biomass data analyses were performed using two-tailed *t test.* All the ANOVA analyses that had three treatment groups (AMF, non-AMF or control and fallow) were also subjected to appropriate post hoc tests to examine the significance of all pairwise combinations using Tukey or Dunn's tests for parametric and non-parametric tests respectively. More details of the statistics are provided in Table 3.2.

## Results

### Growth traits

The results from the field experiment revealed that AMF inoculated plants were significantly taller (29.6%) than non-inoculated plants (Mann-Whitney U test; U=1343, $P=0.0160$) (Figure 3.2a, Table 3.1). No significant difference was found for the number of fully opened leaves between the treatments (Two-tailed *t test*; $P=0.3505$) during the mid-season of plant growth (Figure 3.2b, Table 3.1). However, plants inoculated with AMF also produced significantly more leaves (Two-tailed *t test*s; $P<0.0001$; $P=0.0002$) than the control plants in the mid-season (Figure 3.2c), and towards the late season (Figure 3.2d). Control plants were significantly denser Mann-Whitney U test; U=55, $P<0.0001$) than plants inoculated with AMF (Figure 3.2e, Table 3.1); while AMF inoculated plants had significantly higher girth by 73.65% and 54% (Figure 3.2f,g, Table 3.1) than the control during the final two development stages (Two-tailed *t tests*; $P<0.0001$).

### Defense traits

Damage assessment in field showed that AMF inoculated plants suffered lower damage by FAW larvae than control plants, during the mid-season of the crop (Mann-Whitney U test, U=1616, $P=0.0045$; Figure 3.3a, Table 3.1). However, there was no significant difference for the number of FAW damaged leaves between two treatments later in the season (Two-tailed *t test*; $P=0.8749$; Figure 3.3a, Table 3.1). Consistent with this, our early season and late season herbivore observations in field also showed that plants inoculated with AMF had lower incidence of FAW ($X^2=4.261$, $P=0.0390$; $X^2=11.30$, $P=0.0008$). However, the third set of data recorded during the late season, shows no significant difference ($X^2=2.079$, $P=0.1493$) for the presence of FAW between both treatments (Figure 3.3b, Table 3.1). Our insect community trapping data also shows interesting trends. The data collected at 40 DAP and 60 DAP was examined and grouped by the major insect orders into damaging herbivores or beneficial insects. In total, we collected ~6400 insects. Our results showed a notable impact of AMF on the insect community composition. No significant results were found for affinity of Coleoptera insects (One-Way ANOVA; *P*=0.7520; Figure 3.3c, Table 3.1) to either AMF inoculated or control; however, we found specialist herbivorous beetles such as the *Diabrotica spp. and Epitrix spp.* (family: *Chrysomelidae*), and some generalist detrivorous beetles (families: *Carabidae* and *Staphylinidae)* that are particularly not harmful for the crops, in the traps. Interestingly, Hemipteran insects displayed a lower affinity to the AMF inoculated plants relative to the control (Kruskal Wallis test; *P*=0.0034; Figure 3.3d, Table 3.1) that included herbivores such as leaf hoppers and shield bugs (families: *Cicadellidae and Pentatomidae*). However, we found both beneficial (families: *Tachinidae* and *Syrphidae)* and herbivorous Dipterans (families: *Cecidomyiidae* and *Bradysia spp.*) in significantly higher numbers on control plants, and fallow when compared to AMF inoculated plants (One-Way ANOVA; *P*=0.0335; Figure 3.3e, Table 3.1). More interestingly, Hymenoptera were found in significantly higher numbers (Kruskal Wallis test; *P*=0.0056; Figure 3.3f, Table 3.1) on the plants incorporated with AMF. Winged beneficial Hymenopterans, largely comprising of the parasitoids in *Braconidae*, *Ichneumonidae,* predatory wasps, and ants, were largely driven towards the AMF inoculated Sorghum-sudangrass. Overall, AMF was positively associated with beneficial insects and negatively associated with damaging herbivores.

Our multivariate statistics with discriminant analyses also reinforced these results. The outer ellipse on the canonical plot (95% confidence level for each mean) clearly showed that the groups separated out without overlapping, showing significant differences and with distinct separation between the beneficial Hymenoptera clustered at the AMF when compared to other groups. Wilks lambda had a value of 0.408 and a $P < 0.0001$, showing significant treatment differences and robustness of the group separation in terms of a direct measure of the proportion of variance in the combination of dependent variables that is unaccounted for by the independent variable (Eigen value= 1.4487, F value=7.23; Figure 3.4a). For second replication, we built a similar plot and conducted analyses but with additional treatment of fallow (mostly infested by weedy grasses). Like the previous model, we found that the AMF treatment separated from the non-AMF and from the fallow treatments, while non-AMF and fallow overlapped their ellipses (Eigen value= 1.3655, F value=11.22; Figure 3.4b). In addition, Wilks lambda had a value of 0.389 and a $P < 0.0001$, clearly showing significant differences in treatments. Taken together, the analyses clearly show that AMF plants varied from both other treatments by attracting higher number of Hymenoptera insects and lower number of other insect groups.

### Seedling germination and establishment in lab

To confirm the effect of AMF on Sorghum-sudangrass under sterilized soil conditions (without the presence of any native AMF in field), we conducted laboratory experiments at different concentrations of AMF inoculum (Figure 3.5, Table 3.2). We found that seeds inoculated with twice the recommended rate of AMF germinated significantly more seedlings than control seeds ($X^2=15.97$, $P<0.0001$). However, we did not find any significant difference between seeds inoculated at recommended rate and control seeds ($X^2=0.0083$, $P=0.9272$). Moreover, we found significant difference comparing half the recommended rate against control seeds, where the control seeds germinated better ($X^2=167.069$, $P<0.0001$). While comparing the germination effects among the different rates of AMF, we found that seeds inoculated at twice the recommended rate of AMF germinated significantly better than seeds inoculated at both the recommended rate and half the recommended rate of AMF ($X^2=55.85$, $P<0.0001$ and $X^2=26.29$,

$P<0.0001$). However, we also found that seeds inoculated with half the recommended rate germinated better than seeds inoculated at the recommended rate of AMF ($X^2=7.883$, $P=0.0050$) (Figure 3.5a). Taken together, our results suggest that AMF in general significantly improved the germination rate of sorghum sudangrass.

However, some of this effect was lost at establishment stage (Figure 5b). Results from seedling establishment (3 weeks after seeding) suggest that seeds inoculated at recommended rate and half the recommended rate of AMF established significantly better than control ($X^2=4.161$, $P=0.0414$; $X^2=4.536$, $P=0.0332$). Moreover, the establishment rates were significantly higher for seeds inoculated with double the recommended rate against control ($X^2=8.228$, $P=0.0041$) (Figure 3.5, Table 3.2).

**Field and lab biomass**

For the samples collected from field, the plants inoculated with AMF had significantly higher shoot dry biomass (Two-tailed *t tests*; $P=0.0128$; Figure 3.6a, Table 3.1) while no significant difference was found for root dry biomass between the two treatments (Two-tailed *t tests*; $P=0.7684$; Figure 3.6b, Table 3.1). Nevertheless, the overall dry biomass was significantly higher for AMF inoculated plants (Two-tailed *t tests*; $P=0.0245$; Figure 3.6c, Table 3.1). Lab experiments conducted under sterilized soil conditions, free of native mycorrhizae, suggest that inoculation with different concentrations of commercial AMF produce seedlings with significantly higher dry biomass (One-Way ANOVA, $P= 0.0006$; Figure 3.6d-f). Tukey's multiple comparisons test suggests that the seedlings with twice the recommended rate of AMF (double concentration) significantly gained more biomass than rest of the treatments (Tukey's multiple comparisons, $P=0.0012$; $P=0.0029$). However, the difference between dry biomass of seedlings with AMF at recommended rate and control showed no significant difference (Tukey's multiple comparisons, *P*=0.9396; Figure 3.6d-f). Also, seedlings with twice the recommended rate of AMF had higher shoot length than seedlings with recommended rate and control treatments (Tukey's multiple comparisons, *P*=0.0013; *P*=0.0299). Shoot length among seedlings at the recommended rate of AMF, half the recommended rate of AMF and control treatments (Tukey's multiple comparisons, *P*=0.1159; *P*=0.7358; *P*=0.6520), and between half the recommended rate and double the recommended rate of AMF were not significantly different (Tukey's multiple comparisons, *P*=0.2578) (Figure 3.6d). Similarly, we did not find any significant differences for root length between half the recommended rate and twice the recommended rate of AMF (Tukey's multiple comparisons, *P*= 0.1454). Additionally, no significant differences were found in root length between the seedings with recommended rate and twice the recommended rate of AMF (Tukey's multiple comparisons, *P*=0.5508). However, seedlings with half the recommended rate had very significantly higher root length than the seedlings with recommended rate of AMF (Tukey's multiple comparisons, *P*=0.0023). Not surprisingly, roots of seedlings with half the recommended rate and twice the recommended rate of AMF were significantly longer than the roots of control seedlings (Tukey's multiple comparisons, *P*<0.0001; *P*=0.0103) (Figure 3.6e). Taken together, our data shows the positive effect of AMF on total biomass of the plants from the field and seedlings from the lab, along with positive concentration dependent effects on plant growth.

### Light microscopy for arbuscules detection

The results for the light microscopy clearly showed arbuscules in the roots fragments of AMF colonized plants from the field, thereby, suggesting a successful colonization of Sorghum-sudangrass roots by AMF fungi. There were little to no arbuscules found in the roots of plants without AMF inoculation (Figure 3.1).

## Discussion

Our findings demonstrate that AMF can provide both overall growth as well as defense benefits to plants against herbivores. Improved growth traits in our study system can be attributed to the increased availability of nutrients by AMF to the host plants (Lynch 1992; Roesti et al. 2006; Smith 2008). In our study, AMF inoculated plants were significantly taller than control plants in resonance with various recent and past studies. For example, Murrell et al. (2019), showed that AMF colonization increases the growth in cover crops (Murrell et al. 2019). Similarly, Bi et al. (2018) recorded increased height in AMF inoculated *Amygdalus pedunculata*, a native tree species used for ecological restoration, than the control plants (Bi et al. 2018). We also recorded significantly higher girth in AMF inoculated plants than the control plants (Figure 3.2f, g), suggesting a positive impact of AMF on plant vigor (Siddiqui et al. 2008). Not surprisingly, AMF inoculated plants grow vigorous over the course of the season (Zangerl and Rutledge, 1996). Young seedlings have been found to invest their resources more towards height increment before diverting it to leaf production (Weiner 1994; Nagashima 1995; Nagashima and Hikosaka 2011), and in our experiments we see that the plants inoculated with AMF had a higher number of leaves than control only post the initial growth stage (Figure 3.2 c, d).

In a recent study (Murrell et al. 2019), AMF inoculated plants were found to invest more in growth and defenses simultaneously. Similarly, in our study, the number of insect damaged leaves during the mid-season and FAW incidence until the mid-season, were significantly lower in the AMF inoculated plants than control (Figure 3.3a, b). AMF possibly alters the chemical composition of plants by changing their nutrient pool (Weiner 1994). Therefore, healthy and nutrient rich (Zangerl et al. 2007; Mithöfer et al. 2018; Formenti and Rasmann 2019) AMF inoculated plants allocated more resources to defend against herbivores, in this case against FAW.

We speculate that AMF inoculated plants either produced defense chemicals to hinder the FAW feeding/ development or activated signaling molecules that mediate defense pathways in the species. Additionally, the negative impact of mycorrhizal-plant association on herbivores through altered carbon: nitrogen ratio has been reported previously (Bryant et al. 1983). They speculated AMF inoculated plants invest higher in carbon-based secondary metabolites, thereby discouraging herbivores (Bryant et al. 1983; Kempel et al. 2010). A recent study confirmed the activation of jasmonic acid signaling pathway in various AMF inoculated cover crops under the attack of European corn borer (*Ostrinia nubilalis*), a polyphagous lepidopteran pest (Murrell et al. 2019), with similar feeding habit as FAW. However, we also found that FAW was able to establish itself and colonized the entire field towards the later crop growth stages, including AMF plants (Figure 3.3a, b). It is possible that AMF helps the crop protect itself against herbivores during the initial establishment stages, thereby allowing the host to allocate more resources for fitness (Formenti and Rasmann 2019) and once established on the non-inoculated plants, FAW eventually damage the AMF plants as well as in the late season.

More interestingly, our insect community trapping experiment recorded twice at 40 and ~60 DAP showed that AMF inoculated plants attracted lower number of harmful Hemipteran and Dipteran herbivores (Figure 3.3d,e) but significantly more natural enemies of Hymenoptera (parasitoids and predators) (Kariyat et al. 2012) than control plants (Figure 3.3f). Therefore, AMF indirectly defends the host plants against herbivores by recruiting more natural enemies, through tri-trophic interactions (Hempel et al. 2009). In fact, a study has documented increased number of hymenopteran insects visiting the mycorrhizae inoculated plants than the control plants (Gange and Smith 2005). However, we found no difference among the number of coleopterans trapped in the treatments including both generalist (predatory and detrivorous) and specialist (herbivorous) beetles. We speculate that constitutive and/or induced volatile compounds produced by the plants from each treatment regulated the movement of both herbivores and beneficial arthropods (parasitoids and predators) (Kariyat et al. 2012). Therefore, AMF possibly helps inoculated plants to alter their volatiles to attract the beneficial insects. However, it is unclear if the increased defenses in the inoculated plants are a resultant of increased nutrients acquisition and consequently more available resources towards the plant defenses or its direct association with the plant roots. In contrast, less vigorous and resource-limited control plants, are possibly more susceptible to herbivory, for example, through reduced induction of defensive plant volatile compounds such as terpenes (Heil 2008; Kariyat et al. 2012). Taken together, our data validates the efficacy of AMF inoculated crops to attract beneficial arthropods and repel damaging insects.

To confirm that the effects observed in our experiments were primarily due to inoculated commercial AMF on Sorghum-sudangrass and not overpowered by natural AMF present in the soil systems (Torrecillas et al. 2011; Berruti et al. 2016), we conducted various laboratory experiments at different concentrations of AMF inoculum under sterilized soil conditions (without the presence of native AMF). Not surprisingly, we found a concentration dependent effect on the germination of seeds inoculated at different rates of AMF: Seeds inoculated with twice the recommended rate of AMF germinated and established better than control seeds. However, seeds inoculated with double the recommended rate performed better than other two AMF rates, which further strengthens the premise of concentration dependent effect of AMF on seedling germination and establishment. These findings confirm the results for growth traits obtained from the field study and the often-documented positive results of AMF on plant growth in various other studies, except Maighal et al. (2016) which showed that AMF negatively affects seed viability in the soil (Maighal et al. 2016). However, very recently, concentration dependent effects of AMF have also been found in *Medicago truncatula* against pea aphids (*Acyrthosiphon pisum*) (Garzo et al. 2018). Notably, more research in this field under both lab and field conditions is warranted- to understand the optimum concentration of commercial AMF to reap both growth and defense benefits. It is quite clear that AMF can successfully alter plant chemistry that can modulate defense responses, possibly through plant volatiles and secondary defense metabolites; an area we are currently exploring.

Recently, AMF inoculated crops were reported to have increased shoot and root dry biomass of 80.8% and 73.6% respectively in a total of 146 and 91 experiments used in their analyses (Berruti et al. 2016). In our study, field grown AMF inoculated plants had greater aerial dry biomass than control (Figure 3.6a) possibly due to acquisition of more nutrients (Roesti et al. 2006). Additionally, for seedlings grown under controlled lab conditions (without the presence of native AMF), seedling growth was found to be positively associated with AMF concentration (Figure 3.6d), even during the initial establishment stages. Consistent with our results, Bi et al. (2018) also recorded increased root and shoot growth in AMF inoculated *Amygdalus pedunculata* trees (Bi et al. 2018). Also, since during initial stage plant tends to invest more energy in elongation rather than secondary growth, (Weiner 1994; Nagashima 1995; Nagashima and Hikosaka 2011) we found similar results to the total dry biomass results for the seedlings grown in lab (Figure 3.6f). Similarly, higher biomass for mycorrhizal wheat has also been previously documented in some studies (Al-Karaki et al. 2003; Zhu et al. 2015)

## Conclusions

Overall, our results suggest that AMF boosts the crop health and vigor. But more importantly, AMF repels damaging herbivores while selectively attracting natural enemies in the initial crucial stages of crop growth and development (Weiner 1994). Our results also show that AMF effects are clearly visible in early stages through germination, establishment, growth and herbivore defenses. The mechanisms underlying these effects warrant immediate and detailed examination.

**Supplementary Materials**: Data S1: Different mycorrhizal species included in the mix, Figure 3.S1: Feeding damage by fall armyworm (*Spodoptera frugiperda)* on Sorghum-sudangrass *(Sorghum x drummondii)*, Figure 3.S2: Schematic of the trap set up for insect community experiment enclosing Sorghum-sudangrass *(Sorghum x drummondii)*, Figure 3.S3: Picture of unique three trap cage set up enclosing Sorghum-sudangrass *(Sorghum x drummondii)* in the field to collect attracted insect community.

**Acknowledgments:** Lindsey Richards, Habraham Lopez and Stephanie Kasper for their immense help with getting the field experiments done, Anwar Garza for providing the land, labor and equipment for the field experiments, Paloma Flores for assisting in lab experiments, Dr. Lekshmi Sasidharan for statistics, and Lili Martinez for assisting in field trap set up and collection.

## Tables and Figures

Table 3.1. Details of statistical analyses to examine the effects of arbuscular mycorrhizal fungi on various growth traits, defense traits, seedling germination, seedling establishment and the insect community dynamics in Sorghum-sudangrass *(Sorghum x drummondii)*. Significant differences are in bold at $P<0.05$.

| ***Trait*** | ***Test*** | ***Test Statistics*** | ***P value*** |
|---|---|---|---|
| Height | Mann-Whitney U test | Mann-Whitney U=1343 | **0.016** |
| Opened leaves<br>Mid-season | Two-tailed *t test* | *t*=0.9372, df=118 | 0.3505 |
| Opened leaves<br>Late season 1 | Two-tailed *t test* | *t*=4.238, df=177 | **<0.0001** |
| Opened leaves<br>Late season 2 | Two-tailed *t test* | *t*=3.861, df=197 | **0.0002** |
| Plant density | Mann-Whitney U *test* | Mann-Whitney U=55 | **<0.0001** |
| Plant girth<br>Late season 1 | Two-tailed *t test* | *t*=5.658, df=177 | **<0.0001** |
| Plant girth<br>Late season 2 | Two-tailed *t test* | *t*=6.310, df=195 | **<0.0001** |
| Number of fall armyworm (FAW) damaged leaves<br>Mid-season | Mann-Whitney U test | Mann-Whitney U=1616 | **0.0045** |
| Number of FAW damaged leaves<br>Late season | Two-tailed *t test* | *t*=0.1576, df=198 | 0.8749 |
| Insect diversity: Coleoptera | One-Way ANOVA | *F*= 0.2867, df = 2, 51 | 0.7520 |

| | | | |
|---|---|---|---|
| Hemiptera | Kruskal-Wallis One-Way ANOVA | Kruskal-Wallis Statistic =11.37 | **0.0034** |
| Diptera | One-Way ANOVA | *F*= 3.634, 2, 51 | **0.0335** |
| Hymenoptera | Kruskal-Wallis One-Way ANOVA | Kruskal-Wallis Statistic=10.36 | **0.0056** |
| Lab: Shoot length | One-Way ANOVA | *F*=5.429, df=3,76 | **0.0019** |
| Lab: Root length | One-Way ANOVA | *F*=11.61, df=3,76 | **<0.0001** |
| Lab: Total Biomass | One-Way ANOVA | *F*=8.545, df=2,53 | **0.0006** |
| Field: shoot biomass | Two-tailed *t test* | *t*=2.571, df=57 | **0.0128** |
| Field: root biomass | Two-tailed *t test* | *t*=0.2958, df=57 | 0.7684 |
| Field: Total biomass | Two-tailed *t test* | *t*=2.311, df=57 | **0.0245** |

Table 3.2. Results of Chi-square tests of multiple pairwise comparisons of seedling germination and seedling establishment among control and three concentrations of AMF. Pairwise comparisons have also been included for fall armyworm (*Spodoptera frugiperda*) incidence over three-time intervals of Sorghum-sudangrass growth (*Sorghum x drummondii)*. Chi square test statistics at respective degrees of freedom (*df*) and *P values* are also included. Significant results with *P*< 0.05 are in bold.

| ***Comparison*** | ***$X^2$, df*** | ***P value*** |
|---|---|---|
| ***Seedling germination*** | | |
| Control (no-AMF) and Standard concentration | 0.0083,1 | 0.9272 |
| Control and Half concentration | 167.069,1 | **<0.0001** |
| Control and Double concentration | 15.97,1 | **<0.0001** |
| Double and Standard concentration | 55.85,1 | **<0.0001** |
| Double and Half concentration | 26.29,1 | **<0.0001** |
| Standard and Half concentration | 7.883,1 | **0.0050** |
| ***Seedling establishment*** | | |

|  |  |  |
|---|---|---|
| Control (no-AMF) and Standard concentration | 4.161,1 | **0.0414** |
| Control and Half concentration | 4.536, 1 | **0.0332** |
| Control and Double concentration | 8.228,1 | **0.0041** |
| Double and Standard concentration | 0.7024,1 | 0.4020 |
| Double and Half concentration | 0.5586,1 | 0.4548 |
| Standard and Half concentration | 0.0082,1 | 0.9276 |
| ***Fall army worm (FAW) incidence in field*** |  |  |
| Early season (30 DAP) | 4.261,1 | **0.039** |
| Late-season I (60 DAP) | 11.30,1 | **0.0008** |
| Late season II (70 DAP) | 2.079,1 | 0.1493 |

Figure 3.1: Light microscopy images of Sorghum-sudangrass *(Sorghum x drummondii)* root fragments. (a) Arbuscular mycorrhizal fungi (AMF) inoculation indicated by presence of blue stained arbuscules in the roots (b) Absence of AMF inoculation indicated by clear root with no arbuscules.

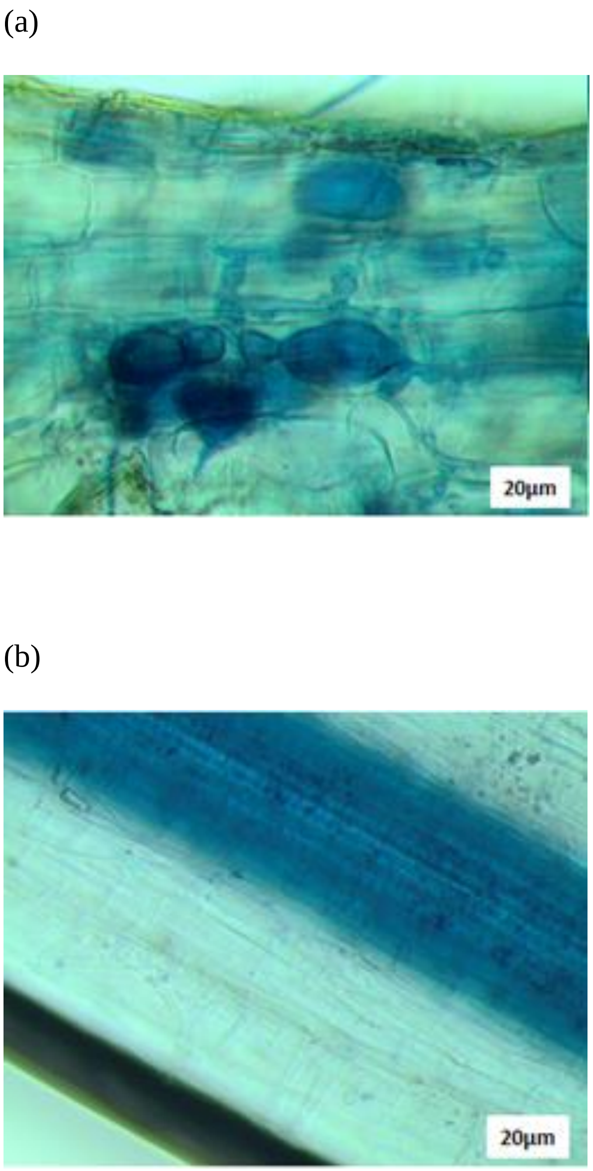
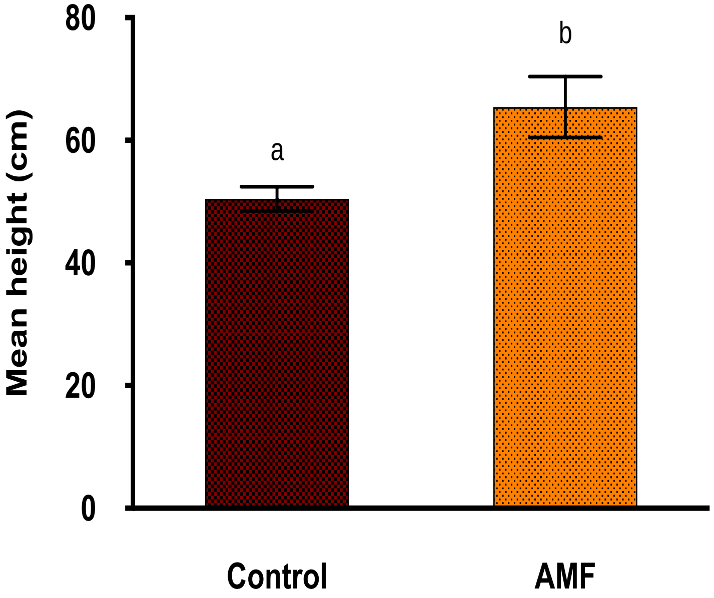
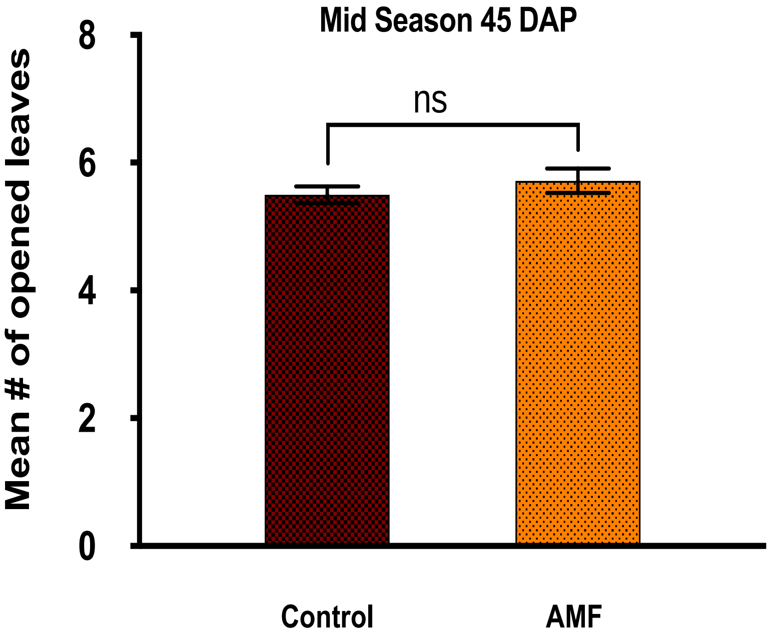


Figure 3.2: Results of growth traits comparisons between control and arbuscular mycorrhizal fungi (AMF) inoculated Sorghum-sudangrass *(Sorghum x drummondii)* in field. (a) mean height (cm); mean number of fully opened leaves at (b) mid-season (45 DAP), (c) at 50 DAP, (d) late season (60 DAP); (e) mean plant density/m$^2$; mean girth (cm) at (f) late season (60 DAP) and (g) late season (70 DAP) are reported**.** Mean and standard error of the results of Mann-Whitney U test of the mean height between plants (y-axis) (in cm), two-tailed *t tests* to examine the number of opened leaves between plants (y-axes) (in cm), Mann-Whitney U tests of mean density of plants (in plants per m$^2$) (y-axis), two-tailed *t tests* data analysis of measurement for girth of plants (in cm) (y-axis) in control and AMF treatment (x-axis) are represented. Statistically significant differences are represented by different lowercase alphabetical letters at $P<0.05$, while ns denotes non-significant results.

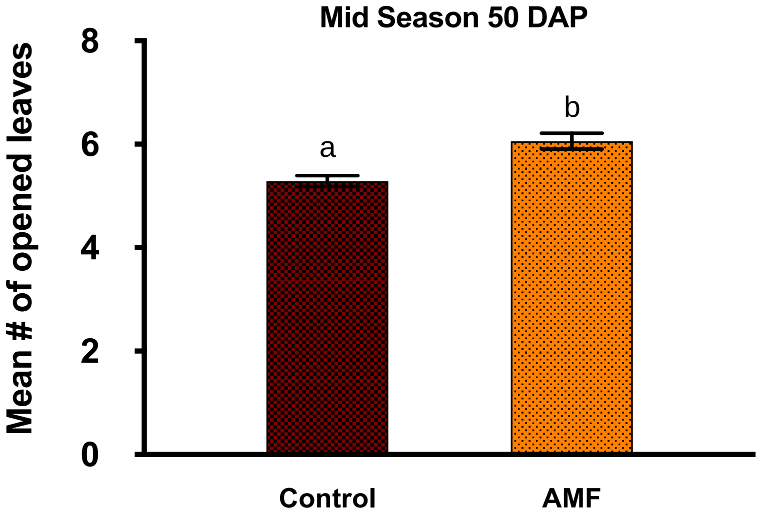
(a)



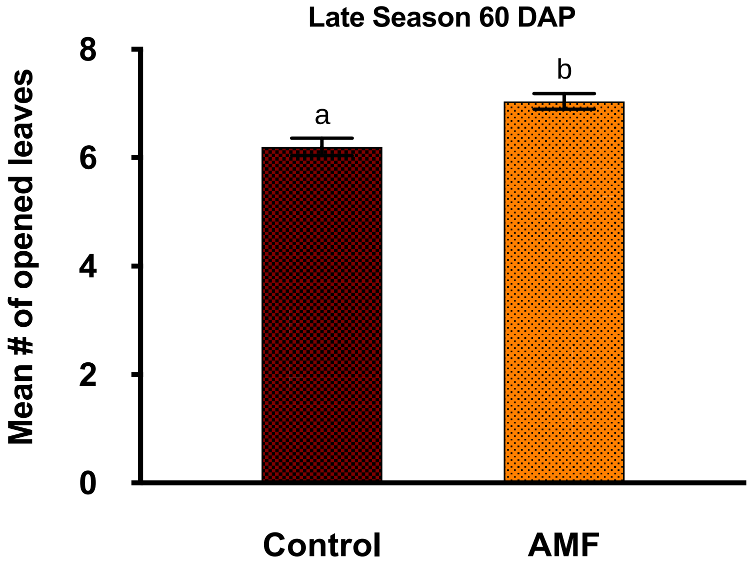
(b)


Mid Season 45 DAP
Mean # of opened leaves
8
6
4
2
0
ns
Control
AMF

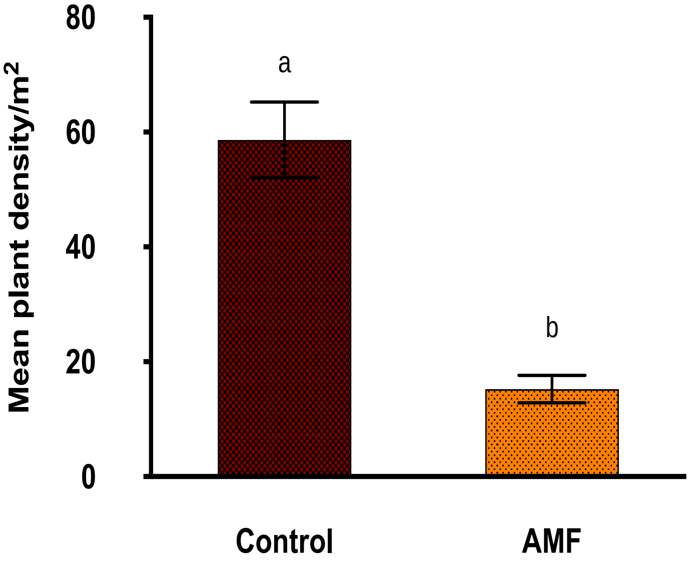
(c)


Mid Season 50 DAP
Mean # of opened leaves
8
6
4
2
0
a
b
Control
AMF

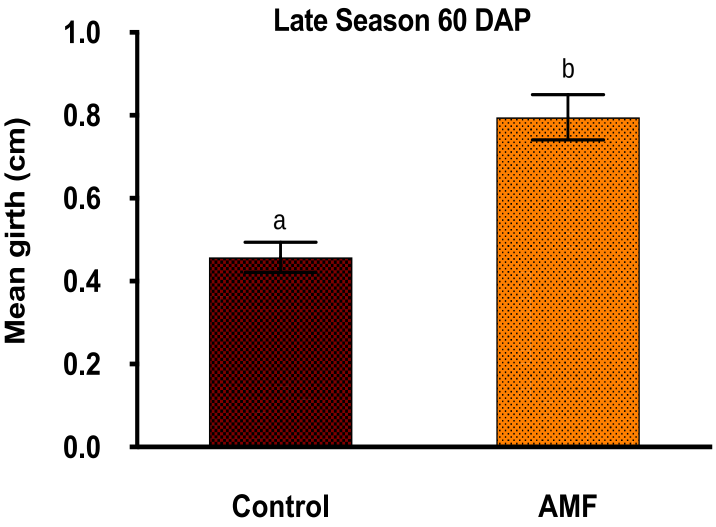
(d)


Late Season 60 DAP
Mean # of opened leaves
8
6
4
2
0
a
b
Control
AMF

(e)


Mean plant density/m²
80
60
40
20
0
a
b
Control
AMF

(f)


Late Season 60 DAP
Mean girth (cm)
1.0
0.8
0.6
0.4
0.2
0.0
a
b
Control
AMF

(g)

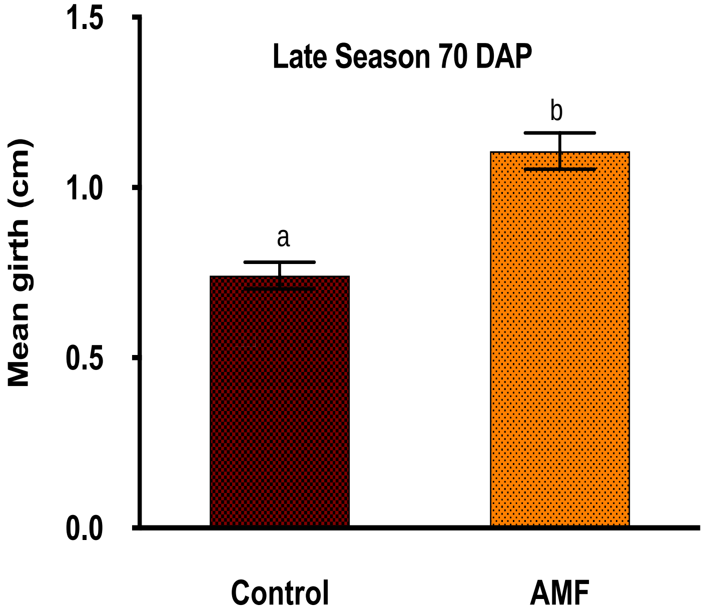
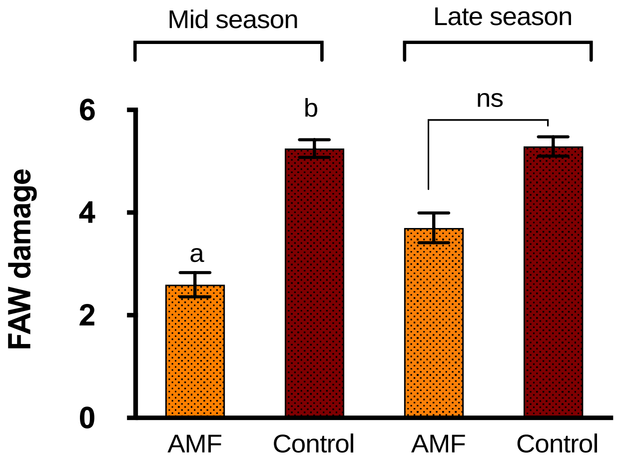
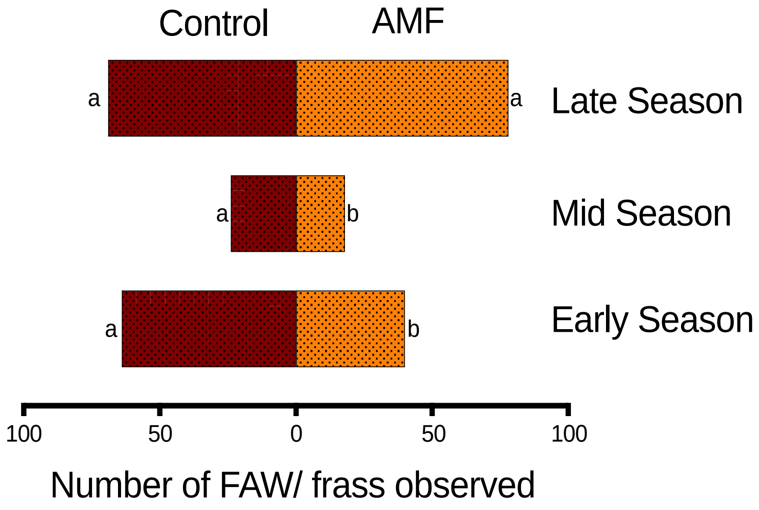

Late Season 70 DAP
Mean girth (cm)
1.5
1.0
0.5
0.0
a
b
Control
AMF

Figure 3.3: Results of defense traits comparisons among different treatments of Sorghum-sudangrass *(Sorghum x drummondii)*. (a) fall armyworm damage; (b) number of fall armyworm / frass observed; (c)-(f) mean number of Coleopteran, Hemipteran, Dipteran and Hymenopteran insects, respectively. Mean and standard error of the results of Mann-Whitney U test and two-tailed *t test* for fall armyworm damage during mid and late season, respectively (y-axis) in control and AMF treatment (x-axis) are represented; One-Way ANOVA tests to examine mean number of coleopterans and dipterans (y-axis) and Kruskal Wallis test to examine mean number of hemipteran and hymenopteran insect diversity (y-axis) in control ,AMF inoculated and fallow plot (x-axis) are represented. Statistically significant differences are represented by different lowercase alphabetical letters at *P*<0.05, while ns denotes non-significant results.

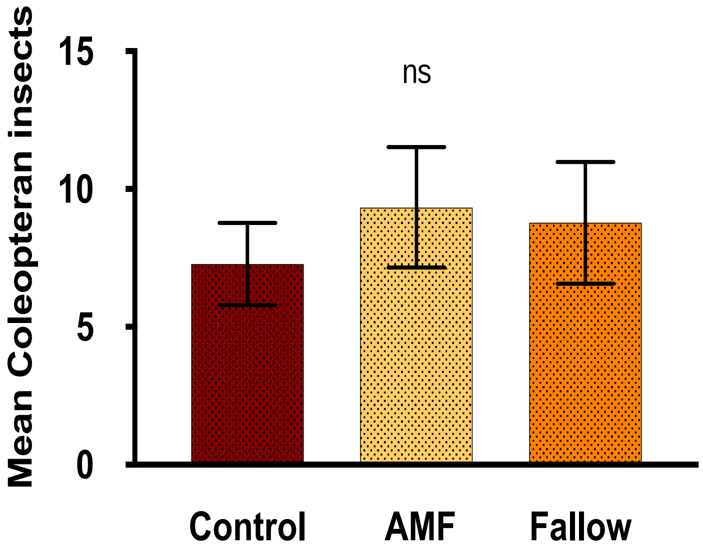
(a)



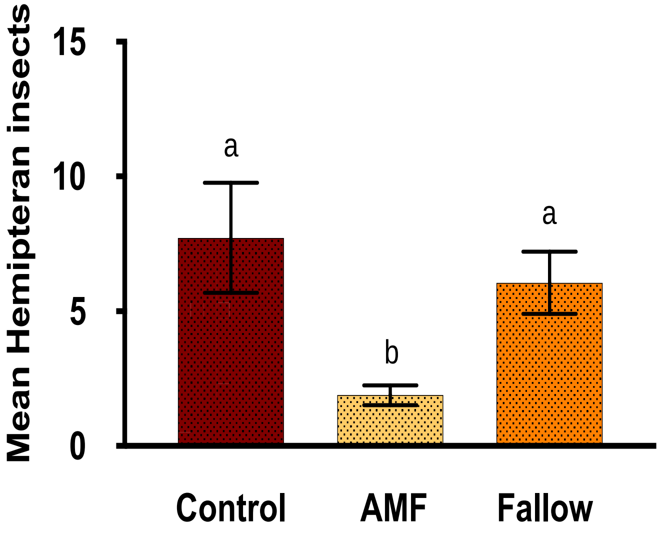
(b)


Control
AMF
a
a
Late Season
a
b
Mid Season
a
b
Early Season
100
50
0
50
100
Number of FAW/ frass observed

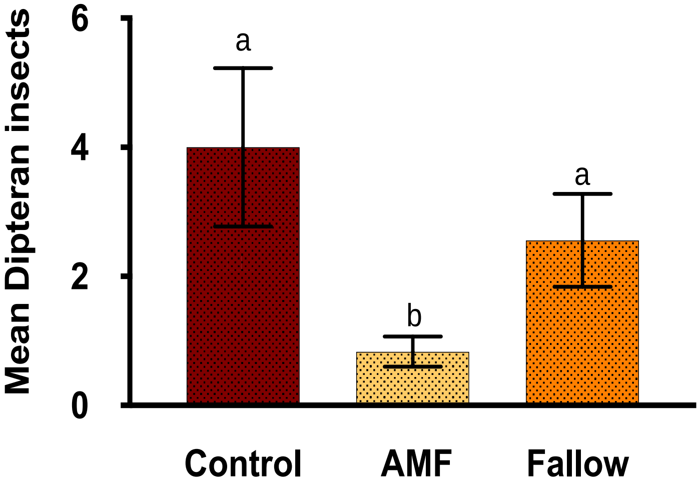
(c)


Mean Coleopteran insects
15
10
5
0
ns
Control
AMF
Fallow

(d)


Mean Hemipteran insects
15
10
5
0
a
a
b
Control
AMF
Fallow

(e)


Mean Dipteran insects
6
4
2
0
a
a
b
Control
AMF
Fallow

(f)

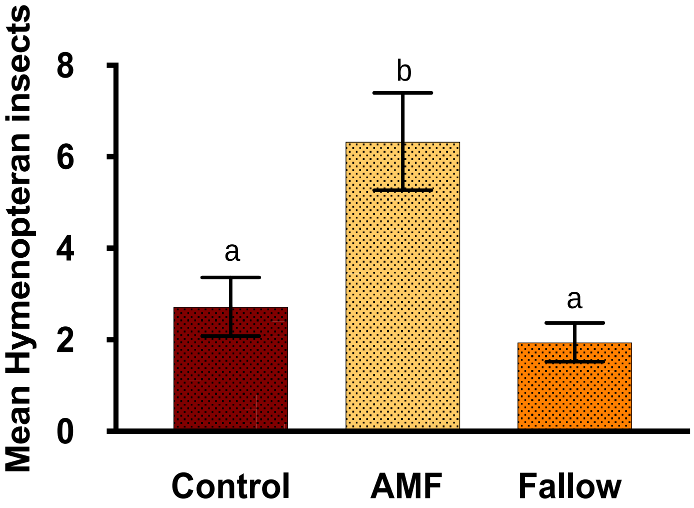
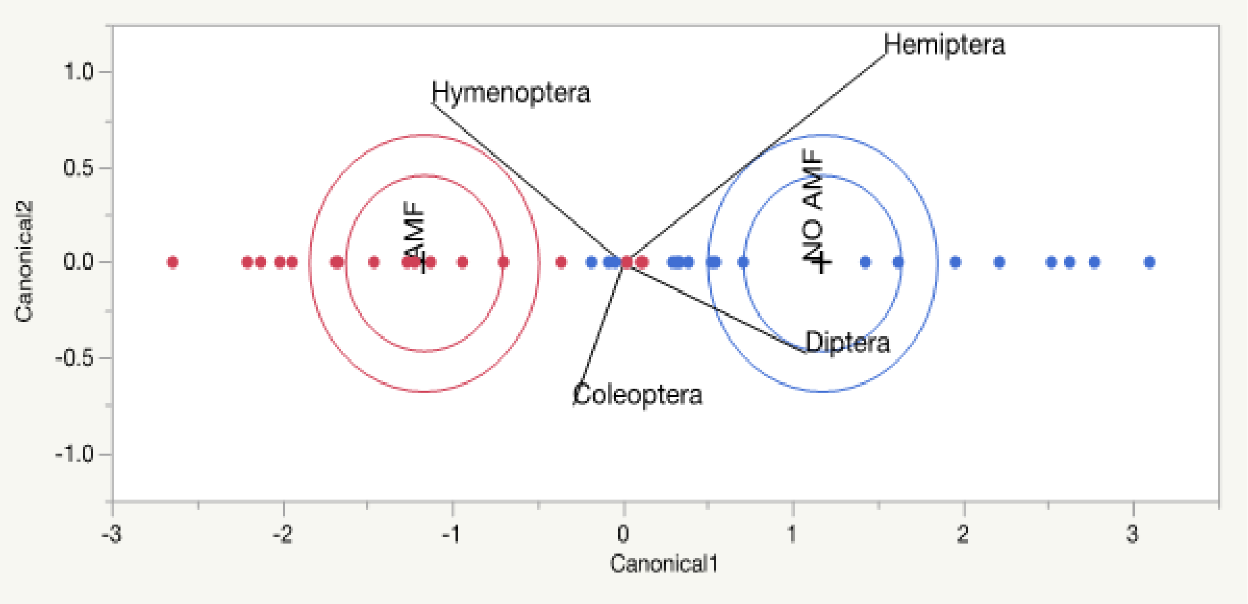


Figure 3.4: Canonical plot depiction of insect community attraction. Canonical plots were constructed for a linear discriminant analysis for the attraction of four insect orders of interest (Hymenoptera, Coleoptera, Diptera, and Hemiptera) with two biplot axes that has the two canonical variables from the linear combination of covariates from the treatment groups and insect orders. The outer ellipse represents 95% confidence interval for each mean and the colored dots represent the treatments. The length and direction of each ray that represents the covariates in the biplot indicates the degree of association of the corresponding covariate with the first two canonical variables. (a) represents the plot for AMF and non-AMF comparisons, and (b) represents the plot for AMF, non-AMF and fallow treatments. Non-overlapping ellipses and canonical details calculated from the overall pooled within-group covariance matrix shows that the treatments differ from each other in their attractiveness towards the insect orders (Table 3.2).

(a)



(b)

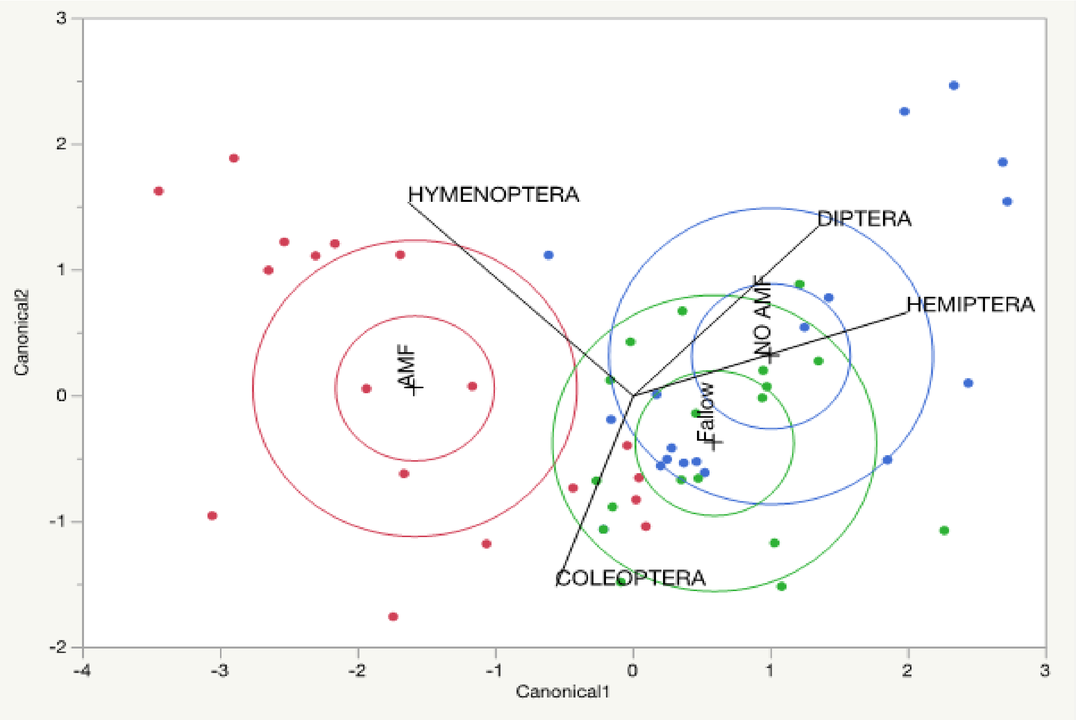
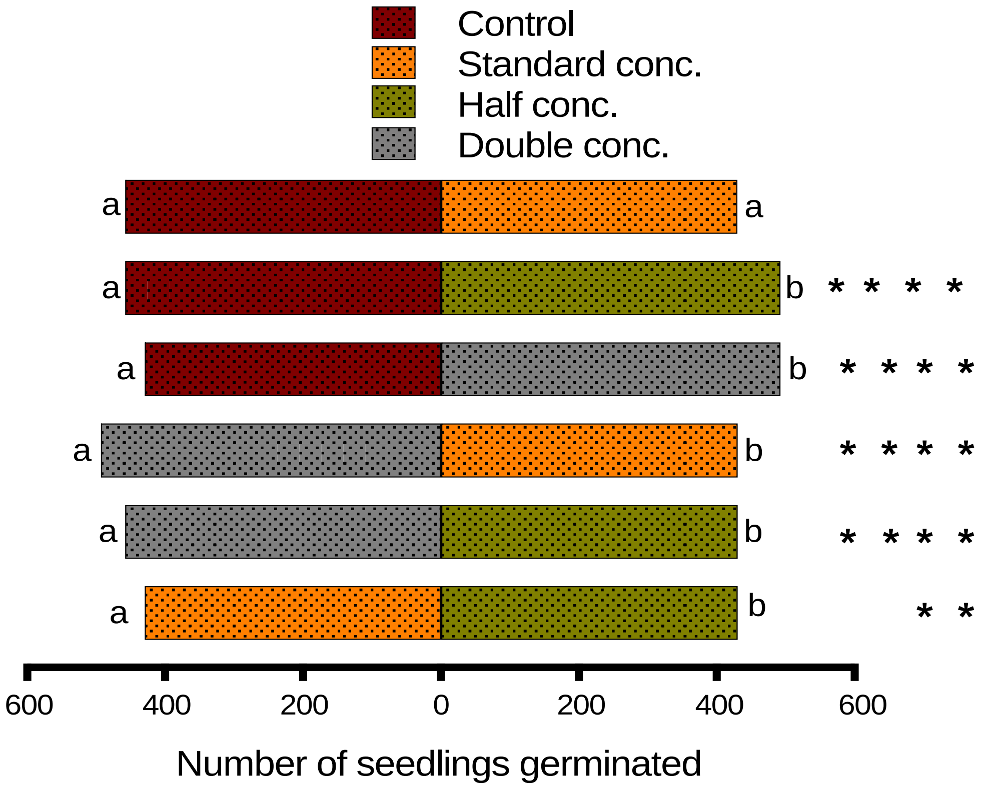
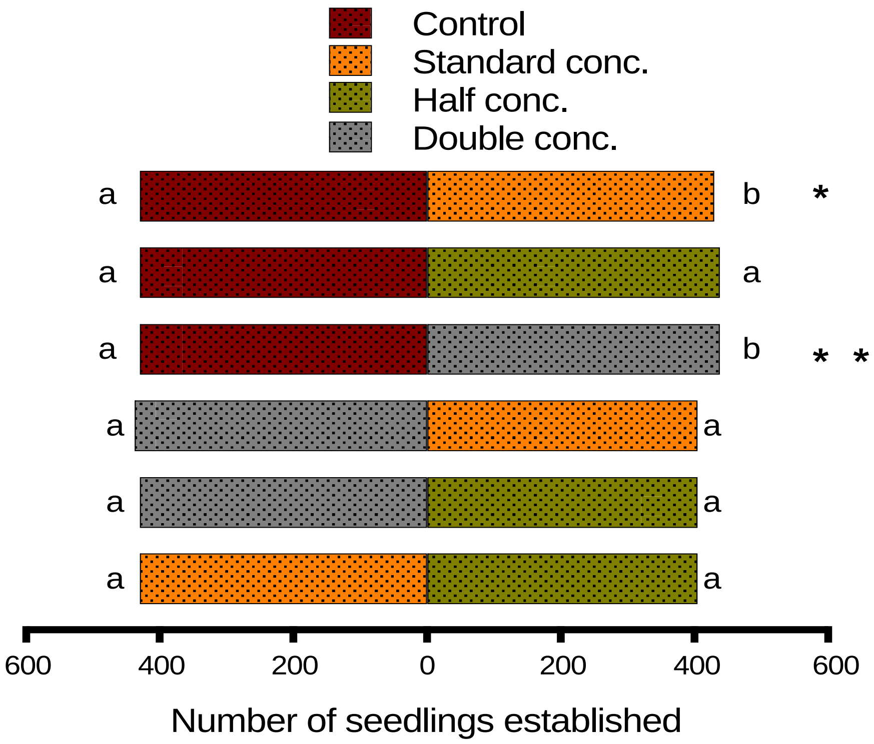

Canonical2
Canonical1
3
2
1
0
-1
-2
-4
-3
-2
-1
0
1
2
3
HYMENOPTERA
DIPTERA
HEMIPTERA
COLEOPTERA
AMF
NO AMF
Fallow

Figure 3.5: Results of separate pairwise comparisons following the Chi-square tests of growth assays conducted in lab ($X^2$). (a) seedling germination and (b) seedling establishment; at three AMF concentration levels and control. X-axis represents the number of seedlings. Different treatments have been represented by different colors in the graph. Significant differences are represented by different lowercase alphabetical letters at *P*<0.05 while asterisks (*) denote the significance at *P≤0.05, **P<0.01, ***P<0.001* and *****P<0.0001*.

(a)



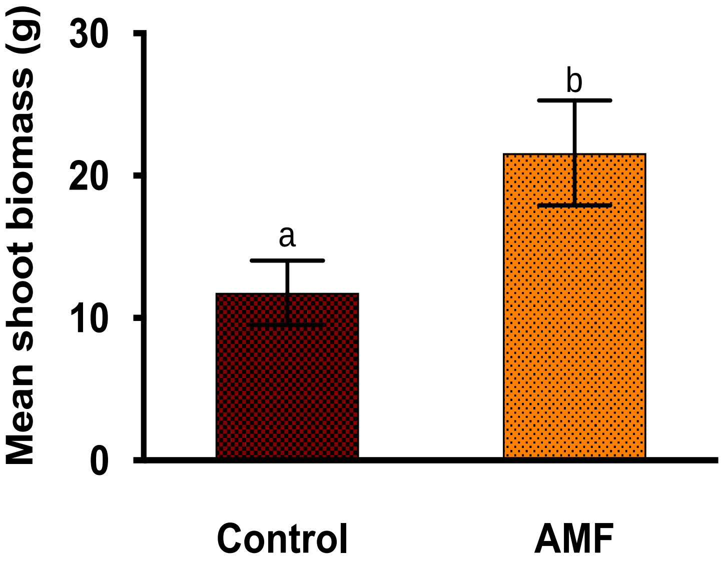
(b)



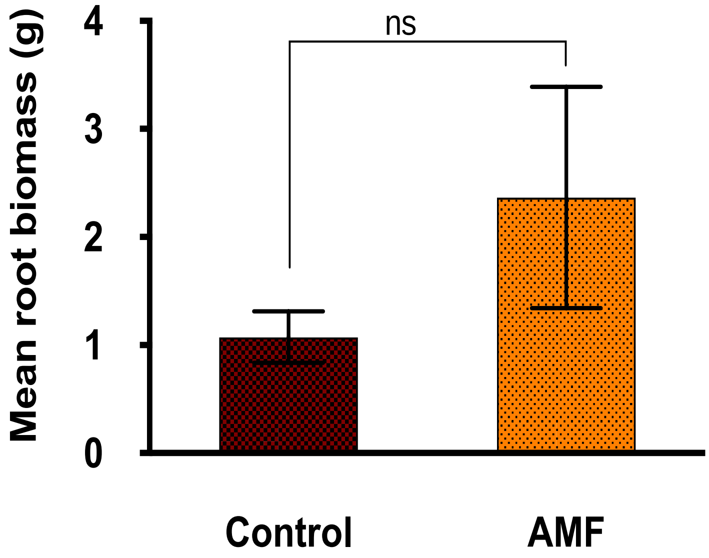
Control
Standard conc.
Half conc.
Double conc.
a
b
*
a
a
a
b
* *
a
a
a
a
a
a
600
400
200
0
200
400
600
Number of seedlings established

Figure 3.6: Results of plant biomass analysis from field collected samples and lab experiments. (a) shoot dry biomass (g), (b) root dry biomass (g) and (c) total dry biomass of the plants (g) from field; (d) shoot length (cm), (e) root length (cm) and (f) total dry biomass of the plants (g) from the lab experiments. Mean and Standard error of the results of the two tailed *t tests* of plant dry biomass data collected from the field experiments (y-axis) and one-way ANOVA of plant dry biomass data collected from the lab experiments (y-axis) at three AMF concentration levels. X-axes and different colors represent different treatments comprising of different AMF concentration levels at which the seeds were inoculated. Significant differences are represented by lowercase alphabetical letters at *P*<0.05, while ns denotes non-significant results.

(a)

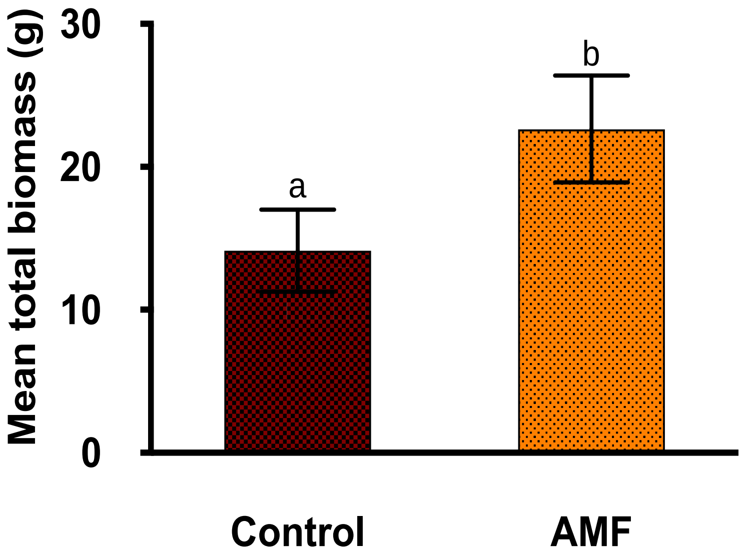


(b)



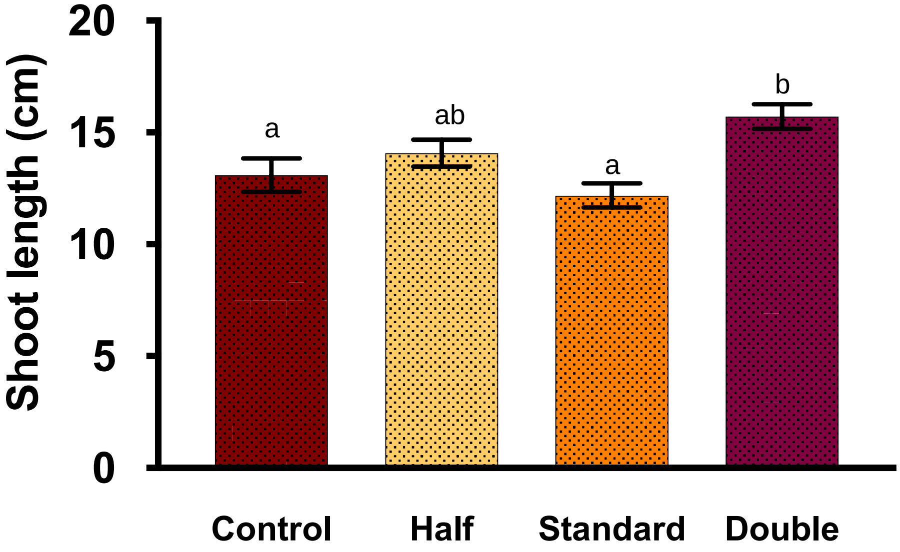
Mean root biomass (g)
4
3
2
1
0
ns
Control
AMF

(c)

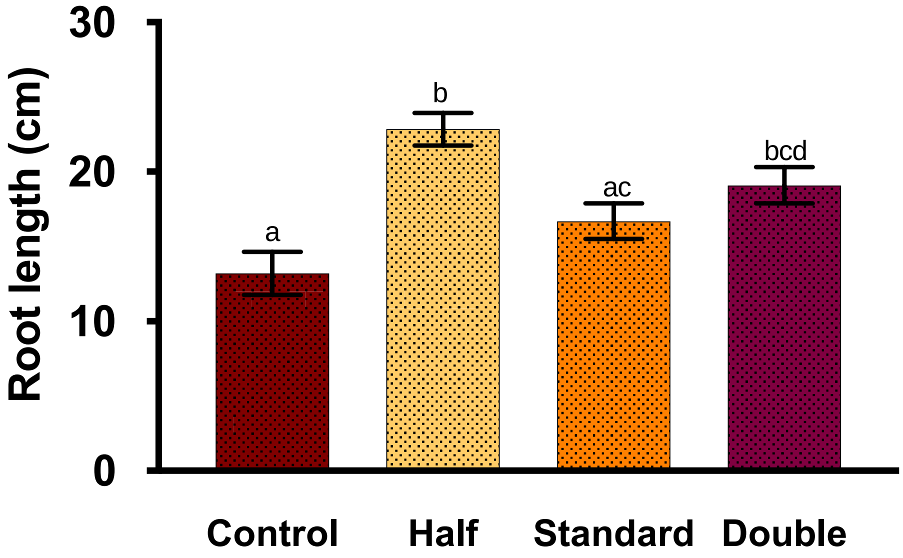


Mean total biomass (g)
30
20
10
0
a
b
Control
AMF

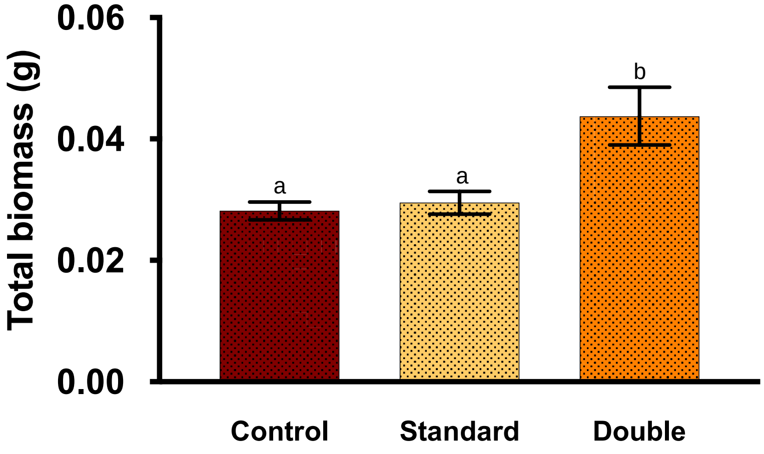
(d)


Shoot length (cm)
20
15
10
5
0
a
ab
a
b
Control
Half
Standard
Double

(e)


Root length (cm)
30
20
10
0
a
b
ac
bcd
Control
Half
Standard
Double

(f)



## Supplementary information

Data 3.S1. Different mycorrhizal species included in the mix.

*Glomus intradices, G. mosseae, G. aggregatum, G. etunicatum, G. clarum, G. deserticola, G. monosporum, Paraglomus brasilianum, Gigaspora margarita, Rhizopogon villosullus, R.luteolus, R. amylopogon, R. fulvigleba, Pisoli-thus tinctorius, Scleroderma cepa, S. citrinum, Trichoderma harzianum and T. konigii.*

Figure 3.S1. Feeding damage by fall armyworm (*Spodoptera frugiperda)* on Sorghum-sudangrass *(Sorghum x drummondii)*. Credit: Jasleen Kaur, UTRGV

Figure 3.S2. Schematic of the trap set up for insect community experiment enclosing Sorghum-sudangrass *(Sorghum x drummondii)*. It is a concept modified from Kariyat *et al*., 2012 (34), drawing modified from Kariyat *et al*., 2018(48); Credit: Annette Diaz, UTRGV

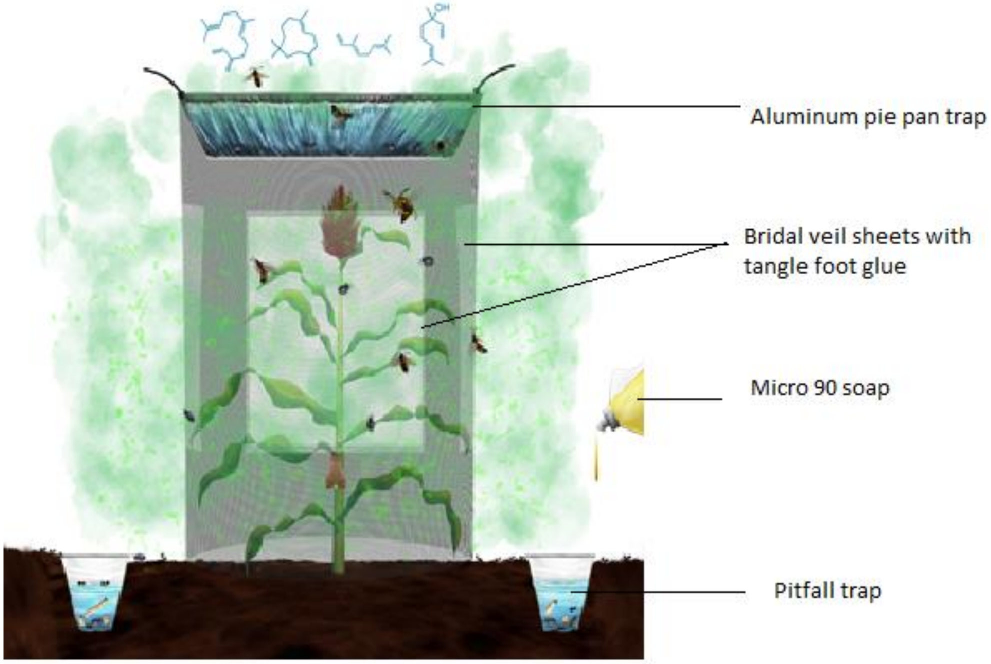


Figure 3.S3**.** Picture of unique three trap cage set up enclosing Sorghum-sudangrass *(Sorghum x drummondii)* in the field to collect attracted insect community.

# CHAPTER IV

# CONCLUSIONS AND FUTURE DIRECTIONS

To the best of my understanding, this thesis is the pioneer study conducted to demonstrate the effects of seed treatment with arbuscular mycorrhizal fungi on the defenses of Sorghum sudangrass (*Sorghum x drummondii;* Family: Gramineae) against insect pests and altered insect community dynamics. Besides, I believe that the following reasons makes these experiments novel: no studies have examined the recruitment of beneficial insects by AMF treated *S. drummondii* and also, no studies have examined whether seed treated AMF enhance growth in lab (no native AMF) at different concentrations. Since *S. drummondi* is a major cover crop in the Lower Rio Grande Valley of Texas and is integral to organic and sustainable agricultural programs, this study will have implications for sustainable management in such agroecosystems.

Most importantly, the experiments were conducted in an organic field owned and operated by local farmers, in South Texas, United States. We found that AMF can impart both growth and defense benefits to the plants. My field experiments show a positive impact of AMF on the growth traits of Sorghum sudangrass, thereby, reconfirming the results of previous studies conducted using AMF. In these traits, we found that AMF can positively enhance plant height, number of leaves, girth and consequently, the total biomass of the plant. It was found that in early season of plant growth, FAW incidence and damage was lower in AMF inoculated plants than control plants but no significant difference was observed later in the season. Therefore, we suggest that early season is the crucial stage when AMF imparts simultaneous benefits to plants for both better growth traits and enhanced defenses against the insect pests. Further, we found greater recruitment of Hymenopteran insects, a group of beneficial insects that includes various parasitoids and pollinators, towards AMF inoculated plants. This suggests that AMF possibly alters the plant chemistry, which causes changes in the herbivore induced plant volatiles (HIPVs) to attract the insects from third trophic level. The results from our lab experiments focused on seedling germination, their establishment and biomass. These experiments also reconfirmed the positive effects of AMF growth traits as observed in the field study, in a controlled environment without the possible confounding effects of native AMF in the field. Besides, we also found dose dependent effects of AMF on plant growth under sterilized soil conditions in lab. Interestingly, the AMF inoculated plants did not reveal any trade offs for resources investment towards growth and defenses during early season in the field study.

Future experiments should be focused on examining the implications of altered plant chemistry due to AMF inoculation, and experiments in chemical ecology can lead to the understanding of underlying mechanisms of multitrophic effects that we found in field. Therefore, we plan to conduct a series of lab experiments using sorghum sudangrass and yellow sugarcane aphid (SCA), another major pest prevalent in the south Texas. In our recent survey, we have observed significantly high numbers of SCA incidence in farmer fields in the region. Therefore, it would be interesting to determine whether alteration of plant chemistry (nutrition/secondary metabolites) in AMF treated plants has any effects on the SCA fecundity rates. Additionally, it will be fascinating to compare the feeding behavior of the aphids on AMF inoculated plants and the control plants through Electrical Penetration Graph (EPG) technique. This technique can provide insight into the feeding patterns of SCA on the plants with or without AMF. Any differences in the results of both population assay and EPG experiments could suggest the presence of secondary metabolites, determining plant defenses against herbivory. Next, it would be interesting to examine whether AMF alters the plant olfactory cues influencing host location and selection for feeding by SCA. Therefore, choice assays with SCA can possibly help to determine if any altered herbivore induced plant volatiles (HIPVs) regulate their host preference. Consequently, the use of push-pull based volatile collection system coupled with Gas Chromatography- Mass spectrometry can help in understanding the chemical composition of the plants. This can help determine any differences in the HIPVs of plants with and without AMF.

Also, defensive roles of the proteins polyphenol oxidases (PPOs) have been widely studied in the past. It has been established that PPOs are the part of certain defense related signaling pathways in plants, activated in response to herbivory.  Therefore, it will be interesting to study whether AMF inoculation helps the plant in the production of PPOs in plants under herbivory. Finally, using RT-PCR, we can determine the differences in transcript levels and expression pattern of the genes involved in plant defense networks (e.g., Jasmonic and salicylic acid pathways) in plants under herbivory in each treatment. I believe, these experiments will help in studying the mechanistic reasons for the results observed in my thesis and further strengthen our understanding of the potential role of AMF in mediating multitrophic interactions.

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